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# Multiple Sclerosis: A Review of The Epidemiology, Pathogenesis, Genetics, And Molecular Characteristics

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## Abstract

MS is an autoimmune and inflammation-mediated syndrome that causes neurodegeneration and makes the sufferer disabled and incapacitated. The clinical patterns and susceptibility to the disease are different in different populations. Since 1990, The occurrence of MS has risen in various geographic areas, particularly in counties of low and middle income, imposing significant health care and economic burden. Most cases of MS are sporadic but 20% are acquired. Familial Multiple Sclerosis (FMS) is characterized by the presence of at least one affected family member, including those related by first, second, or third-degree connections to the pro-bands. It is estimated to have a global prevalence of approximately 12.6%. The prevalence of MS ranges widely among Arabs and is lower than in Western countries but the rate is expected to increase over time and thus required the attention of the scientific world. The etiological features of the disorder are still unknown and extensive genetic level research is still required to make progress in understanding the causation of the disease. Furthermore, future investigations should include well-designed clinical trials examining the efficacy and safety of licorice-derived drugs in MS patients to bridge the gap between preclinical discoveries and clinical applications. These trials can provide useful insights into the real-world applicability of licorice-based treatments, perhaps influencing their incorporation into routine MS treatment procedures. This study will provide insight for future researchers who are willing to work in the area of medical genetics.

**Keywords:** Autoimmune Disease, Epidemiology, Genetics, Multiple Sclerosis, Pathogenesis

## 1. Introduction

Multiple Sclerosis (MS) is a persistent autoimmune inflammatory condition that affects the central nervous system, leading to various neurological impairments. The disease causes abnormalities that disrupt the nerve impulse and results in various neurological disorders. The patients become incapacitated or disabled due to uncontrolled nervous system deterioration. Their mobility is compromised and they require constant care (Alonso & Hernán, 2008). Most of the MS cases are sporadic but 20% are hereditary (Ramagopalan et al., 2011).FMS is characterized as a subtype of MS where individuals with the condition have at least one family member, including those related by first, second, and third-degree connections, as well as relatives of pro-bands, who are also affected by MS (Hader & Yee, 2007).

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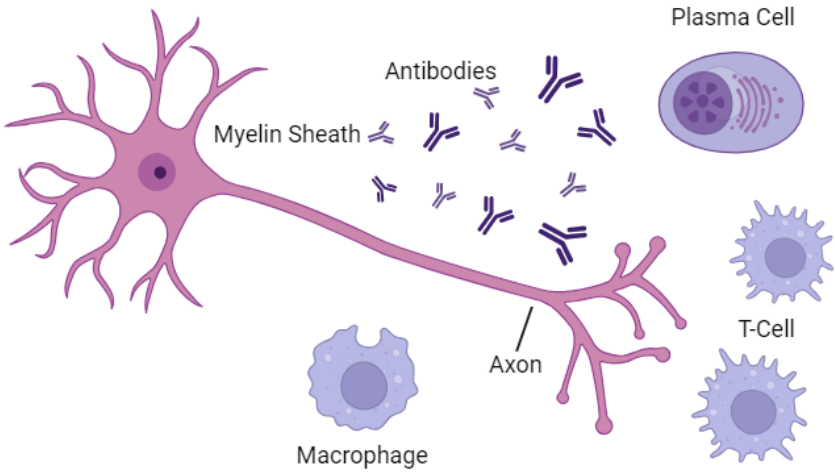


**Figure 1** Autoimmune attacks on Myelin

MS is a common inflammatory neurological condition, affecting approximately 33 individuals per 100,000 worldwide. This disease presents distinct clinical patterns and susceptibility factors that vary among different populations. Notably, since 1990, the prevalence of MS has seen a substantial increase, particularly in low and middle-income countries, resulting in considerable healthcare and economic burdens (McGinley, Goldschmidt, & Rae-Grant, 2021). The earliest documented case of FMS dates back to 1933, as reported by Curtius. The prevalence of MS demonstrates significant variations between countries, with higher rates observed in Europe and North America, exceeding 100 cases per 100,000 population, while lower rates are seen in Sub-Saharan Africa and Eastern Asia, with only 2 cases per 100,000 population (Vidal-Jordana & Montalban, 2017). The worldwide FMS prevalence was reported at 12.6% according to a recent meta-analysis and systemic review of FMS occurrence. This study has also shown that there is a great diversity in prevalence among different countries such as 32.7% in Saskatchewan, and 2% in Hungary. Another study investigated that there is a higher FMS risk in males than females in Iran (Vidal-Jordana & Montalban, 2017).

The Arab population is predominantly spread across two major continents, Asia and Africa, comprising a total of 315 million individuals. Additionally, there are approximately 12 million Arabs residing in North America and Europe (Morgan, 2008). Within the same geographic region, other non-Arab ethnic groups such as Kurds, Black Africans, and Berbers also reside (Ahmed 2017). It's important to note that data on MS incidence is available primarily for specific Arab populations, including Jordanian, Saudi, Palestinian, Iraqi, Omani, Libyan, and Kuwaiti populations (Hajjej, Almawi, Arnaiz-Villena, Hattab, & Hmida, 2018).

A review spanning from 1975 to 2007 revealed varying MS incidence rates among different Arab populations. For instance, in the Arab-born population residing in Greater Jerusalem, the incidence of MS was 0.7 cases per 100,000 individuals per year. In contrast, the incidence rate in Kuwait was higher at 2.08 cases per 100,000 population per year (Sinniah et al., 2014).

The prevalence of MS ranges widely among Arabs and is lower than in Western countries. The rate is expected to increase over time. The clinical pattern of MS is comparable to “Western countries” but there are problems with diagnostic testing and optic-spinal disease features.

Furthermore, the data on MS prevalence in Arab immigrants and Arab- Americans in Europe could be helpful to understand the migration effects from lower to higher risk areas (Benamer, Ahmed, Al-Din, & Grosset, 2009).

The precise pathogenesis and etiology of the disease remain elusive, given that it involves intricate interactions between environmental and genetic factors. Numerous studies have pointed to a combination of genetic, infectious, and environmental factors as potential contributors (Ghasemi, Razavi, & Nikzad, 2017). Moreover, the prevalence of MS tends to rise following adolescence, peaking between the ages of 25 to 35. Genetic predisposition is recognized as a significant factor in the development of MS, and it is noteworthy that females are more frequently affected by the condition than males (Loma & Heyman, 2011).

Genetic factors contribute to approximately 30% of the susceptibility to this disease. Over 100 genes and gene loci have been associated with MS . Numerous studies have demonstrated a familial tendency for MS, as well as a high level of concordance among twins and relatives of affected individuals (Thorsby & Lie, 2005). While some genetic variations have been examined among family members of MS patients, the results have not provided definitive evidence regarding the specific genes involved in the disease (Wolters & Wijmenga, 2008). The complex etiology of MS has been recognized by researchers, who also note that a number of genes, including proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), and myelin basic protein (MBP), may be susceptible. These genes are regarded as potential genetic loci for vulnerability to FMS. However, their direct linkage to the disease remains a subject of controversy and requires further investigation to pinpoint the exact genetic contributors (Amos, Driscoll, & Hoffman, 2011).

Beyond genetic factors, MS onset is influenced by various environmental factors. These include factors such as smoking, the Epstein-Barr virus (EBV),

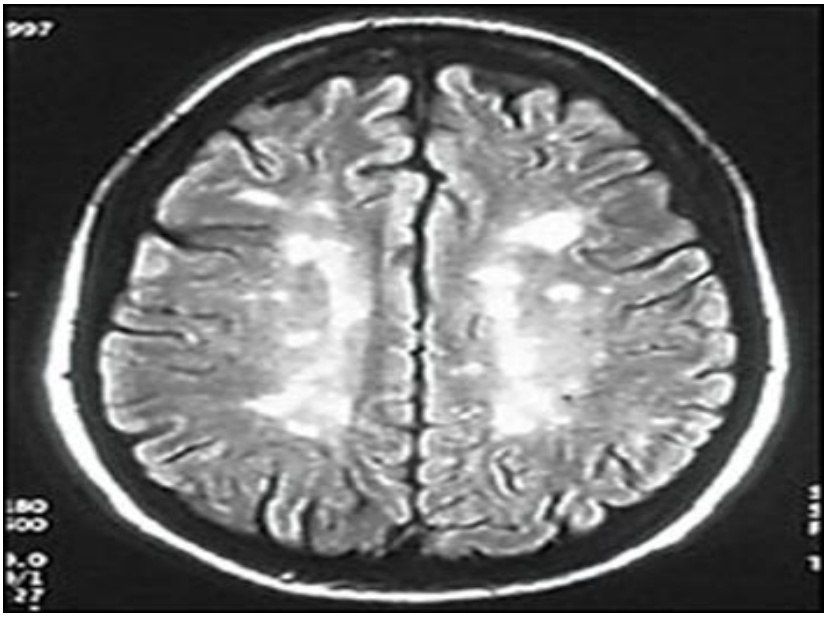

**Figure 2** Fluid attenuation inversion recovery (FLAIR) transverse section image of the brain with multiple sclerosis. The lesions are shown in high signal white matter and periventricular region (Double Inversion Recovery Brain Imaging at 3T: Diagnostic Value in the Detection of Multiple Sclerosis Lesions, 2007)

and fluctuations in serum vitamin D levels. Moreover, environmental elements such as obesity and exposure to infectious agents also play a role in MS development (Mechelli et al., 2010). Diagnosing MS relies on the detection of lesions that are both spatially and temporally distributed within the central nervous system. It's important to note that relying solely on a single diagnostic test is not a reliable approach for identifying MS due to the possibility of other medical conditions presenting with similar symptoms (Handel, Giovannoni, Ebers, & Ramagopalan, 2010).

Since there are still limitations and areas to overcome to open new horizons in research toward medical success in the prevention and cure of this disorder. Novel research is needed for the identification of causative variants that contribute to the development of the disease via the latest and defined research methods and optimum research design (Wingerchuk, 2011).

## 2. Pathogenesis and clinical features

The pathophysiological characteristics of MS can be divided into two phases: (1) inflammatory, and (2) neurodegenerative as shown in Fig. 1 (Steinman 2001). The pathogenesis mechanism and autoimmune attack on the myelin leading to MS is shown in the figure 1 below. The most common clinical symptoms of MS involve loss of balance and coordination, bladder dysfunction, numbness, mood changes, weakness, cognitive dysfunction, pain, fatigue, and vision impairment (P. S. Rommer et al., 2019). The figure 2 below represents the fluid attenuation inversion recovery (FLAIR) transverse section image of the brain with MS.

## 3. Genetics study of MS:

A Genetics study of MS revealed that it is a polygenic disease like asthma disease and diabetes. which suggests that certain underlying mechanisms beyond DNA assist the onset of disease (P. Rommer & Zettl, 2022). A wide spectrum of factors falls within this category, encompassing environmental elements such as viral infections, smoking habits, and dietary factors, as well as epigenetic factors that include histone modifications, non-coding RNAs, and DNA methylation. Furthermore, the causative factors of MS involve numerous genes and pathways (Kister, Bacon, & Cutter, 2020).

T cells may assault brain cells directly if their T cell receptors are altered or mutated. T lymphocyte activation is a two-step procedure. The contact between an antigen presented by the MHC on the surface of antigen-presenting cells (APCs) and the T cell receptor (TCR) results in the first signal. The second signal is produced when co-stimulatory receptors on T cells are bound by ligands on APCs. T cells undergo apoptosis in the absence of this second signal, essentially blocking the start of an immune response (Hecker, Bühring, Fitzner, Rommer, & Zettl, 2021). Through analysis, it has been observed that the G allele in rs231775A > G of the CTLA-4 gene is linked to a higher susceptibility to MS only in individuals with the HLA-DRB115:01 genotype. It's important to note that studying the CTLA-4 gene alone, without considering HLA-DRB115:01, does not confer susceptibility to MS. Further analysis has shown that heterozygotes with AG have a 1.5 times higher risk of MS than individuals with the AA genotype, while GG individuals have a 2-fold higher risk than those with the AA genotype (Wagner et al., 2015). Signal peptides play a crucial role in transporting the protein to the lymphocyte surface, and even slight alterations in a signal peptide can affect the intracellular transport of the CTLA-4 molecule, influencing its accessibility on the surface of T cells. A missense mutation (Thr>Ala) results in changes to the overall signal peptide structure, directly impacting the inhibition of T cell activation via CTLA-4 (Borysewicz-Sańczyk et al., 2020).

### *3.1. Familial Occurrence Of MS*

It has been observed that consanguinity is prevalent in the Arab population and

FMS is quite common in several countries, however, there is an obvious shortage of beneficial population-based genetic studies in the Arab population. The genetic basis of MS was laid with the identification of familial aggregation in the 1980s (Rodríguez-Sánchez et al., 2023). The contribution of genetics to MS is raised from both familial and twin clustering studies. Both identical and monozygotic twins have been observed to showcase significantly greater clinical concordance rates (25% to 30%) than fraternal or dizygotic twins (3% to 7%), a difference that has a possible impact on the less penetrance of this ailment, that is, the lower chance that a particular genotype would express in MS. 15% TO 20% MS patients has demonstrated familial occurrence of MS that is much more than the prevalence in general population (Eichhorst et al., 2019).

The risk is greatly increased for first-degree relatives of MS patients and is estimated to be 3%. Particularly, siblings have a 4% risk, whereas parents and children have a 2% risk. This risk is 10 to 30 times greater than the age-adjusted risk in the general population, which normally varies from 0.1% to 0.3%, and is three times higher compared to second- and third-degree relatives, who have a 1% risk (Agre et al., 2020). Complete siblings are more vulnerable than half siblings, and the risk increases when both parents have MS. However, adopted people and step-siblings of index cases run the same risk as the general populace. As a result, there is a nonlinear link between the degree of genetic material shared with the sick family member and the probability of FMS recurrence. Monozygotic twins had a recurrence risk of 18.2%, whereas siblings have a risk of 2.7%, resulting in a sibling relative risk (s) of 16.8 according to a thorough meta-analysis of over 500 papers (Hu et al., 2019).

One study from Iran reported that MS is prevalent among sisters whereas the father-son relationship has the lowest association (Salehi, Almasi-Hashiani, Sahraian, & Eskandarieh, 2020). Mohammed et al. found that parental consanguinity is more common in FMS patients and reported evidence that the increasing prevalence of MS in Saudi Arabia could be multifactorial (Evans, Levasseur, Cross, & Piccio, 2019).

According to research, the NLRP1 gene possesses a mutation (Gly587Ser) that may be the cause in the families studied, pointing to an autosomal recessive genetic tendency. These variations demonstrated elevated IL-1B gene expression, active cytokine production, and general immunologic pathway activation linked to NLRP1. This variant comprises altered amino acid residues of NACHT, an evolutionarily highly conserved domain, that was required for the correct NLRP1 inflammasome assembly and oligomerization (Tupik, Nagai-Singer, & Allen, 2020). The pathogenic variant of NLRP1 can be validated by the reported genetic associations and continuous activation of NLRP1 inflammasome that directly lead to the constant overproduction of mediators such as IL-1β as produced in MS and malignant melanoma tissues. IL-1β is involved in different types of inflammatory and neurodegenerative processes that occur during MS, evidence also suggests that IL-1β is involved in the migration of activated T cells across the blood-brain barrier. IL-1β is usually present in CNS lesions and its concentration increases in CSF fluid in MS patients (Muela-Zarzuela et al., 2023).

### *3.2. Candidate Gene Studies*

The initial population studies investigating hereditary factors in MS laid the foundation for identifying genetic contributors. However, the early phase of association studies was hindered by numerous small-scale investigations that lacked the statistical power needed to identify genuine associations. The MS gene database (msgene.org) serves as a comprehensive historical repository containing over 700 genetic studies pertaining to MS. Despite the limited

confirmation of genetic findings in MS studies, only a handful of authentic associations have been established. Notably, the human leukocyte antigen (HLA) gene cluster located on chromosome 6p21 has emerged as the most significant locus in MS research, both through candidate studies and microsatellite markers (Vilariño-Güell et al., 2019).

In a study conducted by Ogawa et al., the association between HLA genes and MS susceptibility was investigated. Their findings confirmed that the most robust association with MS was linked to HLA-DRB115:01. Through a stepwise conditional analysis, they were able to identify HLA-DRB104:05, HLA-B39:01, and HLA-B15:01 as independent factors contributing to MS susceptibility. This reaffirmed prior research that had already established a strong connection between HLA genes and MS, with HLA-DRB1*15:01 having the most substantial impact on susceptibility to the disease. Importantly, both class I and class II HLA genes were found to independently confer susceptibility to MS (Szymczak, Colli, Mamula, Evans-Molina, & Eizirik, 2021). Furthermore, the study identified two specific MS risk factors associated significantly with HLA-DRB1*15:01 and a particular amino acid, phenylalanine 9, at HLA-DQβ1. The exact mechanisms by which these variants are formed or exert their effects remained unclear. Nevertheless, based on their validation efforts, the study suggested that HLA-DQβ1 Phe9 plays a causal role in HLA gene variants contributing to MS (Kaufmann et al., 2022).

### *3.3. Genome-Wide Association Studies (GWAS)*

The first genome-wide association study (GWAS) on people with MS was carried out by the International MS Genetics Consortium (IMCGC), and the results were published in 2007. In addition to a replication cohort made up of 2,322 MS patients and 2,987 healthy people, this study included 931 family trios (Wen & Yu, 2023). In the original GWAS, a variable known as rs12722489, located in the first intron of IL2RA, was identified as a significant genetic connection in MS that was not associated with the major histocompatibility complex (MHC). The interleukin-2 receptor, which plays crucial roles in several immunological pathways, is encoded by the IL2RA gene. More recently, the largest genetic study on MS to date, which included 47,351 patients and 68,284 controls, identified 233 genome-wide loci that increase the chance of getting MS (Afrasiabi et al., 2019).

MS has been linked to 17 genetic variations discovered through whole-exome sequencing, according to genome-wide association studies. Of these 17 variations, they found one in the TRIOBP gene that was significantly different from the average person without MS and multiple sclerosis. The amino acid Ala>Ser was substituted as a result of this missense variation (Chr22: 37723520G>T, Ala322Ser, rs201693690), which was present in exon 7 (Ahangari et al., 2022). Another research, which was reported in ExAC, discovered a connection between it and schizophrenia and deafness. which was described in ExAC, identified a link between it and deafness and schizophrenia. Compared to the ExAC research, the allelic frequency of this variation in MS patients was much higher (19% vs. 0.05%). The development of the risk of MS may be linked to the existence of this variation. Despite the fact that the TRIOBP gene is associated with the control of cell migration and proliferation, aberrant protein aggravation can result in persistent mental disease (Shepard, Cline, Hinds, Jahanbakhsh, & Prokop, 2019).

### *3.4. Rare Variants*

The attainment of GWAS in the discovery of common variations has opened doors for the detection of rare variants. Exome sequencing has been used to investigate MS cases from 43 families that had four or more index cases and identified a rare pathogenic variant in the CYP27B1 gene that led to total loss of

gene function in one of the participants. However, extensive replication and specific sequencing research failed to reproduce the results (Goris, Vandebergh, McCauley, Saarela, & Cotsapas, 2022). One more research discovered that a mutation p.Arg415Gln in NR1H3 leads to an extreme and progressive mendelian form of MS. No confirmation was possible in research of 36,538 controls and 32,852 cases. Furthermore, one research that used the Exome Aggregation Consortium (ExAC) discovered numerous individuals with p.Arg415Gln in NR1H3 but no testified MS or relevant disorder. A handful of studies have been reported that identified rare pathogenic variants in the Arab region through exome sequencing (Hočevar, Ristić, & Peterlin, 2019). One paper performed a replication analysis of non-HLA rare MS pathogenic risk variants in the Kuwaiti population of 170 cases and 311 controls. The researchers studied the association of a set of risk variants of MS and authenticated the outcomes in replication research of a uniform Kuwaiti background. Till 2018, no evidence has been found related to the monogenic MS, and at-length investigation via exome sequencing methods needs to be used. Any associations in MS for rare pathogenic variants are still to be proven (Villarreal et al., 2021).

# 4. Environmental factors

## *4.1. Relation to Infections*

Different infections have been implicated in the pathogenesis of MS, usually in constant association with past infections of Epstein-Barr virus (EBV) (Ahmed, Aziz, et al. 2019). The relationship between EBV and MS has been studied in Kuwait and both have mentioned conflicted results. One report has shown no relation between EBV and MS, and the other one exhibited a substantial association (Baker, Amor, Kang, Schmierer, & Giovannoni, 2020).

Measles, a member of paramyxovirus, is quite contagious. The transmission of the virus occurs via direct contact or through airborne droplets and affects the air tract, with consequent extends to the entire body. Three studies have been conducted, two of them were carried out in Saudia, and one in Kuwait. The studies from Saudia showed that measles make individuals more susceptible to MS. In contrast, the report from Kuwait showed no association between measles and MS (Maroufi, Mortazavi, Sahraian, & Eskandarieh, 2021).

For human herpesvirus-6 (HHV-6), one report conducted in Kuwait showed no relevance to MS risk (Maroufi et al., 2021). The same is the case for mumps reported in a study from Kuwait. For varicella-zoster virus (VZV), three studies were conducted, one in Kuwait and two in Saudia, and all three of them have reported no association with MS risk (Alluqmani, 2023).

## *4.2. Role of Vitamin D*

The concept of vitamin D as an explanation for the latitude gradient was first proposed by Goldberg in 1974. In humans, the primary source of vitamin D is exposure to ultraviolet B (UVB) radiation from sunlight (Gombash, Lee, Sawdai, & Lovett-Racke, 2022). It has been studied in a retrospective observational report of about 10,709 individuals in Saudi clinics and hospitals, that 83.6% of the cases were deficient in vitamin D (Gombash et al., 2022), which is quite high considering its contribution towards MS risk (Gombash et al., 2022).

Correction of vitamin D levels might influence the cognitive activities in MS cases, apart from its function in bone health and immune defenses. Recent research has revealed that while vitamin D plays a role in the latitude effect associated with sunlight exposure, it is not the sole mediator, but rather an associated risk factor for MS . Vitamin D's immunomodulatory effects on the immune system have implications for its impact on MS risk, as do the persistence and severity of Epstein-Barr virus (EBV) infection (Galoppin et al., 2022).

One study has reported an inverse relationship between EBV DNA load and

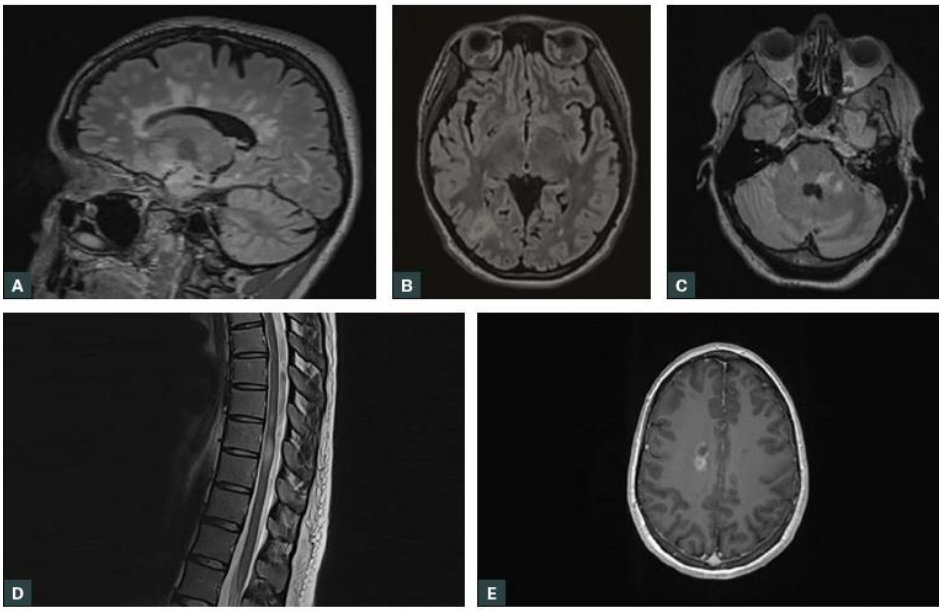


**Figure 3** A Typical multiple sclerosis magnetic resonance imaging finding from several patients (Travers et al., 2022)

serum 25(OH)D levels in individuals without MS. However, this study did not provide significant evidence to support an increased risk of MS due to interactions between vitamin D deficiency and EBV infection. It suggests that while both vitamin D and EBV infection may be relevant factors in MS, their combined effects in increasing MS risk are not well-established (Brütting, Stangl, & Staege, 2021).

### *4.3. Smoking*

In a study of a North American cohort, more than 50% of MS patients were smokers and the patients are heavier smokers than the general population of smokers, and they keep on smoking even after diagnosis of the disease. It is also common in people of the Middle East and North Africa, particularly in males, having a prevalence of about 50% in several countries of MENA (Hanaei, Sahraian, Mohammadifar, Ramagopalan, & Ghajarzadeh, 2021).

Sundstrom et al. examined plasma cotinine levels as a measure of tobacco exposure and found an increased risk in females with passive cigarette smoke exposure. One meta-analysis observed the impact of smoking on dose response on MS risk, but the authentication methods did not appear to be reliable. It has been studied that pregnant smokers do not increase the risk for the progression of MS in the exposed unborn child, however, in another case it turned out to make the child more susceptible to MS (Miclea, Bagnoud, Chan, & Hoepner, 2020).

### *4.4. Dietary Intake and Supplements*

Both in vitro (conducted in controlled laboratory environments) and in vivo (conducted in living organisms) studies have indicated that lower sodium intake and reduced intracellular sodium concentrations can have a protective effect on the immune system. However, the findings of a recent case-control study suggest that there is no significant association between dietary sodium intake and the risk of developing MS . These results imply that the relationship between dietary sodium and MS risk may not be straightforward and require further investigation (El-Salem et al., 2021).

### *4.5. Impact of Pollution*

The relationship between air pollution and MS is still not fully understood. However, there is evidence suggesting a potential connection. Exposure to particulate matter in the air can initiate an inflammatory response in the lungs, leading to increased levels of systemic inflammation and inflammatory cytokines. Prolonged exposure to air pollution has also been linked to damage to the blood-brain barrier and neuro-inflammation (Abbaszadeh et al., 2021).

Several studies have indicated a significant association between MS and exposure to specific air pollutants, including carbon monoxide, lead, and sulfur dioxide. These findings suggest that air quality and pollution levels may play a role in the development or exacerbation of MS, although further research is needed to fully elucidate the mechanisms and extent of this association (Bergamaschi et al., 2022).

## 5. Diagnosis

Diagnosing MS relies on the presence of lesions that are disseminated both in space and time within the central nervous system. Relying solely on a single diagnostic test is not considered reliable for identifying MS, as various other medical conditions can present with symptoms similar to those of MS, making it necessary to consider multiple clinical and diagnostic criteria for an accurate diagnosis (McGinley et al., 2021). Magnetic resonance imaging is a highly sensitive detection method for silent MS plaques. The clinical findings of imaging are included in the diagnostic criteria proposed by investigators. It explains the characteristics of MS lesions, such as location (infratentorial, spinal, juxtacortica), number (nine or more), and lesion enhancement using a contrast medium (Solomon et al., 2023).

A brain MRI is considered a confirmatory diagnostic tool for MS (Figure 2). It helps identify lesions in areas with high signal intensity, such as the spinal cord or cerebral white matter. MRI provides detailed structural information about these lesions in regions including the cervical cord, craniocervical junction, and posterior fossa. It plays a crucial role in visualizing the pathological changes associated with MS and is a valuable tool for diagnosing the condition (Yamout et al., 2020).

Other confirmatory tests include cerebrospinal fluid analysis in which higher IgG concentration is found in MS patients. Other blood tests involve the determination of thyroid hormone level, vitamin B12, erythrocyte sedimentation rate anti-neutrophil antibodies, and T-lymphocytes, but these tests are not specific as they can mimic other disease conditions (Jakimovski, Awan, Eckert, Farooq, & Weinstock-Guttman, 2022).

A. periventricular lesions (FLAIR sequence); B. right parieto-occipital juxtacortical lesion (FLAIR sequence); C. Lesions in the brainstem and cerebellum (FLAIR sequence); D. Spinal cord lesions in the thoracic cord (T2-weighted turbo spin echo sequence); E. Gadolinium-enhancing lesion (T1-weighted sequence).

## 6. Treatment

Treatments of MS include agents for immune suppression and immune modulation since the 1980s. These conventional treatments usually do not inhibit inflammation in the central nervous system (Hauser & Cree, 2020).

### *6.1. Symptom Specific Treatments*

Symptom-specific treatment is significant to enhance life quality in younger and older MS patients. For example, a neurogenic bowl is treated with dietary fiber intake, adequate hydration, and bulk-foaming agents. Neuropathic pain is treated with specific serotonin uptake inhibitors, anticonvulsants, and tricyclic antidepressants (Brownlee, Bourdette, Broadley, Killestein, & Ciccarelli, 2020). Hydrotherapy also has the potential for pain management. Muscle spasticity is treated by Baclofen, which is a first-line agent and helps to decrease alpha motor neuron activity (Chisari et al., 2021).

### *6.2. Disease-Modifying Agents*

Disease-modifying agents are used to sustain and preserve a healthy immune system by suppressing the T-cells cascade responsible for axonal damage and demyelination. The Food and Drug Administration (FDA) has approved seven agents for the treatment of MS . These include teriflunomide, dimethyl fumarate, glatiramer acetate, natalizumab, fingolimod, mitoxantrone, and interferon beta (Otero-Romero, Ascherio, & Lebrun-Frénay, 2021). These diseases modifying therapies are effective only if they are started in an earlier stage of disease progression. The limitation of disease modification include reactions at the injection site, inflammation, edema, leukopenia, suicidal thoughts, neutralizing antibodies, elevated liver enzymes, and influenza-like symptoms (Rostami Mansoor & Ghasemi-Kasman, 2021).

The application of new potent agents having a high potential to inhibit inflammation within the central nervous system is introduced. These new drug agents are associated with adverse effects even death. Therefore, no effective treatments for the disease progression stage are available (Amato et al., 2020).

## 7. Conclusion

In conclusion, MS MS remains a complex and multifaceted disorder with significant variations in clinical patterns and susceptibility among different populations. The rising prevalence of MS, particularly in low and middle-income countries, underscores the urgent need for increased attention and research efforts to address the growing health care and economic burdens it imposes. The familial component of MS, represented by FMS, reveals the involvement of genetic factors in disease predisposition, with a global prevalence of approximately 12.6%. Despite extensive research, the exact etiological factors underlying MS remain elusive, emphasizing the ongoing necessity for rigorous genetic investigations to unravel the disease's causative mechanisms. Notably, the prevalence of MS within Arab populations, while currently lower than that in Western countries, is anticipated to rise, necessitating heightened scientific focus and collaboration to understand and manage this emerging challenge. Comprehensive genetic studies, including whole exome sequencing followed by confirmatory Sanger sequencing, hold promise in identifying rare pathogenic variants that contribute to the disease's development. These advancements not only shed light on the underlying molecular pathways driving MS but also pave the way for the discovery of novel biomarkers and potential therapeutic targets.

In the quest to decipher the intricate interplay between genetics, environment, and disease manifestation, ongoing efforts are crucial for enhancing our understanding of MS. The insights gained from unraveling the genetic underpinnings of MS through meticulous research will undoubtedly contribute to more accurate disease prediction, improved diagnostic methods, and the development of innovative treatments. As we continue to delve into the intricacies of MS, the scientific community's collaborative endeavors hold the promise of improving the lives of those affected by this challenging disorder.

## 8. Declaration Statements

***Protection of Privacy:*** Not Applicable

***Ethics Approval:*** Not Applicable, as no humans or animals were utilized in this study.

***Conflict of Interest:*** There is no conflict of interest among the authors, all the authors have contributed equally in this work.

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***Authorship Contribution Statement:*** A.A designed, proposed and supervised this study, B.A performed the literature review

and I.A proofread and revised the manuscript.

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# Emerging Perception of Activity Cliffs: A Brief Review

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## Abstract

Activity cliffs (ACs) can be characterized as the collection of structurally similar molecules with significant differences in their potencies. Such molecules are of core importance in medicinal and computational chemistry as any minute change in their structure greatly influences their biological action. They play an important role in the optimization during drug discovery and can be analyzed by structure-activity relationships (SAR), but the factors like the molecular representation, selection of the data sets, and the descriptor used greatly affect the end results. Due to these factors, ACs were thought to be a rarity as the concept is contrary to the similarity property principle (SPP), which forms the basis of quantitative structure-activity relationship (QSAR) modeling and likeness based strategies. Today, the data available on activity cliffs has been refined as well as increased a lot. In this review, we have described literature ranging from 1988 to 2021 and highlighted the factors that are important in analyzing ACs and selecting the data sets for the analysis. Moreover, several strategies including matched molecular pairs (MMP) have been developed. MMP is mostly used for finding similar molecules but having a different group(s) responsible for the change in their potency. Furthermore, the role of ML (machine learning) in ACs has also been discussed as it could further refine the analysis of ACs by developing various logarithms and minimizing the faulty results.

**Keywords:** Activity cliffs; Computational chemistry; Matched molecular pairs; Quantitative structure-activity relationship; Similarity property principle

## 1. Introduction

The groups of molecules having high structural similarities but different potencies are called activity cliffs (ACs) (Maggiora, 2006). Not only are they intriguing, but also are of prime importance in medicinal and computational chemistry and have remained under discussion for the last three decades, representing the details of SAR-discontinuity (Silipo & Vittoria, 1991; Stumpfe & Bajorath, 2012; Stumpfe *et al*., 2014). Early determination of activity cliffs and an accurate understanding of the activity landscape (AL) are indispensable for the progression of computational models designed for the prediction of the activity of molecules (Guha & Van Drie, 2008; Guha & Van Drie, 2008). AL is used to characterize SAR (structure-activity relationship) by considering two or three dimensions in which chemical space is predicted as 2D projection and potency of compounds as the third dimension, thus making AL similar to the geographical maps that can be apprehended easily (Wassermann *et al*., 2010; Waver *et al*., 2010; Peltason & Bajorath, 2010). If any insignificant

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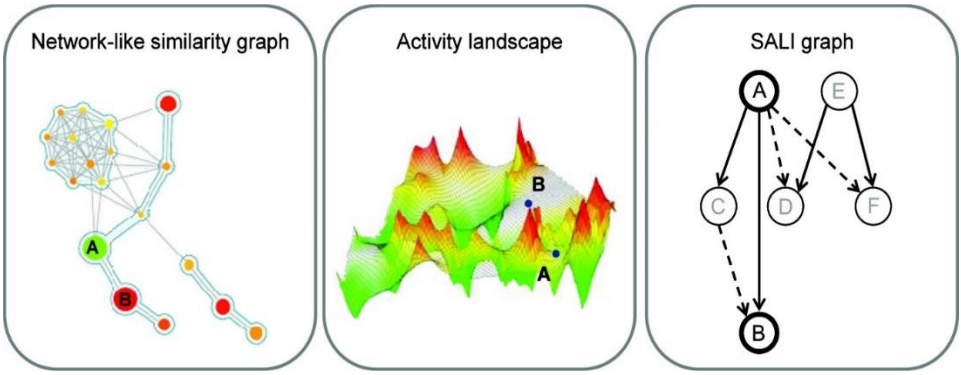


**Figure 1** AC can be represented by three methods such as network-like similarity graph, AL and SALI graph. In the first graph, compounds are represented by nodes whereas pairwise similarity relationship is denoted by edges. In the second graph (AL), chemical space between compounds is represented as 2D projection whereas the third dimension is added to represent the potency of the compounds. In the third method (SALI), compounds are denoted by the nodes and edges representing ACs of different magnitudes. Reproduced with permission from Ref. (Stumpfe & Bajorath, 2012). Copyright © 2012, American Chemical Society.

change in the chemical structure of compounds alters biological activity moderately, they are represented by smooth regions, and such compound mapping areas correspond to continuous SARs (Peltason These have proved to be advantageous for QSAR modeling and likeness-based strategies for analytical tools based on SPP (similarity property principle) (Guha & Van Drie, 2008; Bajorath *et al*., 2009; Johnson & maggiora, 1990). Contrarily, discontinuous SARs refer to the canyon like regions formed when a little modification in the chemical structure of the compounds has a drastic impact on their potencies, giving rise to activity cliffs (Waver *et al*., 2010). In other words, ACs are the form of discontinuity in SARs which is the basis of the lead optimization ((Waver *et al*., 2010; Dimova *et al*., 2013).The reliability of the data under consideration is very important before the interpretation of the SARs. Moreover, the size of the data sets is also crucial as data sets with few compounds are harder to study for activity cliffs compared to the ones consisting of a large number of compounds (Medina-Franco, 2013). The activity of these compounds can be studied efficiently against single- or multi-targets leading to single-and multi-target activity landscapes (Wassermann *et al*., 2011). A set criterion has been established and is used for the analysis of ACs quantitatively to determine high structural similarity and difference in activity. For instance, medicinal chemists use empirical rules that are beneficial in converting the qualitative AL data into quantitative one. However, empirical rules are beneficial unless misused as Lipinski's rule of five that overlooked the limitations of the rules (Faller *et al.*, 2011; Ganesan, 2008). Moreover, network-like similarity graph (NSG), structure-activity landscape index (SALI) and SARI (SAR index) can be employed to quantify SARs, and analyze ACs, as represented in the Figure 1 (Stumpfe & Bajorath, 2012; Peltson & Bajorath, 2007; Hu & Bajorath, 2012) SALI have been proposed by Guha and Van Drie to assess the biochemical SAR model and is derived from examining the activities of specific interactions that don't variate linearly with linear property changes (LeDonne *et al*., 2011).

## 2. Role of molecular structures in ACs

Molecular structures are not only crucial for obtaining actual ACs but also for drawing efficient SAR analysis. If the placement of bonds, protonation or tautomer formation is not proper, the results will be faulty. The type of descriptor can also affect the results. For instance, 2D molecular representation

showing two molecules as the same cliff can show different activity if analyzed by 3D molecular representation, thus making the existence of ACs obscure. This deviation can be explained by using the concept of stereo-isomerization according to medicinal chemists. It is accepted now that actual ACs can be determined by 3D methods compared to 2D approaches (Yongye & Medina-Franco, 2012). Moreover, multiple molecular representation approach is best for minimizing the faulty cliffs in which final results are obtained by considering common conclusions (Yongye & Medina-Franco, 2012; Medina-Franco *et al*., 2009). Additionally, the concept of "data set modelability" has been introduced by determining the effect of ACs on the working of QSAR models (Hu *et al*., 2012).

## 3. Estimating similarity between molecules

Although the interpretation and detection of activity cliffs are challenging, medicinal chemists can easily extract useful information for analyzing SARs. According to Bajorath *et al.* molecular representation is an essential aspect of AL modeling. Furthermore, practical and interpretable results of the SARs are associated with the correct interpretation of ACs (Golbraikh *et al*., 2014; Agrafiotis *et al*., 2011). The importance of molecular representation has raised many questions like, is there any descriptor that can explain the appearance of ACs? Is there any particular depiction of chemical space that can be beneficial in investigating SAR related to any target? Is there any method for the better representation of AL modeling and for the identification of factual ACs? These questions are not difficult to answer. The response to the first question is that the descriptors used for the interpretation of activity cliffs must provide the information of variables that can be helpful in determining the unknown behavior of compounds regarding activity. For instance, finger-print representation can be used to detect cliffs serving as a process for the structure-based interpretation of activity cliffs (Mendez-Lucio *et al*., 2012). If we talk about the chemical space, with the emergence of new targets and molecular libraries, chemical space can be expanded but many efforts are under process (Nguyen *et al*., 2009; Lopez-Vallejo *et al*., 2012). The last concern can be addressed considering the approach proposed by Hu *et al.* explaining which substructure relationship must be preferred on computed similarity values (Yongye *et al*., 2012). The concept of matched molecular pairs (MMPs) and MMPs-cliffs made the interpretation of results easy from chemical perspective. The main challenge is to establish an interpretable way for the determination of faulty changes in three-dimensions used in ligand-target recognition (Yongye *et al*., 2012; Agrafiotis *et al*., 2011).

## 4. Methods for the identification of activity cliffs

Many computational protocols have been reported in various publications to apply the concept of AC and their identification. Predominantly, molecular fingerprint and Tanimoto similarity coefficient referred to as molecular graph descriptors are used to calculate similarity values to determine similarity between compounds on the basis of 2D similarity molecular representation (Stumpfe & Bajorath, 2012). Another method for the identification of ACs is matched molecular pair (MPP) formalism which analyze the pair of compounds based on the molecular substructure differing at only a specific site and yield objectively significant chemical explanation (Rabal & Oyarzabal, 2012). Another conscientious approach is based on 3D structures to determine ACs in which the bound ligands represent drastic activity difference despite spatial similarities in the complex structure with a protein of interest (target) as shown in Figure 2 (Rabal & Oyarzabal, 2012).

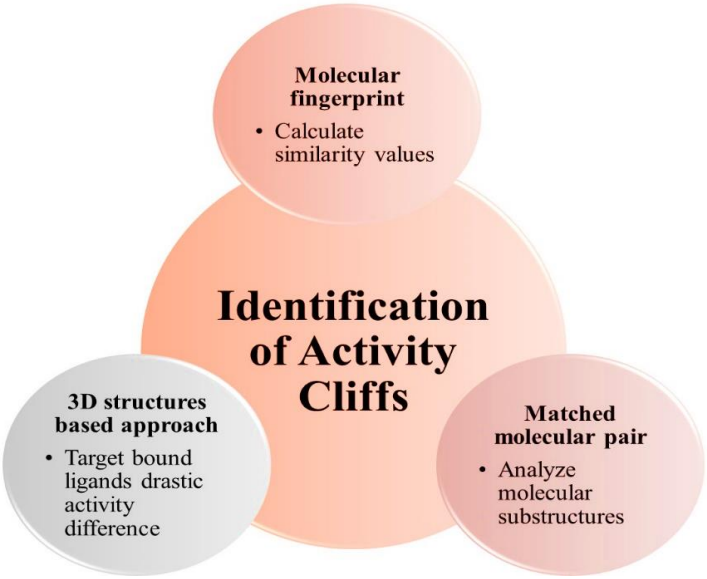


**Figure 2** It illustrates the methods for the identification of activity cliffs (ACs). The ACs can be identified by molecular fingerprint, matched molecular pair and 3-dimensional (3D) structure based approach

## 5. Convolutional neural network in forecasting activity cliffs

Recently, convolutional neural networks (CNN) have acquired catbird-seat in chemical informatics and drug designing because specific features from the image data can be obtained by deriving CNN models from 3-dimensional images of ALs and 2-dimensional images of molecular graphs (Lopez-Vallejo *et al*., 2012). Molecular matched pair (MMP) concept and its implementation is an ingenious logic for the well-ordered determination of structurally similar pairs with different potencies that are referred to as AC's (Bajorath, 2017).The use of MPPs for the representation of AC's, leads to the establishment of MPP-cliffs which are developed by the pair of compounds having potency difference but functional against the common target.[22] These MPP-cliffs predict AC's at various levels that is initiated from the utilization of support vector machine, based on compound pair-based kernel function and fingerprint representation. This support vector machine is used to distinguish between MPP-cliffs from MPPs having no or small variation in potency (Thapa *et al.,* 2020). It is a machine learning algorithm that works by constructing hyper-plane H to segregate the training data of two classes via adjusting distance between the classes in space (Iqbal *et al*., 2021). Subsequently, the methodologically simpler prediction of MPP-cliffs is carried out by implementing condensed graph of reaction formalism (Heikamp *et al*., 2012). Moreover, support vector regression is employed for the quantitative prediction of Potency difference generated by MPPs (Blaschke *et al*., 2021). MPP-cliffs are distinguished from MPPs by the fingerprint features that work by tracking back to the original compounds establishing accurate AC predictions by describing the critically crucial structures (Hussain *et al*., 2010).

## 6. Molecules-in-molecules fragmentation based method

Molecules-in-molecules (MIM) is referred to as the multi-level hybrid energy fragmentation approach based on the supposition that chemical properties are not largely influenced by the groups far off from the area of interest (Thapa *et al*., 2020; Hu *et al*., 2014). The basic principle of MIM is comprised of four steps namely (1) generation of non-overlapping small

fragments of larger molecules by fragmentation (2) devising overlapped primary substructures utilizing local interactions between fragments (3) employing inclusion-exclusion principle for forming derivative subsystem and (4) assessment of the energy of large molecules by adding the independent energies of sole substructures (Hu *et al*., 2014) In 2020, Raghavachari and co-workers have used quantum mechanical (QM) investigation to disclose the formation of ACs by using MIM fragmentation method. The main advantage was the reduced computational cost of QM estimations and calculations. Moreover, MIM method can also identify critical residues by residue specific energy decomposition analysis to distinguish between two ligands. Therefore, MIM is considered as an ideal method to apprehend ACs (Thapa *et al*., 2020).

## 7. Activity cliffs in drug discovery

ACs are important in drug discovery because a small change in the structure of a particular compound greatly influences its biological activity (Stumpfe *et al*., 2014; Thapa *et al*., 2018). In medicinal chemistry, they play an important role in early phase drug discovery for finding the determinants of high interest in hit-to-lead. Although Acs are crucial in determining SARs information, medicinal chemists encounter many hurdles in the preparation and analysis due to the duality of Acs (Bajorath, 2019). The duality of Acs is similar to the potency difference and similarity parameters for defining Acs and effect their analysis, application and perception, thus making them controversial (Bajorath, 2019). The extent of expertise of medicinal chemist to handle Acs, the computational method for the identification of Acs and the variation in the SAR discontinuity meaning while optimization of lead can result in the duality of Acs (Bajorath, 2017; Bajorath, 2019).

## 8. Strategies for finding ACs

There exist several strategies for the identification of ACs in databanks (Waver *et al*., 2010). ChEMBL is widely used database for finding the data sets that are formed with time (Stumpfe *et al*., 2013). One of them is the SALI strategy which identifies ACs by comparative scale method because the scale is not definite. SALI has a drawback as it identifies shallow or pseudo cliffs at a particular cutoff value (Waver *et al*., 2010). Some rules were set by professionals to identify the ACs. For example, a molecule must have activity in nanomolar range, an already determined likeness measure is performed, and the activity difference between two molecules must not be less than 100 fold (Waver *et al*., 2010). Matched molecular pairs (MMPs) concept has also been utilized for finding similar molecules that are distinct at some point. For example, the type of ring or an R group which is determined by the implementation of in-house algorithm as proposed by Hussain and Rea (Hussain & Rea, 2010). The characterization of ACs can be performed by various methods like presence of different R-groups and interactions between them, and 3D likeness determined by X-rays during ligand target interactions (Aguayo *et al*., 2014). It must be considered that compound data sets chosen should have reported $K_i$ values (Stumpfe *et al*., 2013).

## 9. Synthetic relevance

In chemoinformatics, molecular fingerprint descriptors form the basis for the calculation of Tanimoto similarity values for defining ACs (Stumpfe *et al*., 2014; Thapa *et al*., 2018). It is hard to understand Tanimoto similarity values as they don't depend on synthetic or analog relationship, thus MMPs (matched molecular pairs) are used to represent ACs as MMPs cliffs but with size-restricted chemical modifications (Hussain and Rea, 2010). However, there is a drawback of MMP cliffs that they are unable to

determine synthetic relationships; therefore, a modified version retrosynthetic MMP cliffs (RMMP cliffs) was proposed in accordance with retrosynthetic rules by which systematic computational framework consisting of exocyclic single bonds in MMPs was substituted with fragmentation. RMMPs have been formed systemically from currently available compounds having activity data of high confidence (Hu and Bajorath, 2018). The main advantage of retrosynthetic bond fragmentation method is the small fragment space occupancy giving rise to smaller number of RMMP cliffs compared to MMP cliffs (Hussain & Rea, 2010; Hu & Bajorath, 2019).

## 10. Target set-dependent differences in activity and optimization of compounds

It is widely considered that potency difference is already set for the determination of ACs but actually potency difference vary depending on the target set (particular pharmacological target and compound's activity class) (Hu & Bajorath, 2019). In order to avoid the ignorance of target set- dependent activity difference and enhancing SARs exploration, ACs need to be redefined. Accordingly, the threshold for AC formation is obtained by measuring the pair of compounds in a particular target set following the AC similarity criterion (Hu & Bajorath, 2019; Vogt *et al.*, 2011). ACs duality may be due to the fact that how compounds are dealt by medicinal chemists or how they are analyzed computationally. As different target sets consist of different compounds from different sources, they require different optimization procedures and efforts to obtain factual ACs (Thapa *et al.*, 2018).

## 11. Coordination and frequency of ACs

When analyzing a data set, number of ACs are obtained in a coordinated manner with huge variation in activities of structurally related compounds. Moreover, a single compound has the ability to form a large number of ACs with different analogs. In AC network, compounds present in a data set are represented as nodes, and pair-wise edges serve as activity cliffs and coordinated cliffs, produced by subsets of compounds, leading to disjoint cluster formation (Stumpfe *et al.*, 2014; Demova *et al.*, 2015). AC clusters are more favorable for the analysis of SARs compared to isolated cliffs because >95% of the cliffs of various data sets are produced in a coordinated manner (Stumpfe & Bajorath, 2015; Stumpfe & Bajorath, 2012). Clusters of ACs often consist of "hubs" with numerous partner compounds forming center of local ACs representing molecules as nodes and such molecules are referred as "activity cliff generators".( Stumpfe & Bajorath, 2012). Frequency of the occurrence of ACs for different data sets has also been found along with the coordination of ACs, and the information has been increased tremendously over time as total number of activity data was doubled from 2011 to 2015 with more than 17,000 MMP cliffs in 2015 (Stumpfe & Bajorath, 2012).

## 12. Determination of potency differences

The measurement of potency differences related to AC production depends on the comparison of experimental values. The validity of AC assignments is certified by assessing the potency difference (in theory) using $K_D$ (dissociation constant) or $K_i$ (assay-independent equilibrium) values. The evaluation of ACs can also be completed formally with increasing potency difference as continuation of pairs of compounds (Hu *et al*., 2012). The use of the constant potency difference is preferred not only in the analysis of ACs, but also for finding ACs in databases (Stumpfe *et al*., 2012; Stumpfe *et al*., 2014). Pair-wise potency difference is lower compared to constant potency difference threshold in analysis of analogs, and is significant statistically. Moreover, potency difference of nearly 100-fold is

usually utilized in AC analysis (Stumpfe *et al.,* 2019). The role of constant potency difference threshold has made computational search for ACs easier, but it doesn't consider class dependent activity difference in the distribution of compound potency which vary greatly in activity class due to similarity relationship between compounds (Stumpfe *et al.,* 2020). AC analysis can further be refined by the class dependent deviation of potency difference threshold. For the determination of class dependent threshold, average of the compound pair-based potency difference distribution plus two standard deviations are statistically performed (Vogt, 2011).

## 13. Activity cliff generations

The evolution of the ACs is contributed to the variation in the manner of addressing similarity and potency difference. This variation played a part in differentiating among the three generations of two dimensional ACs, also known as molecular graph based ACs (Stumpfe et al., 2019; Stumpfe *et al.,* 2020). First generation of ACs is classified as a separate group using constant potency difference threshold in all the activity classes and similarity measures based on sub-structures Stumpfe *et al.,* 2020). In 2015, they (first generation ACs) were reported based on ChEMBL release 20 by extracting 48,244 compounds having $K_i$ values and were active against 746 targets. MACCS structural keys, MPP formalism and extended connectivity fingerprint with bond diameter 4 (ECFP4) were used to identify first generation activity cliffs with $\Delta pK_i \geq 2$, where $\Delta pK_i$ represents potential difference threshold (Perez *et al.,* 2015;Stumpfe *et al.,* 2020; Stumpfe *et al.,*2017) Second generation of ACs came into existence because of capturing single substitution site of structural analogs (R), and MMP cliff formalism with varying potency difference threshold depending upon activity class. Their search was initiated in ChEMBL release 23 from which 212 activity classes having potential for AC formation were identified (Hu *et al.,* 2018). These 212 activity classes yielded 16,096 class dependent RMMP-cliffs having $\Delta pK_i$ between 1 and 2.5 (Hu *et al.,* 2019). However, 11,773 RMMP-cliffs were obtained in 195 classes when the $\Delta pK_i$ was $\geq 2$. Moreover, 145 RMMP-cliffs containing inactive compounds that are obtained from screening assays in PubChem were also identified from the eight activity classes with available screening data (Hu *et al.,* 2019). Furthermore, single or multiple substitution analog pairs belonging to same series gave rise to third generation ACs with activity class dependent potency difference threshold (Stumpfe *et al.,* 2020). They were 16,454 analog series-based ACs in ChEMBL release 24.1 having class-dependent potency difference threshold. Only 25.6% of 4204 instances were third generation ACs with multi-site cliffs while others contained a single site for substitution (Stumpfe *et al.,*2019*).*

## 14. Activity cliffs containing privileged substructures

The concept of privileged substructures (PS) was introduced by Evans et al., representing non-class specific compounds with specific biological activities (Evans *et al.,* 1988). They have been a center of attention in pharmaceutical research for long. It has been found that the activity cliffs comprising selected PS exhibited huge improvements in efficacy of ligands (Hu & Bajorath, 2020).

## 15. ACs, chemoinformatics and machine learning

QSAR studies are promoted due to continuous SARs and discontinuous SARs have negative impact. Several machine learning (ML) methods have been developed to assist chemoinformatics (Aguiar *et al.,* 2013; Rose, 2013), and are involved in the classification and generalization of data and help in making findings logical (Rose, 2013).However, the ML methods need improvement as they just observe the "rolling hills" as the key signs for the determination of small ACs,

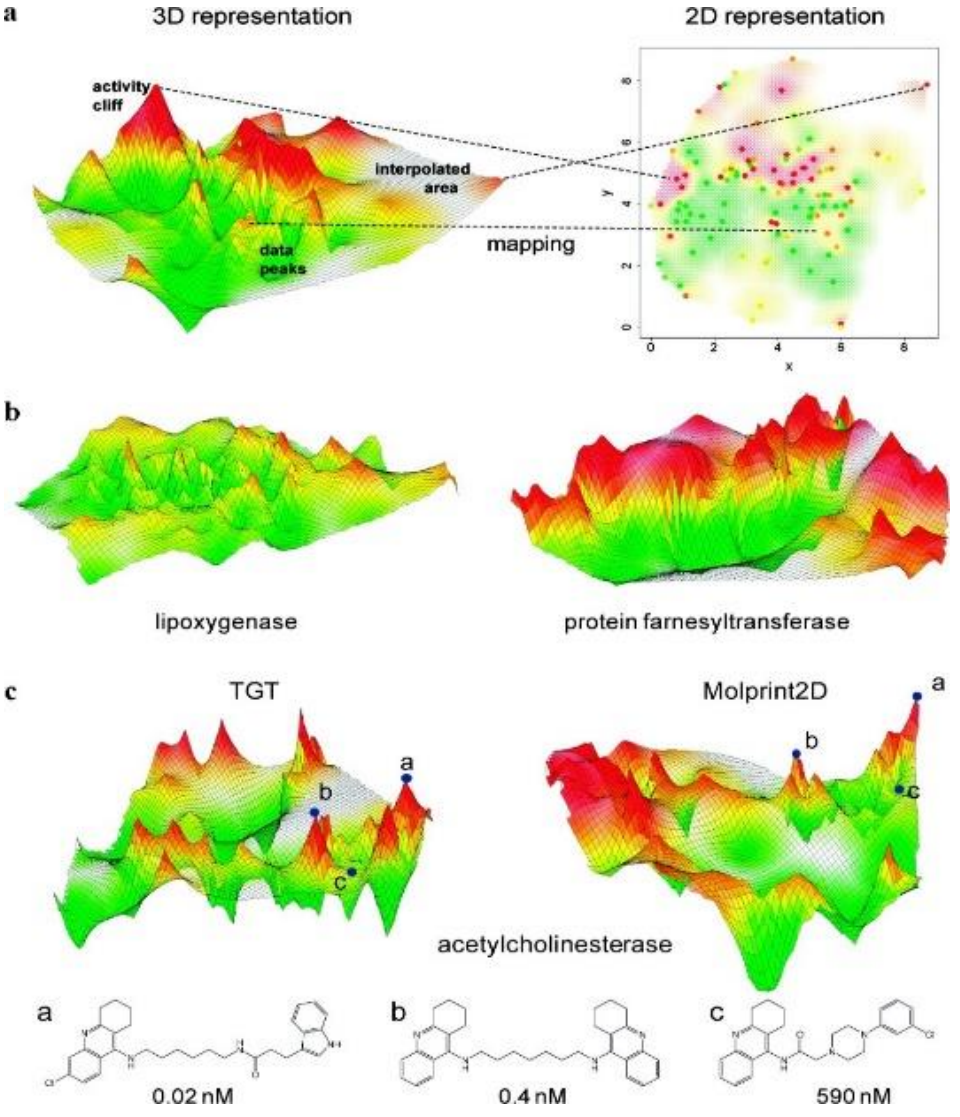


**Figure 3** A comprehensive representation of 3D AL. (a) A comparison between 2D and 3D activity landscape formed by dimension reduction for the analysis of a set of 112 acetylcholinesterase inhibitors, represented as MACCS fingerprints. (b) It represents the 3D AL analysis of a set of lipoxygenase inhibitors (252 inhibitors) and protein farnesyltransferase inhibitors (146 inhibitors) based upon Molprint 2D fingerprint representation. AL for lipoxygenase is indicating SARs continuity while AL for protein farnesyltransferase is representing SARs discontinuity. (c) TGT and Molprint 2D fingerprint are used for the analysis of ACs of acetylcholinesterase inhibitors that were used in Figure (a) but here just three compounds are mapped with their reported potencies. It shows that the ACs can be formed or not depending upon the molecular representation. Reproduced with permission from Ref (Wassermann *et al.*, 2010). Copyright © 2010, American Chemical Society.

thus reducing the forth coming forecast steadiness (Guha, 2011). The determination of the occurrence of ACs in an area of ML and explaining the bad effects of ACs is an important task. However, a pre-processing innovative filtering approach (PRISM) was proposed by Smith and Martinez, and according to a study, ISMs represent estimations to an extra precision. This extra precision is not shown by all the ISMs but a huge correlation can be shown between ACs and


**Figure 4** It enlightens the reasons behind the poor prediction of activity cliffs and their possible solutions.

ISMs. In AL-modeling, ISM has the ability to perform similar incidences with different names in an area. Moreover, ISM can swing to the EL (elementary landscape) that can be renowned by outliers and noise. Three current outliers were discovered by Smith and Martinez that helped in equating PRISM by mediating discovery principles and noise reduction. Pre-removal of the cases determined by PRISM can help to enhance the precision of classification related to the instrument learning computation formed by initial data groups. Thus, removing the ISMs from ML procedures will make the suitable categorization difficult in advance training certificates (Guha, 2011). Moreover, Weka, a data mining suite, is used for the formation of a computational model and provides maximum flexibility for the frameshifting sites when analyzing a new data set for the analysis of ACs (Frank *et al.*, 2004).

## 16. Local vs universal molecular likeness

It is necessary that the medicinal chemists use different calculations to analyze ACs in addition to employing different approaches for determining the likeness of compounds. Scalar molecular caption is responsible for regional similarities that involve the determination of molecular design by topological, functional and constitutional attitude and is novel to researchers working on chemoinformatics who are specialists of QSAR and drug-based pathway. On the other hand, medicinal chemists utilize all the molecular illustrations (2D chemical space depiction) based on fingerprints like MACCS keys (Rogers & Hahn, 2010), as represented in Figure 3a, which involves a comparison between 3D and 2D illustrations based on MACCS fingerprint. Furthermore, Figures 3b and 3c show comparison between the ACs of lipoxygenase and protein farnesyltransferase inhibitors and the ACs formed by TGT and Molprint 2D for the same compounds, respectively (Rogers & Hahn, 2010).

## 17. Reasons for poor prediction

The restriction in the prediction is not closely linked with the methodology but it is retaled with the data. A number of descriptors and statistical approaches are available that are used to validate and predict the data sets probably by cross validation. However, all the data sets can't be predicted due to some reasons. These reasons for poor predictivity involve the absence of activity related features in descriptors, inability of QSAR suitability in complex relation between descriptors and activity, a huge variation in the experimental uncertainty of observed activities among molecules and the formation of more or steeper activity cliffs (Figure 4) (Sheridan *et al.*, 2020).

## 18. Solutions for poor predictivity

If large uncertainty in observed values in dataset is the issue, routine multiple measurements of molecules to cancel its effect in the average and reduction in the assay experimental error can also

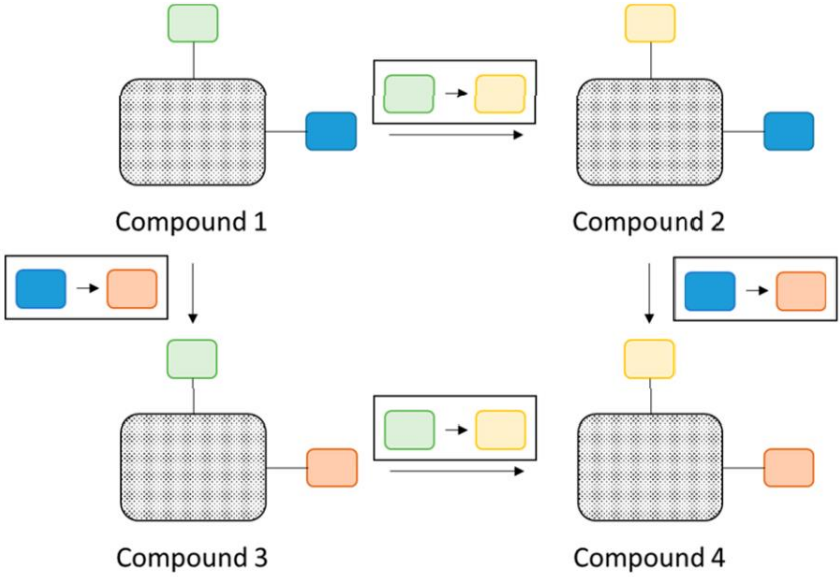


**Figure 5** A diagrammatic representation of nonadditivity analysis known as double transformation cycles. It consist of four compounds that are attached by identical transformations; whereas, the colored blocks in the diagram are representing different functional groups. Reproduced with permission from Ref (Kramer *et al*., 2021). Copyright © 2019, American Chemical Society.

ameliorate the issue. The activity cliff metrices that can't differentitate the 'real' ACs which may be revealed by reducing the uncertainty to a minimum level (Sheridan *et al.*, 2020). According to the literature, the elucidation of ACs is possible by investigating the way molecules bound with receptors with various poses. If it is possible, none QSAR model alone can interfere with the unpredictability of ACs no matter what method or descriptor is used (Figure 4) (Sheridan *et al.*, 2020).

## 19. Nonadditivity analysis

The determination of potential SAR outliers, nonadditivity (NA) and upper limit estimation of data set experimental uncertainty are the contributions of NA analysis. The term nonadditivity refers to the varied results obtained by the fusion of two new fragments as compared to the sum of their individual effect (Kramer, 2019). In case of linear SAR, NA can be an issue; but, if it is used intentionally, NA can occupy a crucial place in drug discovery. Additionally, NA analysis can help apprehend possible experimental noise and provides deep structural insights (Gogishvili *et al.*, 2021). Nonadditivity in compounds can be caused by the variation in hydrophobicity and hydrophilicity, residual mobility, internal hydrogen bonds and clear conformational changes which causes NA above 2 log units (Baum *et al.*, 2010; Kramer *et al.*, 2015). The calculation of NA is done by double transformation cycles that consist of four compounds linked by two indistinguishable transformations as shown in Figure 5. However, some experimental uncertainty is present in the values measured for each compound and the addition of these uncertainties provide apparent and false NA. Thus, this save the time of the researcher from finding explanations for the false non existing effects (Kramer, 2019; Cockroft & Hunter, 2007; Fischer *et al.*, 2007).

## 20. Conclusions

ACs have been found to be the heart of medicinal and computational chemistry as they play a conspicuous role in drug discovery. A large number of ACs have been recognized and studied by SARs

analysis utilizing AL modeling. The occurrence of ACs limits the use of QSAR and likeness-based strategies, which support the concept that 'structurally similar molecules are also potentially identical'. Today, ML has been used for the accurate analysis of ACs and plays a role in optimization for the lead-compound in drug designing by forming various algorithms. Despite all the efforts till now, many algorithms are still required to further ameliorate the analysis.

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# Numerical Modelling and Rugged Techniques for De-orbiting of LEO Space Debris

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## Abstract

According to the statistics of Space Surveillance Network (SSN), approximately 200 on-orbit and 54 strident events occurred which smashed the debris into smaller segments. It is essentially needed to detect deliberated hypervelocity impacts, accidental collision between satellite and explosion of satellites that can form potential a debris cloud. Space debris constitutes failed spacecrafts and upper stages of rockets etc.) occupies substantial space at all the altitudes of LEO, MEO and GEO, which may pose a threat to operational and future missions. There are multiple techniques to mitigate the threatening space debris via targeted deterrent measures, such as passivation measure or active de-orbiting. This paper presents a numerical modeling technique for identification of debris clusters based on density distribution, A/m ratio and explosion velocity. The breakup events of Fengyun-1C, NOAA-16, Iridium 33 and Cosmos 2251 have been considered for dynamic analysis on the basis of real-time orbital data. The debris population is presented in term of their classical orbital elements using statistical tools. A rugged technique is devised for the removal of millimeter and centimeter level debris fragments. It is a cost-efficient yet effective approach, implemented with aid of a Hoover Capturing System (HCS). This system is composed of a current carrying conductor which produces a magnetic field similar to that of a bar magnet. The target fragments would be captured by HCS on close approach of the system. Afterwards, HCS would be transported to a low parking orbit where it would dispose of the junk through a suitable window of opportunity. Ultimately, fragments would be burnt on reentry into lower altitude by atmospheric drag. This technique is very rugged, cost-efficient and can be utilized for frequent cycles.

**Keywords**: Debris, Active Debris Removal (ADR), Hoover Capturing System (HCS), Atmospheric Drag, and De-Orbiting

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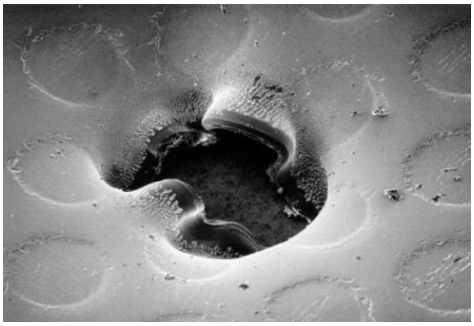

**Figure 1** 3mm paint flake damage the spacecraft (Stansbery 2009).

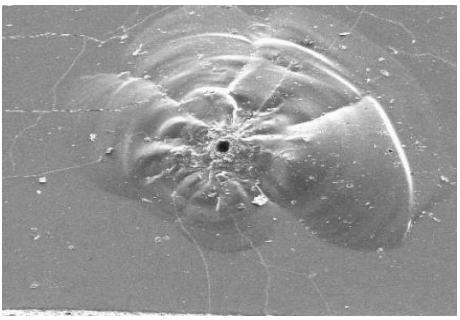

**Figure 2** 0.1mm Aluminum debris damage the window of the spacecraft (Stansbery 2009).

## 1. Introduction

Space debris is a form of unavoidable space junk being produced by high demanding space missions. There have been more than 630 events which include break ups, explosions, collisions and uncharacteristic events resulting in fragmentation (Figure 1 and 2). It is evident from the literature that about 500,000 debris pieces larger in size of 1 cm are present (Lemaître, 2019). Similarly, about 135,000,000 greater than 1 mm, over 300,000 fragments greater than 10 cm and fragments up to 1m or greater have been tracked in LEO (400- 2000 km), MEO (2000-36000 km) and GEO (36000 km), orbiting at a velocity of several $kms^{-1}$ (Stansbery 2014). Most of the debris is produced as result of space activities of major space actors like United States, Russia, China, and Europe etc. The operational satellites and debris have been monitored by the Air Force Space Command (AFSPC) with aid of 25 tracking Radars and optical telescopes. As Radar is not powerful enough to penetrate the altitude above several hundred kilometers; consequently, the optical sensors are of critical importance in debris surveillance and archiving (Klima, Bloembergen, Savani, Tuyls, Hennes, & Izzo 2016), (Hamilton, Blackwell, McSheehy, Juarez & Anz-Meador, 2017).

According to United States Strategic Command (USSTRATCOM), since the launch of first artificial satellite (Sputnik-I), there are about 300,000 debris particles have been tracked in LEO (Maxwell, 2009). The diameter of these particles is greater than 10 cm with approximate mass of 1kg. Space Surveillance Network (SSN) tracks about 31630 objects on a regular basis and catalogues these objects as Two-Line-Element (TLE) (Stottler, 2015). It is estimated that particles less than 10 cm are untraceable due to the sensor's sensitivity constraint. Nowadays, space debris is major threat to continuously increasing space activities and new missions as well. The collisions with operational satellites cannot be prevented at the time of trajectory corrective maneuvers due to the presence of these small debris. The distribution of debris varies in LEO, MEO and GEO due to the high solar drag; however, most of their fragments are found in LEO at 800-850 km altitude due to high concentration of SSO satellites for Earth imaging and communication satellites in polar orbits (Silha, Schildknecht, Hinze, Utzmann, Wagner, Willemsen & Flohrer, 2014).

The space objects are classified as payloads (functional satellites), rocket bodies, debris fragmentation and unknown debris at various orbital altitudes. Due to the incredible buildup of space debris, it is a challenging job for NASA to figure out this entire debris in space (Liou 2011). Therefore, NASA dedicates a latest 1.3 m optical Meter Class Autonomous Telescope (MCAT). The MCAT is deployed at Johnson Space Center, Orbital Debris Program Office (JSCODPO) with four primary operational modes. It can precisely produce and process excess data

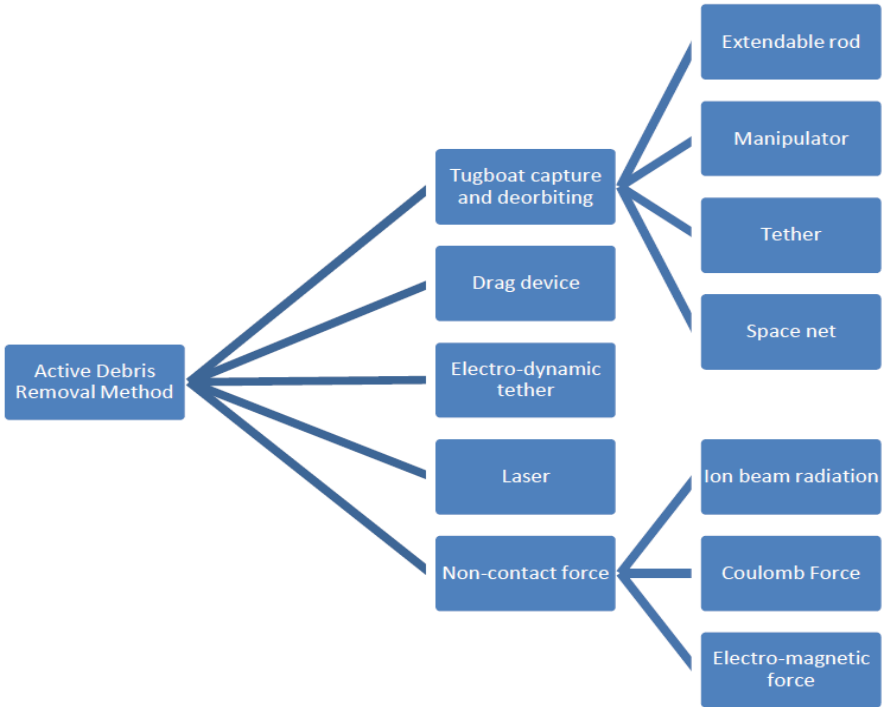


**Figure 3** Active debris removal methods

volumes with astrometry and automated re-acquisition (Lederer, Stansbery, Cowardin, Hickson, Pace, Abercromby, & Alliss, 2013).

As it is well established fact that debris is a high potential risk for space activities. Therefore, aim of this paper is to investigate the numerical modeling techniques for debris location and identification. It also discusses Active Debris Removal (ADR) technique for small fragments (less than or equal to 10 cm) depicted in Figure 3. The structure and design of ADR system is discussed in detail and the paper is divided into following sections: Section 2 describes the physical properties of debris fragments, i.e. density and Area to mass (A/m) ratio of debris objects. The size, A/m ratio and expulsion velocity of all the debris fragment is not same due to difference in their physical properties and altitude levels.

The orbital position of debris in terms of keplarian orbital elements (semi-major axis, eccentricity, inclination, RAAN, mean anomaly), velocity distribution and uncertainty associated with position and velocity in explained section 3. The uncertainty in position and velocity is caused by various factors that influence the motion of objects in orbit. These factors include drag of the atmosphere, gravitational effect due to geopotential, solar and lunar forces and Solar Radiation Pressure (SRP). Section 4 and 5 explains de-orbiting technique for debris removal and design/specifications of the service platform. There are various methods which are currently being under analysis for ADR. These methods are illustrated in Figure 3 (Liou, 2011), (Rezunkov, 2013). The ADR method, is compelled with multiple limitations and works under several assumptions such as technological constraints may impact the efficiency with a finite range, potentially restricting its effectiveness at orbital ranges. High resource and energy requirements could limit to conduct multiple removal missions simultaneously. Finally, mission duration and End of Life (EOL) also pose challenge

**Table 1** Magnetic properties of materials

| Material | Magnetic ordering | Magnetic susceptibility, $cm^3mol^{-1}$ | Density, $gcm^{-3}$ |
|---|---|---|---|
| Aluminum | Paramagnetic | $+16.5 \cdot 10^{-6}$ | 2.70 |
| Titanium | Paramagnetic | $+153.0 \cdot 10^{-6}$ | 4.506 |
| Nickel | Ferromagnetic | - | 8.9 |

to stand out in a cluttered orbital environment.

## 2. Physical properties of debris

Nowadays, lightweight materials have been adopted as a standard in aerospace industry for design and development of spacecrafts. The main advantage of lightweight materials is the reduction of spacecrafts dry mass to lessen mission launch cost. However, highly dense and materials are mostly used in manufacturing of a spacecraft. Desired strength is attained with high efficiency structural materials (aluminum and titanium etc.) at normal temperatures. Meanwhile, nickel and ferrous alloys are mostly optimum for extreme thermal environment materials as these are the best high temperature metals. Iron is a ferromagnetic material and its alloys also demonstrate magnetic properties. A material whose value of density is greater than zero, that kind of substance is known as paramagnetic. The magnetization of a paramagnetic material is higher than that of free space. On the other hand, the substance is diamagnetic if its density is less than zero (Deshpande, Green & Zarnecki, 1993). The magnetic properties of materials are depicted in Table 1.

The collision and explosion events that have been analyzed in this paper are discussed as follows:

***Fengyun-1C:*** This satellite had undergone a hypervelocity projectile impact on January 11, 2007. The mass of this satellite was approximately 880 kg. As a result of this impact, almost 2000 fragments bigger than 5 cm and 1000 fragments bigger than 10 cm that have been cataloged SSN. Most of these fragments are present at LEO altitudes in high concentration (Pardini & Anselmo, 2011).

***Cosmos 2251 and Iridium 33 Collision:*** In 2009, these satellites suffered a collision which caused Cosmos 2251 to generate 1669 fragments and Iridium 33 to produce 627 fragments (Pardini & Anselmo, 2011).

***Explosion of NOAA 16:*** It was a meteorological satellite, launched in Sun Synchronous Orbit (SSO) at an orbital height of about 850 km. It undergone break up because of battery explosion on November 25, 2015. Almost 400 pieces greater than 10 cm have been catalogued, though it produced an unknown amount of tiny un-trackable debris. There are only 53 fragments which have been catalogued on 5 December 2015. The NOAA 16 has produced maximum number of high-density fragments (steel) and other solid fragments of low-density material. The remaining debris is considered to be formed of moderate density materials, i.e. aluminum or solid electronics. A very small amount of low-density material is present in NOAA-16 cloud. The analysis reveals that fragmentation event of this satellite consisted of only 2% (36kg) of the total satellite dry mass (Tan, Reynolds & Schamschula, 2017). If we consider some expected discrepancies because of structure and energy, we can compute the quantity of blasted pieces having size of equal of greater than 1m using expression below (Wang, 2010):

$$N(L_c) = 6L_c^{-1.6} \quad (1)$$

Where, N is the number of exploded fragments and Lc is the fragment size.

### 2.1 Density distribution of debris fragmentation

The density distribution of debris fragment is mainly dependent on its material properties. In our analysis, we have considered that the fragments are composed of same material and therefore have density within the range of 1500 $kgm^{-3}$ to 9000 $kgm^{-3}$. One the other hand, a honeycomb structured fragment might have approximately 150 $kgm^{-3}$ of density. Moreover, there exists a relationship between density and A/m ratio of the fragmentation. Materials with high density like titanium, some aluminum alloys and steel occupy a low value of A/m ratio (approximately 0.001 - 0.1 $m^2\ kg^{-1}$). Conversely, low density materials like Multi Layer Insulation have high levels of A/m ratio (1 – 10 $m^2\ kg^{-1}$) (Sorge, Peterson & McVey, 2017), (Johnson, Krisko, Liou & Anz-Meador, 2001).

The characteristic density of fragment under analysis is determined with an assumption that it is spherical in nature. The mathematical equation that well represents the fragment density is given as follows: (Wang, 2010):

$$\rho_c(d) = 92.937(d)^{-0.74}\ (kg/m^3) \quad (2)$$

Where, ρc is the debris characteristic density and d is the diameter of debris fragment.

### 2.2 Size and A/m ratio distribution of debris fragmentation

The debris region / cloud formed by on-orbit impact and explosion is modeled by JSCODPO since 1970. This debris modeling is based on the physical properties of size and velocity distribution. We have utilized three methods, which we think are responsible for producing fragments. The data has been analyzed to determine their mass distribution (Bess, 1975).

a. Hypervelocity (3.0 - 4.5 $kms^{-1}$) projectile impact with satellite wall.
b. Explosion of high Intensity.
c. Explosion of low Intensity.

It must be noted that the quantity of fragments produced by hypervelocity impact projectile obeys power law. On the other hand, fragments formed from the explosions of high intensity and low intensity obey exponential law. It has been studied that the fragments produced due to hypervelocity projectile has mass up to to $10^{-7}$ grams and the mass of the fragments produced fragment produced due to explosion is 10 milligrams. Meanwhile, the velocity of fragments formed due to hypervelocity impact is 10 $ms^{-1}$. On the other hand, velocity of the fragments produced due to low intensity and high intensity explosions is about 100 $ms^{-1}$ and 3 $kms^{-1}$, respectively (Bess, 1975). The size limit for catalogued objects in LEO is approximately 10 cm. For statistical analysis, we have considered 1000 fragments of Fengyun-1C, Cosmos 2251, NOAA 16 and Iridium 33 produced in various events.

A/m ratio of fragment is defined by Ballistic coefficient and expressed as a product of A/m ratio (cross section) and drag coefficient with the following equation (Hakima, Bazzocchi & Almstrom, 2022):

$$B = D_c \frac{A}{m}\ (m^2/kg) \quad (3)$$

Where, B Ballistic coefficient, Dc is drag coefficient and A/m is Area to mass ratio (cross-sectional). The parameter B is computed by SGP 4 spacecraft orbit propagation model (Hoots & Roehrich, 1980). We can estimate the cross-sectional area from Radar Cross Section (RCS), as this data is not given in Satellite Situation Report (SSR). Therefore, system description is acquired from Space-Track (RCS Legend, 2021). The parameter B is calculated from B-STAR parameter, as mentioned in Two Line Element (TLE) for the entire catalogued objects (CelesTrack, 2023). B-STAR actually provides the magnitude of atmospheric drag on an object (debris in our case). It is mathematically written as:

$$B^* = \frac{\rho_0 B}{2} \quad (4)$$

$$B^* = \frac{D_c \rho_o A}{2m} \quad (5)$$

Where $B^*$ is B-STAR, its unit is inverse of earth radii (Earth $Radii^{-1}$). Further details of B* and TLE can be

obtained from CASTOR (Satellite Tracking and Optical Research, 2020); ρo represents atmospheric density. It has a value of 0.1570 kg Earth Radii $m^{-2}$. If we consider $D_c$ = 2.2, The value of B* is obtained from TLE which allows us to estimate A/m ratio. By re arranging the equation (5), we may get (Satellite Tracking and Optical Research, 2020):

$$\frac{A}{m} = \frac{2B^*}{\rho_o D_c} \quad (6)$$

After determining the number of fragments from each sample (cluster), we may obtain a distribution of the sum of debris pieces having mass equal to or greater than a specific quantity. The A/m ratio of pieces has a direct link to orbital life/decay rate which means that higher A/m ratio results in smaller orbital life.

## 3. Collisions estimation and modeling of debris fragmentation

To determine the effect of collision, we need to find the ratio of relative kinetic energy of the object with small mass to that of large mass object. It gives the idea that the collision was catastrophic or not. This ratio of large mass object to small mass object gives us the information whether the collision was appalling or non- appalling. If this ratio is $\geq 40 Jg^{-1}$ then it is an appalling collision or otherwise it would be a non-appalling collision. When it comes to the phenomena of the number of fragments produced, power law is applied to a hypervelocity collision. An optimum estimation of cumulative number of fragments in terms of size (Lc) can be well established by NASA Breakup Model (EVOLVE 4.0). The mathematical expression for size estimation may be written as (Johnson, Krisko, Liou & Anz-Meador, 2001):

$$N(L_c) = 0.1(M)^{0.75} L_c^{-1.71} \quad (7)$$

Where Lc is fragment size (meters), M is the combined mass of both objects (kg). In case of catastrophic collision, the expression may be written as:

$$M' = m_L + m_s \quad (8)$$

Incorporating the value of M, the Equation (6) may be written as:

$$N(L_c) = 0.1(m_L + m_s)^{0.75} L_c^{-1.71} \quad (9)$$

Where, mL is mass of larger object and mS is mass of smaller object. On the other hand, if the collision is non-catastrophic, M is the product mass of small body (kg) and collision speed ($kms^{-1}$)

$$M = m_s \times v_c \quad (10)$$

In this case, equation (7) may be represented as:

$$N(L_c) = 0.1(m_s v_c)^{0.75} L_c^{-1.71} \quad (11)$$

The dry mass of cosmos 2251 was 900 kg and that of Iridium 33 was 556 kg. The collision of these satellites took place at 770 km altitude. The Iridium 33 was an operational communication satellite and cosmos 2251 was debris at the time of impact. Both satellites were orbiting the globe with velocity of 7.5 kms-1and collided with over 10 kms-1 velocity. The debris produced because of this collision was within range of massive objects to dust particles. Iridium produced about 550 fragments of iridium and 1,300 fragments of cosmos were produced that were larger than 10 cm diameter. According to NASA Breakup Model, cosmos 2251 produced 840 fragments larger than 10 cm, 43220 pieces larger than 1cm and 2.22e6 fragments greater than 1mm. The iridium 33 generated 580 fragments bigger than 10 cm, 30 fragments larger than 1cm and 100 fragments larger than and 1.54e6 (Wang, 2010). Iridium 33 fragments has higher A/m as compared to Cosmos 2251, which would decay in a short life span. The reason for high A/m ratio of iridium is correlated with the structural composition of its two solar panels.

As we know, RCS gives the size approximation of the debris pieces and is not suitable for the estimation of fragments less than 10 cm. Ting Wang model is adopted for the estimation of size distribution of iridium-cosmos cloud. This model presents an effective valuation of fragment size to their cumulative number. The size distribution of Iridium and Cosmos shows that most of the fragments are larger than 10 cm in size (Wang, 2010). Table 2 shows the physical properties of

**Table 2** Physical properties of Cosmos 2251, Iridium 33, and Fengyun 1C from NASA breakup modell

| NORAD ID | Name | A/m $m^2kg^{-1}$ | RCS, $m^2$ | $m_s$ kg | $N_c$>10 cm |
|---|---|---|---|---|---|
| 34333 | Cosmos2251 | 0.00395 | 0.0354 | 0.2224 | 25 |
| 34398 | Cosmos2251 | 0.00383 | 0.0317 | 0.2561 | 41 |
| 34657 | Iridium 33 | 0.01430 | 0.0136 | 0.0403 | 8 |
| 34858 | Cosmos2251 | 0.07307 | 0.0137 | 0.0509 | 13 |
| 30455 | Fengyun 1C | 0.00287 | 0.0282 | 0.7377 | 113 |
| 30687 | Fengyun 1C | 0.01038 | 0.0195 | 0.0740 | 29 |
| 30980 | Fengyun 1C | 0.00724 | 0.0127 | 0.0486 | 26 |
| 31998 | Fengyun 1C | 0.01006 | 0.0118 | 0.0428 | 23 |
| 36697 | Fengyun 1C | 0.00048 | 0.0093 | 0.2787 | 88 |

the fragments created from the collision. These calculations are based on NASA Breakup Model. Because of the tracking constraint of SSN radar, only objects of 10 cm or larger size can be measured.

In case of Fengyun-1C, the size and A/m ratio distribution is unique from other identical events. About half of the pieces are made of light material and above hundred fragments have A/m greater than 1 $m^2$ $Kg^{-1}$. These fragments constitute the pieces of spacecraft's thermal insulation and solar panel. If we apply NASA model, we conclude that satellite with a mass of 960 kg would generate more than 900 fragments which are less than 10 cm in size (Pardini & Anselmo, 2007). The cumulative size distribution of Fungyun-1C fragments is below 13 cm. This is because of the lack of sensitivity of the SSN sensors to tiny fragments. The Haystack Radar measurements further reveal that Fengyun-1C has created unusual quantity of fragments as compared to other similar and average hypervelocity. The size distribution of Fengyun-1C size shows deviates from single power law distribution. There is another prominent property associated with the Fengyun-1C fragments is the high A/m ratio (0.1 $m^2kg^{-1}$ and higher). The Fengyun-1C spacecraft was composed of two solar panels having a dimension of 1.5 m × 4 m. Each of the solar panels was insulated with 13 $m^2$ Multi-Layer Insulation (MLI). It is thought that high A/m ratio fragments are resulted from the breakup of solar panels, MLI and light weight plastic material etc. (Liou, J & Johnson, 2009).

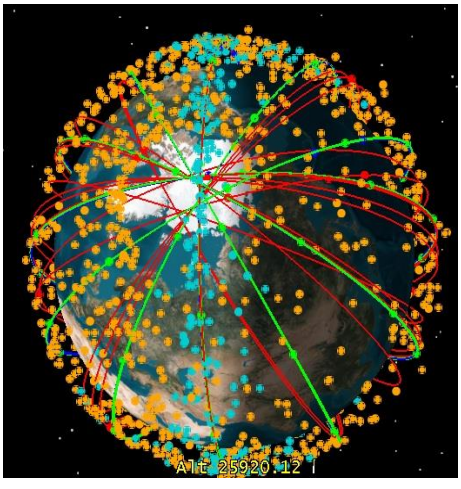


**Figure 4** Visualization of orbits of Cosmos 2251 and Iridium 33 debris fragmentation.

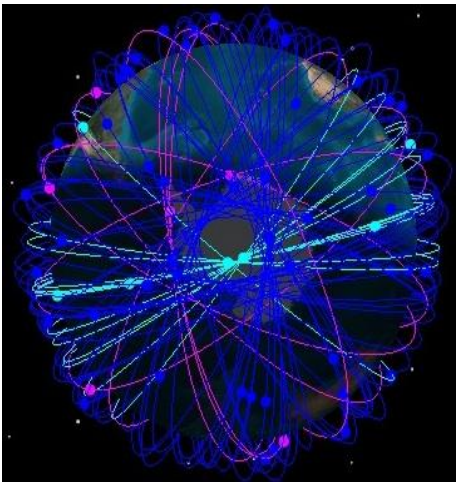

**Figure 5** Visualization of orbits of Fengyun 1C debris fragmentation.

## 4. Simulation and statistical analysis of debris fragmentation in terms of keplarian elements

The simulation analysis has been performed with the help of dynamic simulator. The orbits are propagated on SGP 4 propagator on the basis of TLE data (CelesTrack, 2023). Approximately 1000 files of the orbital data of Fengyun-1C, Cosmos 2251, Iridium 33 and NOAA 16 have been incorporated in the simulator as shown in Figure 4 and 5 to analyze debris population of Fengyun 1C, NOAA-16, Cosmos 2251 and Iridium 33 in term of their orbital elements (semi-major axis, inclination, RAAN, eccentricity, argument of perigee and mean anomaly). The results of simulation analysis are represented in graphical form in Figure 7 to 10.

The most important distribution is

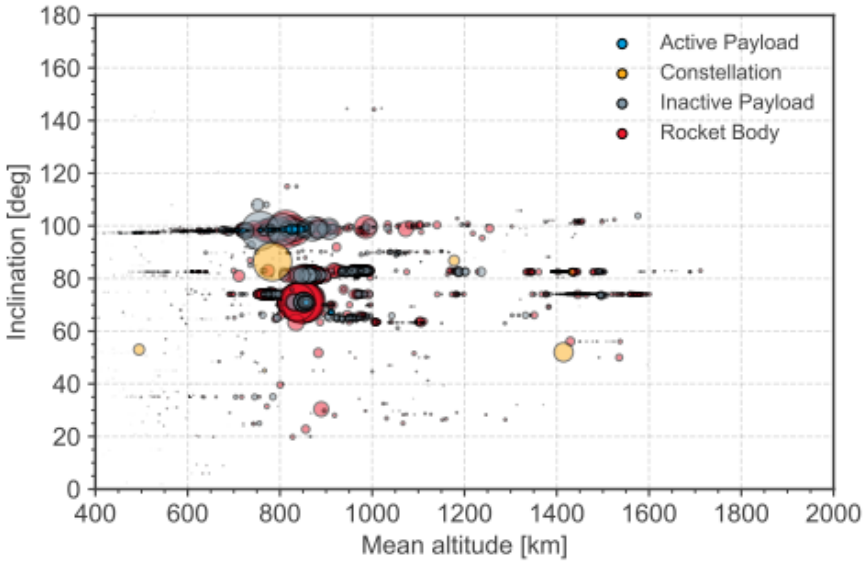


**Figure 6** Debris cataloged by ESA (Annual Space Environment Report 2022) at multiple altitudes and inclination angles, courtesy ESA (ESA, 2022).

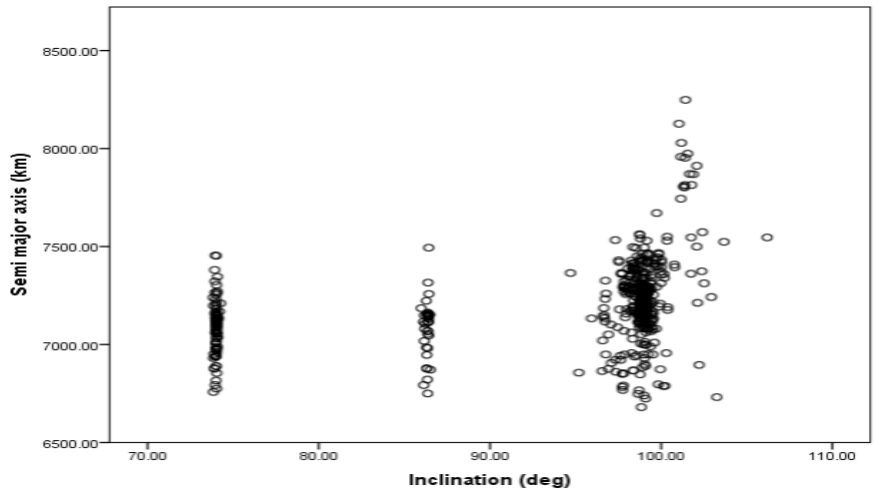


**Figure 7** Representation of debris distribution as a function of inclination and semi major axis.

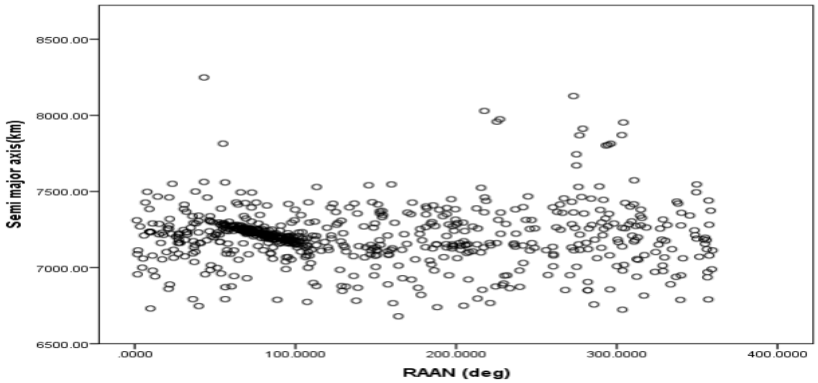


**Figure 8** Representation of debris distribution as a function of RAAN and semi major axis.

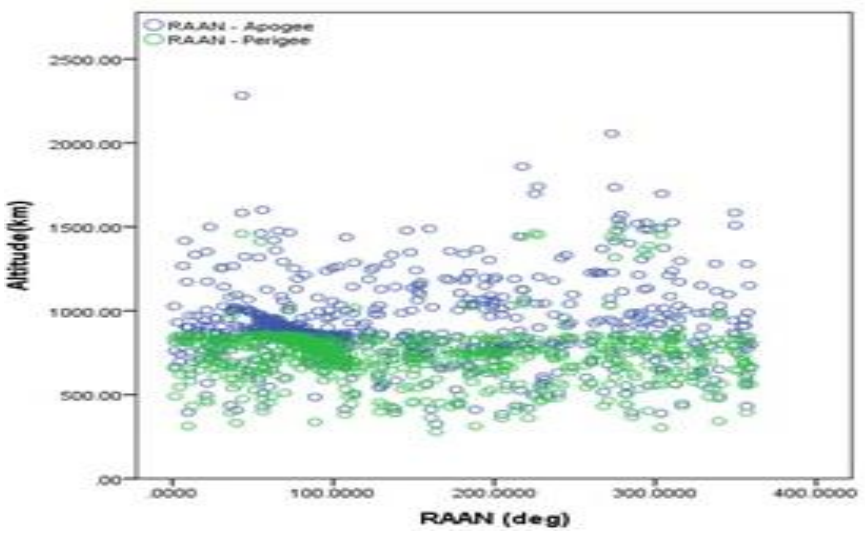


**Figure 9** Representation of debris distribution as a function of RAAN, perigee altitude and apogee altitude.

depicted in Figure 5 which identifies the debris concentrated areas as a function of inclination and semi-major axis (altitude). The plot reveals that fragments are concentrated on semi major axis of range between 6800 and 8000 km which correspond to altitude range of 422 km and 1622 km at an inclination close to 1000. The distributions also reveal a fact that debris from various events (collision or explosion) formulate into clusters. Consequently, the ADR task from numerous fragmentation events becomes feasible and convenient with a single mission design and operation.

There is another orbital parameter along with inclination which defines the orientation of an orbit. This orbital parameter is RAAN. RAAN and semi-major axis distribution is also of critical importance to precisely locate the debris cluster. This distribution is presented in Figure 9 which shows that most of the debris is concentrated within RAAN bin of 50.8 o to 94.6 o corresponding to the semi-major axis range of 6800 and 8000 km. The debris cluster within this range of altitude and RAAN is extremely suitable for the ADR. The critical regions with debris cluster in terms of altitude and inclination are 850 km and 71º, 1000 km and 82º and 800 km and 98º respectively, corresponding to RAAN bin of 50.8 o to 94.6 o. It must be noted that for a single

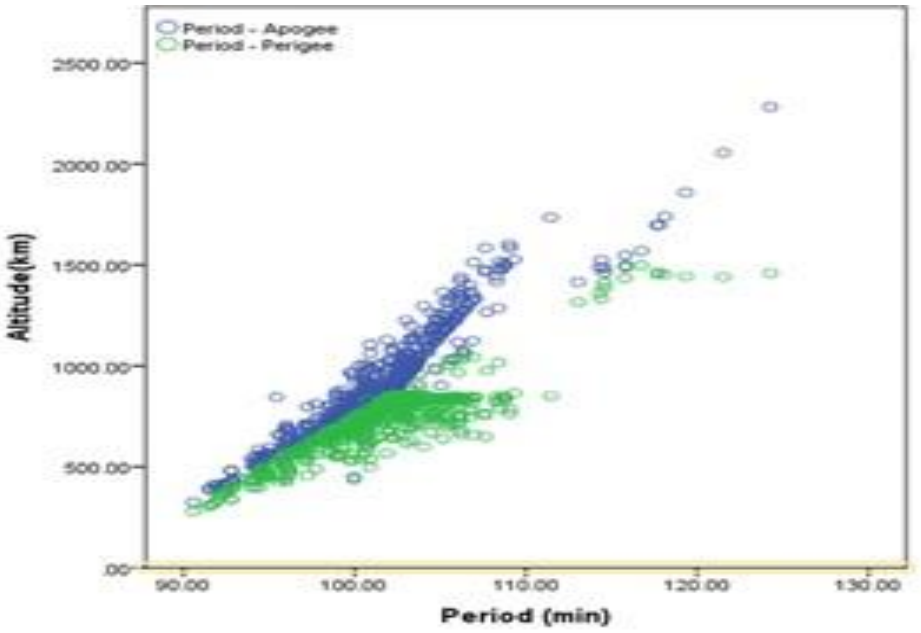


**Figure 10** Representation of debris distribution as a function of orbital period, perigee altitude and apogee altitude.

inclination, position of the fragments is different in each bin of RAAN, we may have to perform multiple rendezvous attempts in a single operation.

The European Space Agency (ESA) has cataloged debris real data (figure 6) at multiple locations which can be correlated to current study (ESA, 2022).

The plot of apogee and perigee altitude against RAAN of debris fragments under against RAAN of debris fragment under analysis is given in Figure 9. This plot also shows that the junk concentrated region lies within RAAN bin of 50.8$^{o}$ to 94.6$^{o}$ corresponding to the altitude of range of 622 and 1122 km. The distribution of fragment apogee and perigee altitude as a function of orbital period is depicted in Figure 10. The overall results of the simulation analysis establish the fact Keplarian elements make it easy to identify the position of the space debris in terms of semi-major axis, inclination and RAAN. However, it must be noted that there are some uncertainties due to the perturbation effects. The magnitude of these uncertainties can be managed with high update rate of orbital data in the simulation.

## 5. Active Debris Removal (ADR) technique

After debris identification, further aim of this paper is to propose cost-efficient debris removal algorithm. The idea is to capture and safely remove debris within the identified fragments from the selected altitude regions. It would be done with a servicing platform, composed of numerous stages. The primary component of this platform would be a Hoover System (HS) and secondary supporting equipment include the Hybrid Rocket Motor (HRM) to perform de-orbiting operation, propulsion system to perform rendezvous maneuver and maintain attitude and communication system for telemetry and command of the space mission (Carmicino & Sorge, 2007). The whole idea is that once the platform approaches the targeted fragments; they would be captured by HS. It would produce electromagnetic attraction force with the help of large solenoids. The whole concept of ADR is discussed in detail as follows:

**5.1 Rendezvous phase**The rendezvous phase begins with maneuvering of servicing platform already inserted and parked in the target (fragment cloud)

orbital plane. The rendezvous phase is subdivided into various stages, discussed as follows:

***Phasing with the target:*** To start rendezvous, various orbital phase changing maneuvers have to be performed to transfer the service platform from parking orbit to targeted debris cluster. These plane-change orbital maneuvers are based on Hohman transfer technique as it is an efficient and energy saving technique. Figure 11 demonstrates the orbit transfers required to first approach, rendezvous, capture and transfer the debris fragmentation to low altitude for re-entry. When the platform reaches the phasing orbit, distance between service platform and debris cluster (target) would decrease to few kms. There can be some uncertainty in cluster location because of the limited accuracy of orbital data (ground track and TLE). The precise position of the target cluster would be achieved with the help of onboard optical sensors like far range camera or star sensor and infrared sensor during of eclipse. Line of Sight (LOS) of the cluster is one of the most critical types of information which would be obtained from a far range sensor to carry out the phasing maneuvers accurately. Similarly, camera imagery combined with GPS receiver data would provide parameters of range and range rate to establish relative movement of the platform with respect to the target. The initial optimum identification of the target would be acquired from optical sensor. Moreover, the time when relative distance between the platform and cluster reduces from kilometer to meter level, near range rendezvous phase would be initiated with the aid of a near range camera and monocular or stereo-vision methods.

***Mid far range rendezvous:*** When the server platform approaches near target, a mid-far range rendezvous is needed to bring the servicing platform near to the cluster. To carry out this rendezvous, technical equipment must be precise to avoid any serious issue in this operation. Technologically verified equipment could be acquired from other similar rendezvous operations, e.g., DEOS mission, RRM mission and Phoenix Program of NASA.

***Close range rendezvous HCS:*** An identification and inspection with target fly

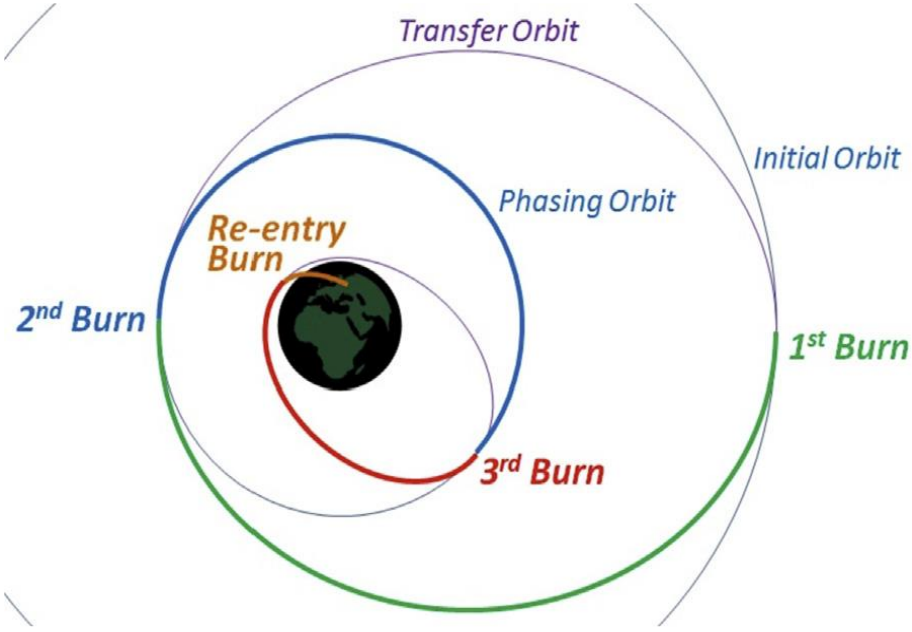


**Figure 11** Illustration Hohmann technique for orbital transfers required for approach, rendezvous and transfer the debris cluster.

**Table 3** Illustration rendezvous stages and detectors for identification and tracking of debris.

| Stages | Maneuver | Sensor | Separation |
|---|---|---|---|
| Phasing | Target Phasing (absolute navigation) | GPS | ~10 km |
| Far-mid range rendezvous | Tracking and prelude identification of the cluster (relative navigation) | Infrared or optical sensor of far-mid range | ~10 m |
| Close range rendezvous | Fly around of HS for identification and inspection (close immediacy navigation) | Infrared or optical camera of close range | ~1 m |

around is needed before capturing the target. This action is necessary for the selection of the finest positions for capture maneuver. A close-range infrared/optical camera would be used for close range rendezvous phase. This phase is of critical importance because it is the final stage of rendezvous and the platform would start to capture the targets with the HCS. Therefore, major challenges would be mitigated with state-of-the-art technology and superior mission planning. This involves the determination of the critical parameters loke relative velocity, angular momentum and energy of the cluster. Meanwhile, synthetic information would also be acquired to compute the relative orientation of the cluster with respect to the platform. For this purpose, several algorithms can be utilized, such as, contour mapping, linearization and detection of edge. A brief description of various stages of rendezvous phase and detectors/sensors for identification and tracking of debris cluster is presented in Table 3.

We suggest a practically feasible methodology for the removal of 10 cm or larger fragments. It is named as Hoover Capture System (HCS) and its operating principle is similar to that of the vacuum cleaner. The only difference is that there is magnetic field instead of air that sucks up all the debris to be disposed of on a later stage. This HCS is a type of transducer, mainly composed of cylindrical solenoids, which are mounted with base of cylinder to generate magnetic field. The solenoid is basically composed of an insulated copper wire that is winded on a metallic core to produce uniform magnetic field. The general behavior of a solenoid is electromagnetic in case when a controlled magnetic field is needed. The power supplied to the solenoid would be generated by the solar panels. The left-hand side of the solenoid acts as south pole and right-hand side acts as north pole, if the current flow direction is clockwise and vice versa. The value of magnetic flux (B) can mathematically be presented by the Ampere law (Lim & Greenside, 2016):

$$B' = \mu_o \frac{NI}{l} \tag{12}$$

In the above equation, $B'$ magnetic flux, N represents total turns in the coil, I is electric current, l solenoid's length and $\mu_o$ is the free space permeability. It must be noted that B has a direct relation to N, which implies that higher value of N would result in higher B. If we consider the case that the coil is wounded across a material with a permeability of μr, the increase in magnetic field can be expressed as:

$$B' = \mu_o \mu_r \frac{NI}{l} \tag{13}$$

An assumption is made that the current is evenly distributed over the surface of a fixed continuous solenoid. We may denote the radius of solenoid as a, length of solenoid as L and its current density with K. The value of B can be calculated using magnetic vector potential component $A_\varphi$ in φ-direction, hence in cylindrical coordinates (ρ,φ,z) (Lim & Greenside, 2016) .

$$\vec{K} = \frac{I}{L}\hat{\varphi} \tag{14}$$

$$A_\varphi = \frac{\mu_o I}{4\pi}\frac{1}{L}\sqrt{\frac{a}{\rho}}\left[\beth k\left(\frac{k^2+H^2-k^2H^2}{k^2H^2}K(k^2) -\right.\right.$$

$$\frac{1}{k^2}E(k^2)+\frac{H^2-1}{H^2}\prod(H^2,k^2)\Big)\Big]_{ↄ_-}^{ↄ_+}, \quad (15)$$

Where,

$$ↄ_\pm = z \pm \frac{L}{2}$$

$$H^2 = \frac{4a\rho}{(a+\rho)^2}$$

$$k^2 = \frac{4a\rho}{(a+\rho)^2+ↄ^2}$$

$$K(m) = \int_0^{\pi/2} \frac{1}{\sqrt{1-m\sin^2\theta}}\,d\theta$$

$$E(m) = \oint_0^{\pi/2} \sqrt{1-m\sin^2\theta}\,d\theta$$

$$\prod(n,m) = \int_0^{\pi/2} \frac{1}{(1-n\sin^2\theta)\sqrt{1-m\sin^2\theta}}\,d\theta \quad (16)$$

In the above equations, last three expressions are complete elliptic integrals of equation 13, 14, and 15. As we know:

$$\vec{B} = \nabla \times \vec{A} \quad (17)$$

Thus magnetic flux density becomes

$$B_\rho = \frac{\mu_0 I}{4\pi}\frac{1}{L}\sqrt{\frac{a}{\rho}}\left[ ↄk\left(\frac{k^2-2}{k}K(k^2)+\frac{2}{k}E(k^2)\right)\right] \quad (18)$$

$$B_z = -\frac{\mu_0 I}{4\pi}\frac{1}{2L}\frac{1}{\sqrt{a\rho}}\left[ ↄk\left(K(k^2)+\frac{a-\rho}{a+\rho}\prod(h^2,k^2)\right)\right]_{ↄ_-}^{ↄ_+} \quad (19)$$

The radial component will be vanished on the symmetry axis, thus the axial field component is

$$B_z = \frac{\mu_0 NI}{2}\left(\frac{\frac{l}{2}-z}{l\sqrt{a^2+(\frac{l}{2}-z)^2}}+\frac{\frac{l}{2}+z}{l\sqrt{a^2+(\frac{l}{2}+z)^2}}\right) \quad (20)$$

As $(\frac{l}{2}- |z| \gg a)$ inside the solenoid far away from the end, the equation becomes $B = \frac{\mu_0 NI}{l}$

### 5.2 Debris capturing and de-orbiting phase

As mentioned earlier, relative position of the cluster would be calculated after close range rendezvous phase. It would be done with the help of near range sensor by utilizing monocular or stereo-vision methods. When the relative location of the target is determined, an adequate amount of magnetic field of the solenoid would become available to attract the small piece of space junk within the cluster. It must be noted that the solenoids should be tough enough to bear the impact of small fragments. Once the entire cluster has been cleared, the system would be powered off so that all the fragments may demagnetize and settle down.

### 5.3 Controlled re-entry phase

After successful rendezvous, now next phase of the operation would be initiated to bring the junk from its present location to predefined parking orbit at 250 km. This altitude would be achieved with the help of a plane change maneuver. Approximately 50 ms-1 energy in terms of velocity would be required to decrease the perigee altitude to 90 km. At this point, the fragments begin to re-enter the atmospheric where they would experience strong heat due to drag and ultimately burnt. The heat intensity is dependent on numerous parameters, e.g. initial velocity, flight path angle and ballistic coefficient etc. It is extremely tough to forecast the accurate re-entry location of the captured junk. This is due to almost 10% prediction uncertainty for the remaining lifetime (Patera & Ailor, 1998).

## 6. Hybrid Rocket Motor (HRM) for transfer/launch of service platform HCS

Hybrid Rocket Motor (HRM) a vehicle to carry the service platform near the debris cluster to initial ADR mission. It is an innovative system with some added advantages like thrust couture, stop-restart ability, enhanced safety systems and cost efficiency. Above all, it has operation capability in dual mode, such that, gas or solid oxidizer and solid-state fuel (DeLuca, Lavagna, Maggi, Tadini, Pardini, Anselmo & Viola, 2014).

### 6.1 Engine design

These days HRM is built with an innovative geometry in which a swirl injection is utilized. This arrangement produces a rotational speed at the surface and axial speed along the center. The main

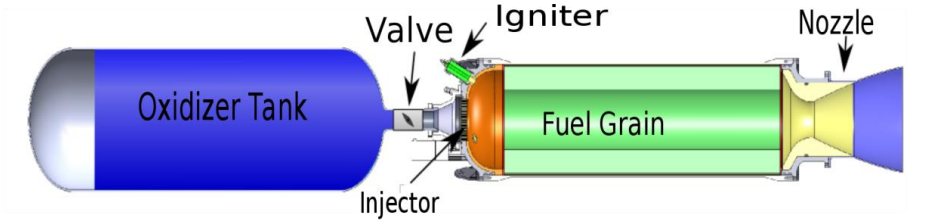


**Figure 12** Fundamental deign of HRM

components include cylindrical solid fuel tank that is connected to the nozzle and oxidizer tank injector via ports. The oxidizer tank contains gas or liquid oxidizer material which flows towards the fuel tank via connected ports. This type of flow provides high stability during combustion process and is also critical for an optimum mixing of oxidizer with the fuel. In HRM, solid fuel is mixed with oxidizer within flame a zone flame. This flame zone is produced at the intersection, which melts and burns the solid fuel. The primary performance factor of combustion is the regression rate, which is a measure of the mixing of solid grain fuel with oxidizer. Therefore, a high quantity of heat flow within flame zone results in a high regression rate. The design of a basic HRM is depicted in Figure 12 (DeLuca, Lavagna, Maggi, Tadini, Pardini, Anselmo & Viola, 2014)..

### 6.2 Oxidizer

The oxidizer selection depends on numerous factors like specific impulse, regression rate, stability, density, and cost etc. As oxidizers, we can use Halogens ($F_2$, $Cl_2$, $Br_2$), but they are sensitive and toxic nature in nature are the major points of concern. For example, $F_2O$ can be an effective selection because it gives a high specific impulse and also lighter in weight. However, it has some drawbacks as it is toxic, unstable, and also corrosive in nature. Moreover, it is also expensive, that's why it is not considered as a potential candidate of oxidizer for this mission design. Liquid oxygen (LOX) can also be seen as an oxidizer candidate because of its high specific impulse. The main disadvantages of LOX oxidizer are high cost, cryogenic nature, and lack of self-pressurizing property. On the other hand, Nitrous Oxide ($N_2O$) is the best and amply utilized oxidizer, which is readily available, self-pressurizing compound and produces a positive heat of formation. If we talk about its physical properties, its has density of $1.22\text{g/ml}$, boiling point of $-88.5\,\text{C}^\text{o}$ and melting point $-90.8\,\text{C}^\text{o}$ (Zakirov, Wan, Shan, Zhang & Li, 2006). It goes through following reaction:

$$\text{N}_2\text{O} \rightarrow \text{N}_2 + \frac{1}{2}\text{O}_2 + 19.61\text{kcal/mol} \quad (22)$$

### 6.3 Fuel

There are numerous options which can be taken into consideration for a fuel of this system. If we consider some properties as a deciding factor, the best fuel is stable and provides high value of specific impulse along with high regression rate. The excessively used hybrid solid fuel is Hydroxyl Terminated Polybutadiene (HTPB). Its density is $0.9494\text{g/ml}$, but when polymerized with Isonate (catalyst), the value of density beccomes $0.9651\ \text{g/ml}$. This is because the process of polymerization causes it to become stiffer, harder and stronger. The main advantage of using HTBP over other fuels is its stability and low cost. Meanwhile, there is also a disadvantage of HTPB that it has a low regression rate (Chluda, 2006).

### 6.4 Oxidizer injection

The regression is influenced by the factor of oxidizer flow rate. This is due to its dependence on the connective heat output from the flame to the grain of fuel. This regression rate can be increased by swirl injection due to swirl velocity component. A recirculation zone is formed in swirl flow and products preheat the combustible mixture in front of the flame combustion and therefore acts as a source

**Table 4** Specifications of proposed HRM components.

| **Component** | | **Density, g/ml** |
|---|---|---|
| HRM Oxidizer | Nitrous Oxide ($N_2O$) | 1.22 |
| HRM Fuel | Hydroxyl Terminated Polybutadiene (HTPB) | 0.9494 |
| Oxidizer Injection | Swirl flow | N/A |

of ignition to incoming mixture. This process is crucial because the flow of swirl actually regulates the combustion.

The flow of Swirl is categorized into two types of flow structures / components, rotational velocity and axial velocity components. The rotational velocity component is directed near surface and axial velocity components is directed along center. In the case of short grain, the average value of regression rate highly depends on the injection of swirl. The centrifugal force of flow of the oxidizer results in an optimum better regression rate of the fuel. We can determine the relation between regression rate and restrained parameters. The average value of regression rate is provided by the following mathematical equation (Chluda, 2006):

$$\dot{r}_{avg} = a_o \overline{G}_{ox}^{n} \quad (23)$$

In the above equation, $\dot{r}_o$ denotes average rate of regression, $a_o$ determines experimental coefficient (grain length is included) of regression rate, $\overline{G}_{ox}$ represents average mass flux rate of the oxidizer and n denotes the exponent of regression rate.

## 7. Conclusion

Numerical modeling technique of the debris fragmentation has been analyzed with the help of real-time case studies of Cosmos 2251, Iridium 33 and Fengyun-1C collisions and NOVAA 16 explosion. Meanwhile HCS based ADR method has been proposed in this paper, which is not only feasible for removal of fragments but also a cost-effective solution for long-term operations. Furthermore, the orbits of aforementioned debris fragments are propagated in dynamic simulator which suggests that the critical regions with debris cluster in terms of orbital parameters (semi-major axis and inclination) are 1000 km and 82º, 800 km and 98º, 850 km and 71º respectively. We need to select an appropriate region where fragments are present in clusters that can be eliminated with a single operation attempt. An ADR technique is based on HRM based servicing platform that is composed of HCS, sensors and cameras to detect and identify cluster. After identification/detention it would be capable of capturing and de-orbiting a debris cluster in an effective manner. There are numerous aspects that can influence the mission design performance such as atmospheric drag, relative orbital speeds of system and the cluster. Furthermore, the design, oxidizer and fuel selection of HRM has also been discussed for this ADS mission.

## 8. Statements and Declarations

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***Conflict of Interest:*** The authors declare that they have no financial or personal relationships that could have inappropriately influenced the work reported in this paper

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# Essential oils and aqueous ethanolic constituents from *Juniperus excelsa* exert antidiabetic effects on Alloxan-induced diabetes in rats

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## Abstract

Diabetes mellitus is a complicated metabolic disorder with 4.4% of estimated prevalence by 2030 which is quite high as compared to 2002 which is 2.2%. This disease is not curable with any of the available anti-diabetic agents in the market, furthermore, several other issues with these agents including cost and side effects are provoking us to look for some newer anti-diabetic agents. The aim of this study was to evaluate the anti-diabetic activity of essential oil and aqueous ethanolic extract of *Juniperus excelsa*, which was selected on the basis of its traditional use. The anti-diabetic activity of aqueous ethanolic extract (100, 200 mg/kg) and essential oil (100, 200mg/kg) of the plant was evaluated in normal and alloxan (150mg/kg, I.P.) induced diabetic rats in acute study as well as in 14 days of chronic study. Hepatic as well as lipid profiles were also evaluated at the end of the study. The results showed a significant reduction in blood glucose levels with crude extract as compared to essential oil, in a dose-dependent manner, in acute as well as 14 days chronic study. Similarly, in hepatic and lipid profile evaluation more significant results were shown by the crude extract than the essential oil. The difference in results for crude extract and essential oil might be due to the presence of different phytochemicals. Although natural products are beneficial for various diseases and are used traditionally they have multiple issues involving oral absorption, bioavailability, and pharmacokinetics that still have room for further exploration.

**Keywords:** *Juniperus excelsa*, Crude extract, Essential oil, Antidiabetic, Hepatic profile, Lipid profile

## 1. Introduction

Diabetes mellitus is among the abnormal metabolic malignancies associated with continued levels of glucose in the blood. The disease is a result of either the inability of the body to produce the required amount of insulin or the efficient utilization of produced insulin. Insulin from the beta cells of the pancreas possesses a central role in the metabolic utilization of carbohydrates. Diabetic patients present a poor control over the varying glucose level in the blood with the food and this poor control over glucose level in the blood leads to various health hazards over the period. Classically, diabetes is classified into two major subsections including type 1 diabetes mellitus and type 2 diabetes

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mellitus. Type 1 diabetes is basically an autoimmune disorder where there is almost negligible production of insulin and it is only managed with an exogenous supply of insulin. alternatively, type 2 diabetes is a kind of metabolic disorder where the cellular components of the body become least responsive to the naturally produced insulin. This least cellular responsiveness leads to the excessive amount of glucose in the bloodstream that in turn triggers the pancreas to produce more insulin. Hyperglycemia, or elevated blood sugar, serves as a distinctive clinical manifestation of diabetes. Symptoms of heightened blood sugar levels include increased thirst, frequent urination, fatigue, blurred vision, and delayed wound healing. Left untreated, diabetes can lead to severe long-term consequences. Prolonged elevated blood sugar levels can cause damage to blood vessels and nerves, escalating the risk of renal and cardiac disease, retinopathy (eye damage), neuropathy (nerve damage) and infections. Effective diabetes management necessitates maintaining blood sugar levels within target ranges through a combination of lifestyle modifications, medications (such as insulin or oral antidiabetic medications), regular physical activity, and a well-balanced diet. Regular monitoring, including blood glucose testing, is crucial to assess and manage the condition effectively (Zaccardi *et al.*, 2015). Its estimated prevalence in 2030 may be around 4.4% which was 2.2% in 2002, affecting 150 million people in the world and by 2025 this figure will reach up to 300 million which will further rise up to 439 million by 2030 (Emam, 2012). According to WHO, by 2030 the diabetic patient will increase up to 114% (Wild *et al.*, 2004). Type-II diabetes, related to improper or incomplete utilization of insulin by cells, is considered as the most prevalent type of DM contributing up to 85%-95% even in developed countries ((Misar *et al.*, 2010). Several drugs for the treatment of diabetes have been discovered but still, this disease is not curable by these agents, furthermore, several side effects, complications, and cost issues are related to these currently available drugs. So we need some alternative therapies, and among them, herbal remedies are the best available alternative to be used as 90% of rural people rely on herbal drugs for the treatment of different diseases. Due to the minimal side effects and low cost the herbal drugs are now widely prescribed (Valithan, 1998). It has been reported in a study that almost 25% of currently available medicines have originated from plants (Kutchan, 1995). Indigenous plants are particularly intriguing because they have evolved in specific ecosystems and have developed natural defense mechanisms against pathogens and environmental stressors. These defense mechanisms often involve the production of bioactive compounds, which can have medicinal properties. Traditional knowledge about medicinal plants and their uses has been passed down through generations within indigenous communities. This knowledge provides valuable insights into the potential therapeutic applications of these plants. Researchers are conducting studies to identify and isolate bioactive compounds from indigenous plants, evaluating their pharmacological activities, and understanding their mechanisms of action. These efforts aim to validate the traditional uses of these plants and explore their potential for developing new drugs or natural remedies. In addition to their medicinal properties, indigenous plants also have cultural significance and play important roles in traditional healing practices. Researchers are increasingly recognizing the importance of respecting indigenous knowledge, engaging with indigenous communities, and conducting research in an ethical and sustainable manner. By studying traditional medicinal preparations of indigenous plants, researchers hope to unlock new treatment options for various diseases and

conditions. This approach also promotes the conservation of biodiversity and the preservation of traditional knowledge, while fostering collaboration and mutual respect between scientific communities and indigenous cultures (Kong *et al*., 2003). Ethnobotanical studies have indeed provided valuable insights into the potential anti-hyperglycemic properties of various plants. These studies involve the documentation of traditional knowledge and the use of plants by indigenous communities for managing diabetes and related conditions. Through these studies, researchers have identified numerous plant species that exhibit anti-hyperglycemic effects, meaning they have the potential to help lower blood glucose levels. It's estimated that around 800 plant species have been identified to possess such properties (Patel *et al*., 2012). The hypoglycemic activities of several indigenous plants, including Momordica charantia (bitter melon), Euphorbia prostrata, Acacia arabica, Onosma echoides, Achyranthes aspera, and Ficus glomerata, have been scientifically evaluated. Momordica charantia (Bitter melon) has been extensively studied for its potential hypoglycemic effects. It contains bioactive compounds that may help regulate blood glucose levels and improve insulin sensitivity. Euphorbia prostrata also known as prostrate spurge, is a plant that has been traditionally used in some cultures for its medicinal properties. Scientific studies have investigated its potential hypoglycemic activities and found that it may help reduce blood sugar levels. However, further research is needed to fully understand its mechanisms of action and determine its safety and efficacy. Acacia arabica commonly known as Babul or Indian gum Arabic tree, has been used in traditional medicine for various purposes, including managing diabetes. Some studies have explored its potential hypoglycemic effects and have reported positive outcomes. The plant contains bioactive compounds that may contribute to its anti-diabetic properties. Onosma echoides, also known as false gromwell, is a plant used traditionally in certain herbal preparations. Scientific investigations have revealed its potential hypoglycemic activities. It may possess compounds that help lower blood glucose levels and improve insulin sensitivity. Achyranthes aspera, commonly known as prickly chaff flower, has a long history of use in traditional medicine for various ailments, including diabetes. Research on this plant has suggested its potential for reducing blood sugar levels and improving insulin sensitivity. Ficus glomerata, also called cluster fig or gular fig, is another plant that has been examined for its hypoglycemic effects. Studies have indicated that extracts from Ficus glomerata may possess anti-diabetic properties and could help regulate blood glucose levels (Muhammady *et al*., 2012; Akhtar *et al*., 1983; Akhtar and Khan, 1985; Akhtar and Riffat, 1986; Akhtar and Riffat, 1988; Akhtar and Iqbal, 1991).

Studies have reported that chemical components from *Juniperus excelsa* include, 4-Terpineol 2.93, Abietatriene 1.13, Camphene 6.00, Fenchene 6.57, Germacrene B 2.21, Limonene 23.42, Myrcene 1.96, α-Pinene 1.77, α-Terpinene 23.85, β-Pinene 1.53, and δ-3-Carene 4.17 % (Göze et al., 2017). Moreover, so far more 48 essential oils have been reported in Juniperus excelsa including 4-Cubebol, 4-Terpineol, Abietatriene, Biformene, Bornyl acetate, Bornylene, Camphene, Camphor, Carvone, Elemol, Fenchene, Furfuryl methyl ether, Germacerene D, Germacrene D, Germacrene D-4-ol, Limonene, Myrcene, Myrtenal, Piperitone, Sabinene, trans-Carveol, Trans-Pinocarveol, Tricyclene, α-Amorphene, α-Campholenal, α-Copaene, α-Cubebene, α-Eudesmol, α-Fenchol, α-Humulene, α-Muurolene, α-Pinene, α-Terpinene, α-Terpineol, α-Thujene, β-Cadinol, β-Caryophyllene, β-Elemene, β-Eudesmol, β-pinene, γ-Elemene, γ-Eudesnol, γ-Terpinene, δ-3-Carene, δ-Cadinene, δ-Cadinene, δ-Elemene and σ-Terpinolene, (Weli et. al., 2014)

*Juniperus excelsa* (*JE*) was selected for anti-diabetic evaluation on the basis of its traditional use as antihypoglycemic in Iran and different regions of Pakistan (Pirani *et al*., 2011). Some other members of Juniperus genus including *Juniperus communis* (Banerjee *et al*., 2013), *Juniperus oxycedrus* (Taviano *et al*., 2013) and *Juniperus phoenicea* (Keskes *et al*., 2014) have also been reported for anti-diabetic activity. *Juniperus excelsa* is known as "East African Pencil Cedar", "Grecian Juniper", "Himalayan Juniper", and "Persian Juniper" (Negussie, 1997.). In Pakistan it is found in three regions Quetta, Ziarat and Kalat (Khajjak *et al*., 2012). Juniper species are used in many forms; such as oil, berries, decoction, infusion, tincture, extractions. Oil, present mostly in berries, is used in food processing, pharmaceutical, and cosmetic industry (Khajjak *et al*., 2012), leaves in the form of decoction and a mixture of leaves and berries are also used, in the form of tea, tea of Juniper is enlisted in German Pharmacopoeia and has a recommendation for its use as digestive aid. In combination with ginger, it is a popular intoxicant (El-Sawi *et al*., 2007), dried heartwood, the stem of this plant, and folk alcoholic beverages containing berries of Juniper are also used for the treatment of hyperglycaemia but this use is not supported by any scientific background. Dried heartwood is used traditionally for colds, UTIs, urticaria, dysentery, hemorrhage, leucorrhea, and in arthritis. The Stem of this plant is used for treating parasitic skin infections, and the root for burns (Ju *et al*., 2008) mature female cones of Juniper are used for flavoring food and pickling meat and in alcoholic beverages, as an antimicrobial agent, in the form of steam inhalant it is used for bronchitis, anti-carcinogenic agent (Kargıoğlu *et al*., 2010).This study was designed with an objective to evaluate the effects of J.E extracts against alloxan induced diabetic rats for the potential anti-diabetic effects that may determine the utility of the plant for further exploration for active ingredient isolation and pharmaceutical formulation preparation in future.

## 2. Material and Method

Male adult white albino rats weighing between 200-300g were utilized. We placed the rats as per the standard protocol in an animal care house for the experimental purpose within the allocated facility in UOS, the Pharmacy department animal laboratory. The stainless-steel cages were used to house the rats, and they were kept under standard laboratory conditions. Standard laboratory conditions typically involve providing a controlled environment to ensure the well-being and health of the animals. These conditions include maintaining specific temperature and humidity levels, proper ventilation, a regulated light-dark cycle (usually 12 hours of light and 12 hours of darkness), and appropriate hygiene practices to ensure cleanliness. Additionally, the animals in laboratory settings were typically provided with adequate food and water. The specific details of the diet and access to water would depend on the study protocol and any specific requirements or restrictions related to the research being conducted. It is worth mentioning that animal studies were adhere to ethical guidelines and regulatory standards to ensure the welfare and ethical treatment of the animals involved. Institutional Animal Care and Use Committees (IACUCs) of the institute UOS, where the time span for daylight was set from 8:00 am to 8:00 pm, a room temperature range of 22-24 ºC with humidity of 55% and all the animals were provided free access to water ad libitum. In our present study, the berries of the plant were collected from Zyarat, Balochistan. After collection, the berries were dried and powdered to facilitate further processing. The powdered berries were then subjected to extraction using an aqueous ethanol solvent in a ratio of 30:70 (30% water and 70% ethanol). The extraction method employed was cold maceration which involves soaking the plant material in the

chosen solvent at room temperature or lower for a specific duration to extract the desired compounds. It is a common method used for extracting bioactive compounds from botanical materials. During cold maceration, the powdered plant material was typically mixed with the solvent in an appropriate container, such as a glass jar or flask. The mixture was then left to stand for a specified period, allowing the solvent to extract the constituents from the plant material. The choice of aqueous to ethanol (30:70) as the extraction solvent was aimed at extracting a range of compounds, including both hydrophilic (water-soluble) and lipophilic (alcohol-soluble) components. Ethanol is commonly used as a solvent in herbal extractions due to its ability to dissolve a broad spectrum of bioactive compounds. Once the maceration period was completed, the extract was filtered to remove any solid residues. The resultant extract, containing the extracted compounds from the plant material was then used for further analyses.

### 2.1. Extraction of essential oil

The essential oils (Es.O) of (*JE)* was obtained by steam-distillation of the plants using a pilot-scale system (Burits and Bucar, 2000).

### 2.2. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was conducted in normoglycemic rats to assess their tolerance to a glucose load in the presence of the crude extract. The OGTT is a commonly used test to evaluate how the body responds to a standardized amount of glucose administered orally. The following steps were adopted; Selection of animals: Normoglycemic rats, which had normal blood glucose levels, were chosen for the experiment. This was to assess the effect of the crude extract on glucose metabolism in the absence of pre-existing diabetes. Fasting period: Before the test, the animals were usually subjected to a fasting period of several hours (usually overnight). This ensures that their blood glucose levels return to a baseline fasting state. The baseline blood glucose levels of the rats are measured before the administration of glucose or the crude extract. This serves as a reference for comparison. Administration of glucose load: A standardized amount of glucose is administered orally to the rats. This was typically done by gavaging a glucose solution directly into the stomach or through oral feeding. Blood glucose measurements: Blood glucose levels are measured at specific time intervals after glucose administration. This is typically done by obtaining small blood samples, often from the tail vein or via other appropriate methods. Evaluation of glucose tolerance: The blood glucose levels at each time point were compared to the baseline levels to assess how efficiently the rats metabolize and regulate glucose to determine the impact of the crude extract on glucose metabolism and tolerance. The studies employed crude extract and (CE) and EsO of *JE* and the method was adopted from (Muruganandan *et al.*, 2005).

### 2.3. Acute hypoglycemic activity in normoglycemic rats

Rats were divided into six groups of five animals in each. Group 1 served as a normal control and was administered with distilled water orally. Group-2 and 3 received 100 mg/kg and 200mg/kg of aqueous ethanolic extract (AqEE) of JE respectively. Group IV and Group V received 100 and 200 mg/kg of EsO respectively. Group VI received 200mg/kg of metformin. The blood glucose level was measured after 0,1,2,4 and 6 hours with a glucometer. All groups were kept on fasting overnight.

### 2.4. Diabetes induction

We injected a single peritoneal injection of 150mg/kg of alloxan monohydrate to induce the diabetes in the animals where alloxan monohydrate was weighed individually for each rat based on their body weight. The specific dosage of 150 mg/kg was determined according to the weight of each animal. Alloxan was then solubilized with 0.2 ml of saline (154 mM NaCl) just before injection. The solubilized alloxan solution was injected

intraperitoneally into the rats that involves the delivery of substance into the peritoneal cavity, the space within the abdomen. After three days after the alloxan injection, the rats' plasma glucose levels were measured. Rats with plasma glucose levels exceeding 200 mg/dl were considered diabetic and were included in the study. After confirming the induction of diabetes in the rats, the treatment with the plant extracts was initiated.

### 2.5. Antihyperglycemic effects in alloxan treated diabetic rats

For this experimental study, we made 6 different groups of animals each containing five animals. First group *i. e* the Group one (G-I) was the untreated diabetic control group. The rats in this group were administered 10 ml of a 5% ethanolic solution which means that the rats in group-I received a solution containing 5% ethanol (ethyl alcohol) in a total volume of 10 ml. The purpose of having an untreated diabetic control group is to provide a comparison for the experimental groups receiving the extracts. By administering the 5% ethanolic solution to the control group, the study may provide the evaluatory analytical effects of the extracts by comparing them to the baseline condition of untreated diabetes. Group-II and Group-III received orally 100mg/kg and 200 mg/kg of AqEE. Group IV and Group V received 100 and 200 mg/kg of EsO. Group VI received 200mg/kg of metformin. The blood glucose level was measured after 0,1,2,4 and 6 hour with glucometer. All groups were fasted overnight (12hr)

### 2.6. Chronic antidiabetic activity in alloxan induced diabetic rats

We divided the experimental animals into 7 different groups and named them as Group I to VII (G-I, G-II, G-III, G-IV, G-V, G-VI, G-VII) and each group contained 5 animals. Our first group was standard control group that represented the healthy, non-diabetic rats. They did not receive any specific treatment and served as a baseline comparison for the experimental groups. Group-II: (The Untreated diabetic control group). These rats were induced to become diabetic (as described earlier) and received

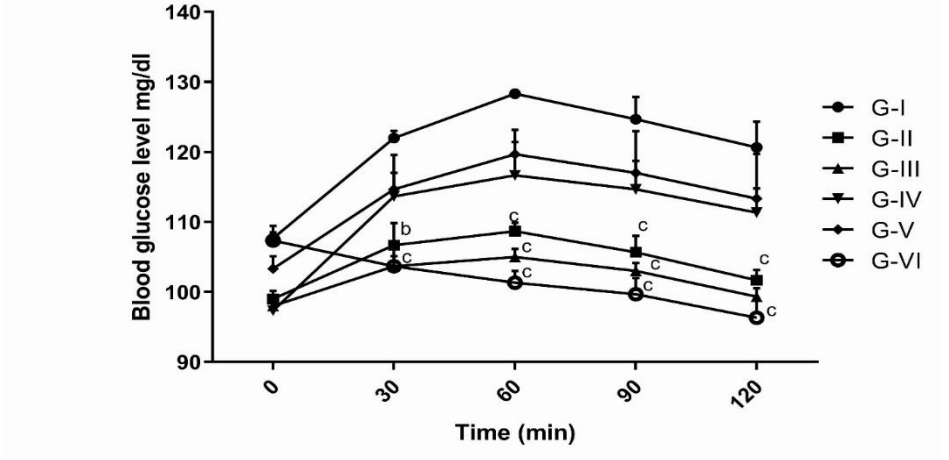


Figure 1 Effect of different groups on oral glucose tolerance test in normoglycemic rats. Values are expressed as mean ± SEM, where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups. G-1= normal control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg), G-V= EsO (200 mg/kg), G-VI= metformin (200mg/kg)

a 10 ml oral dose of a 5% ethanolic solution. This group helped to assess the effects of the vehicle (ethanolic solution) on diabetic rats. Group-III: was given AqEE of the plant with a dose of 100mg/kg, next group of animals (G-IV) was treated with 200 mg of Aquas ethanolic extract of the preparation while G-V and G-VI were treated with an additional oral dose of EsO of 100 and 200 respectively along with 200 mg of AqEE. Group VII received 200mg/kg of metformin. The blood glucose level was measured after 1st, 7th and 14th day with glucometer. All groups were fasted overnight (12hr). At the end of experiment rats were scarified and blood was obtained to evaluate lipid profile and hepatic profile.

### 2.7. Collection of blood sample

Rats were placed in restainers and tail was cleaned with cotton swab dipped in spirit. The tail was pricked with needle and pressed to get blood. First drop of blood was wasted and next was poured on glucometer strip to find the plasma glucose level. After taking sample the tip was again pressed with spirit to avoid any type of infection. To measure cholesterol, LDL, HDL and hepatic profile the blood was obtained at the end of the study by scarifying the animal.

### 2.8. Separation of serum

We obtained the samples of the blood from the animals in sample gel clot activation tubes for the analyses of complete cholesterol profile including low density lipid proteins and high-density lipid proteins as well as for the other enzymatic activities of the liver. Clot activator gel tubes contain an inert gel, typically composed of silicone or other similar substances, that promotes the clotting process the tube was helpful to separate the serum, which is the liquid portion of blood, from the formed clot and other cellular components. Briefly, the

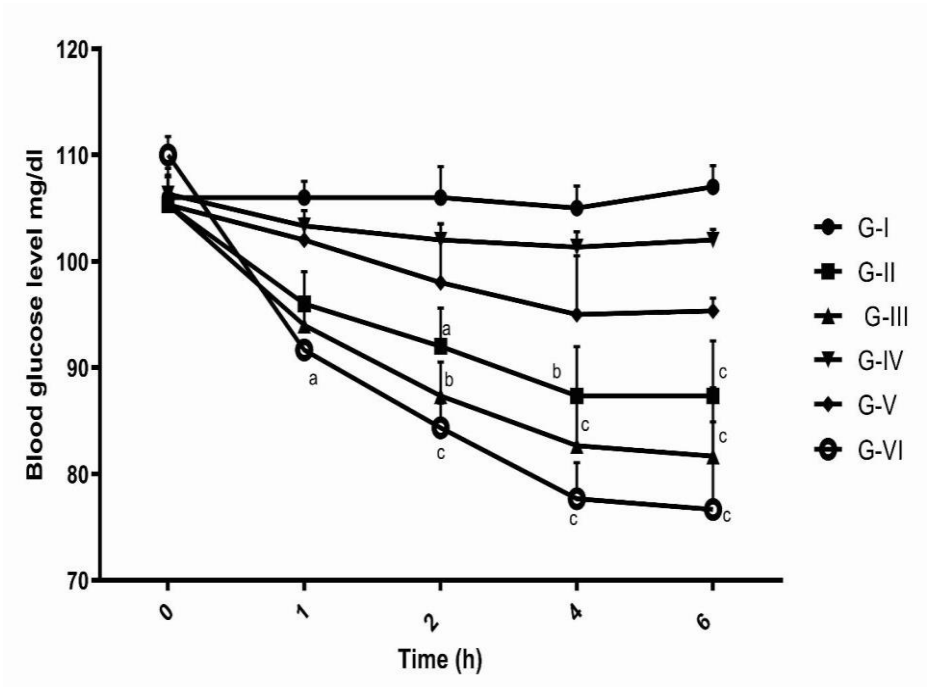


Figure 2 Variations in glucose levels in normoglycemic rats. Values are expressed as mean ± SEM, where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups G-1= normal control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg), G-V= EsO (200 mg/kg), G-VI= metformin (200mg/kg)

blood samples were collected from the rats using clot activator gel tubes. After the blood was collected in the gel tubes, the clotting process was initiated. The clot activator in the gel tube triggers the coagulation cascade, leading to the formation of a clot. Once the clot was formed, the blood samples were subjected to centrifugation at 2000 RPM for 10 minutes. During centrifugation, the formed clot and other cellular components (such as red blood cells and white blood cells) were settled at the bottom of the tube, while the serum, which contains the liquid portion of blood along with dissolved substances, rose to the top. After centrifugation, the serum was carefully separated from the rest of the components by pipetting or decanting the clear, yellowish serum layer without disturbing the underlying clot or cellular material. The separated serum was then used for the estimation of various parameters, such as total cholesterol, LDL, HDL, and hepatic profile. These data provided results about the lipid profile and liver function, which were relevant in assessing metabolic and hepatic health as described earlier by Hakkim and colleagues (Hakkim *et al*., 2007). In order to conduct further analyses freshly isolated and obtained blood serum was transferred in serum cups or apendrof tubes and were place in cold place for future usage.

### 2.9. Statistical analysis

The resultant data were presented as mean ± SD where the significance levels between the groups were analyzed via ANOVA for cholesterol, low density lipids, high density lipids and hepatic enzyme values from different groups. A P value of less than 0.05 was rated as level of significance difference to further conclude the study results.

## 3. Results

Aqueous ethnolic extract of *JE* showed significant dose dependent effect on oral glucose tolerance test. At 40 minutes the 200mg/kg of extract showed more

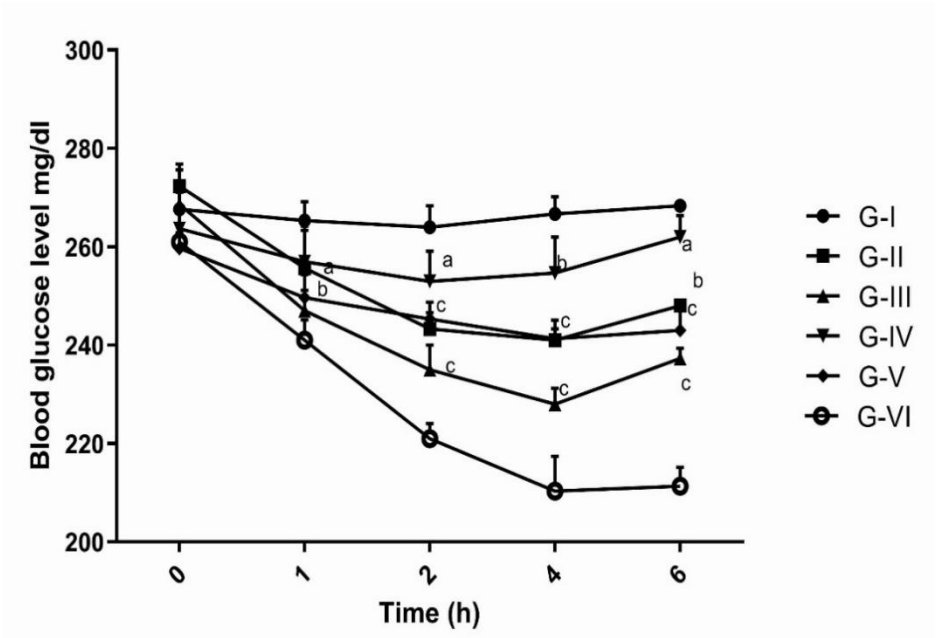


Figure 3 Variations in glucose level in diabetic rats. Values are expressed as mean + SEM,where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups. G-1= diabetic control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg), G-V= EsO (200 mg/kg), G-VI= metformin (200mg/kg)

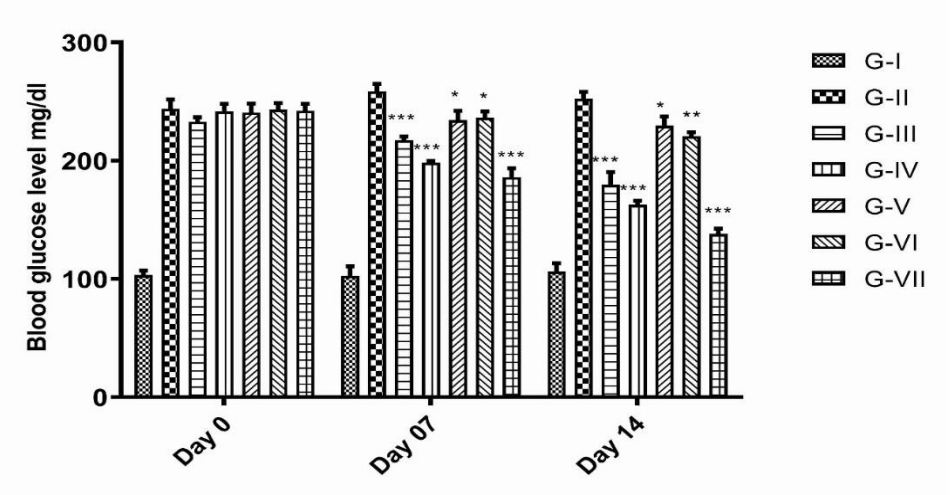


Figure 4 Effect of different groups on blood glucose level (mg/dl) in alloxan-induced diabetic rats in 14 days study. Values are expressed as mean ± SEM, where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, G-II= Diabetic control, G-III= CE (100 mg/kg), G-IV=CE (200 mg/kg), G-V= EsO (100 mg/kg), G-VI= EsO (200 mg/kg), G-VII= metformin (200mg/kg)

significant result than with 100mg/kg, but after 80 and 120 minutes the results were comparable for both of the doses with level of significance P<0.001. The group VI, treated with the standard drug metformin also showed significant results with P<0.001. The group IV, treated with 100mg/kg and V, treated with 200mg/kg of EsO of *JE* did not show any significant result as showed in figure 01.

### 3.1. Acute hypoglycemic activity

After one hour, no group, except the group treated with metformin, showed significant results (P<0.05). This suggests that only the metformin-treated group exhibited a statistically significant effect compared to the control or other treatment groups at this particular time point. In the 2nd hour of the study, Group II treated with 100 mg/kg of the extract showed a significant result with a level of significance of P<0.05. Group III treated with 200 mg/kg of the extract showed a more significant result with a level of significance of P<0.01 while Group VI treated with 200 mg/kg of metformin showed the most significant result with a level of significance of P<0.001. The next observation was made after 4 to 6 hours where, Group II treated with 100 mg/kg of the extract at the 6-hour time point. Group III treated with 200 mg/kg of the extract at the 4-hour and 6-hour time points. Group VI treated with 200 mg/kg of metformin at the 4-hour and 6-hour time points. In all these findings, the results were highly significant, with a level of significance of P<0.001, indicating a robust and consistent effect of the treatments on the measured parameters. Interestingly group IV (100 mg/kg of EsO) and Group V (200 mg/kg of essential oil): did not show any significant results at all. The data obtained from these groups did not exhibit statistical significance compared to the control or other treatment groups at any of the time points evaluated (Fig. 2).

### 3.2. Acute antidiabetic activity in alloxan induced diabetic rats

The group III was administered with 200mg/kg of crude extract and VI treated with 200mg/kg metformin showed comparable significant result with P<0.001 and group II treated with 100mg/kg CE and group V treated with 200mg/kg EsO showed comparable significant reduction on blood glucose level with P<0.05.

### 3.3. Chronic antidiabetic activity in alloxan induced diabetic rats

Crude extract of JE (Group III and Group IV): Both Group III, treated with 100

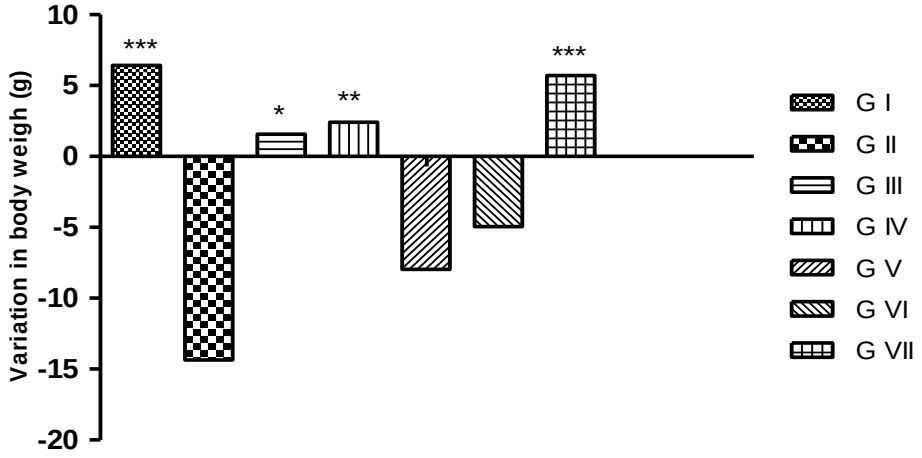


Figure 5: The effect of 14-days treatment with various groups on body weight (g) after alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the diabetic control (G-II) and other groups. G-1= normal control, G-II= Diabetic control, G-III= CE (100 mg/kg), G-IV=CE (200 mg/kg), G-V= EsO (100 mg/kg), G-VI= EsO (200 mg/kg), G-VII= metformin (200mg/kg)

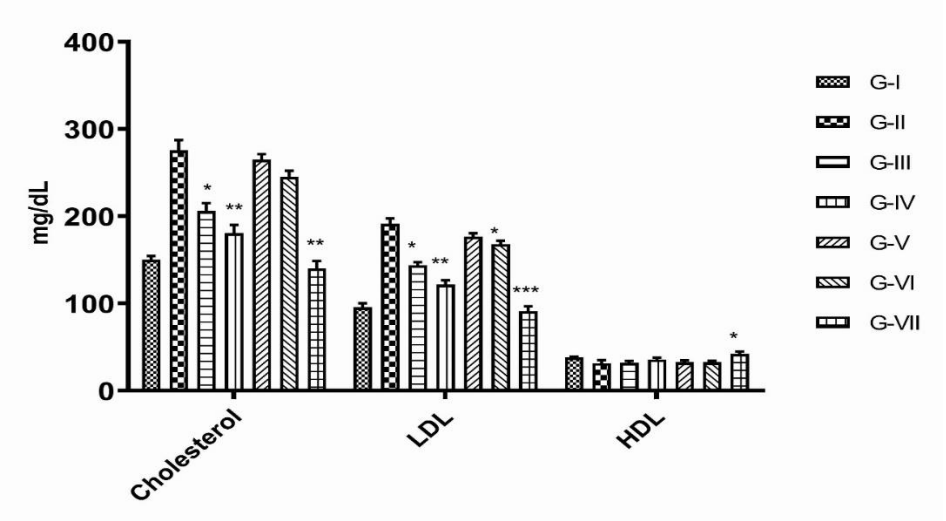


Figure 6 The effect of 14-days treatment with various groups on total lipid profile in alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, G-II= Diabetic control, G-III= CE (100 mg/kg), G-IV=CE (200 mg/kg), G-V= EsO (100 mg/kg), G-VI= EsO (200 mg/kg), G-VII= metformin (200mg/kg)

mg/kg of the extract, and Group IV, treated with 200 mg/kg of the extract, showed a significant reduction in blood glucose levels after 7 days and 14 days of treatment. The reductions in blood glucose levels were highly significant, with a level of significance of P<0.001. This indicates a strong and consistent effect of the JE extract on lowering blood glucose levels. Metformin (Group VII): Group VII, treated with metformin at a dose of 200 mg/kg, also exhibited a significant decrease in blood sugar levels at the 7th and 14th day of treatment. Similar to the JE extract-treated groups, the reductions in blood glucose levels in the metformin-

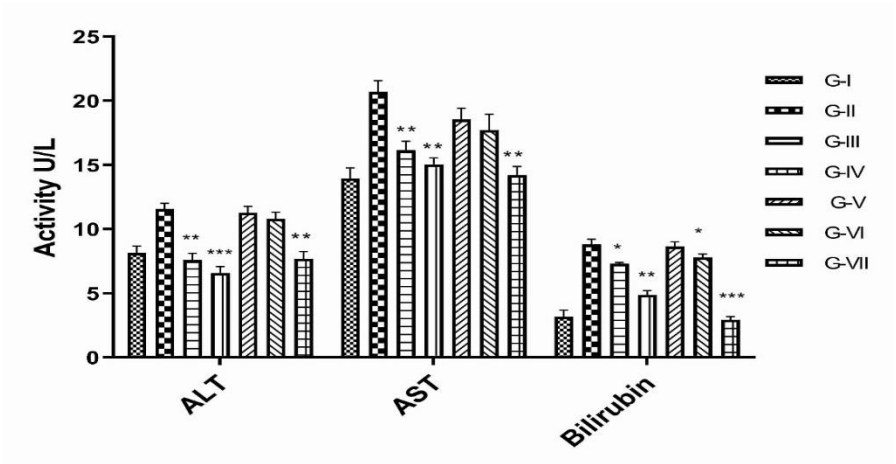


Figure 7 The effect of 14-days treatment with various groups on Hepatic profile ( ALT, AST and bilirubin) after alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, G-II= Diabetic control, G-III= CE (100 mg/kg), G-IV=CE (200 mg/kg), G-V= EsO (100 mg/kg), G-VI= EsO (200 mg/kg), G-VII= metformin (200mg/kg)

treated group were highly significant, with a level of significance of P<0.001. Essential oil (EsO) (Group V and Group VI): Group V, treated with EsO at a dose of 100 mg/kg of body weight, showed a reduction in blood glucose levels at the 7th day of treatment with a significance level of P<0.05. Group VI, treated with EsO at a dose of 200 mg/kg of body weight, exhibited a more significant reduction in blood glucose levels at the 7th and 14th day of treatment, with a level of significance of P<0.01 as shown in figure 4.

### 3.4. Effect of *juniperus excelsa* on body weights

Aqueous ethanolic extract of *JE* showed significant effect on body weight of rats. In group III treated with 100mg/kg and group IV treated with 200mg/kg the gain in weight was 1.57% and 2.42% with level of significant is P<0.05 and P<0.01 respectively. In normal control there was gain in body weight with 6.40% as compare to diabetic control as shown in figure 05.

### 3.5. Effect of juniperus excelsa on lipid and hepatic profile

In light of the results, our study indicates that aqueous ethanolic extract and EsO of *JE* have good antidiabetic activity. AqEE of *JE* exhibited more significant antihyperglycemic activities than essential oil, in alloxan-induced hyperglycemic rats. They can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile.

## 4. Discussion

The selected plant *JE*, has traditionally been used for hypoglycemic activity in different region of Pakistan (Pirani et al., 2011). However, as far as no ascertained scientific details, the study was planned to rationalize its traditional claim, and carried out with AqEE and EsO of plant in alloxan induced diabetic rats. Alloxan has dose dependant diabetogenic effect, for moderate diabetes induction a dose of approximately 150mg/kg is sufficient (Szkudelski, 2001). Superoxide free radicals are formed by redox cycle of alloxan and its reduction products that is dialuric acid (Kakkar et. al., 2013). These radical are converted into hydroxl radical after formation of an intermediate product, hydrogen peroxide. These free radical cause destruction of beta cells and induce diabetes. Fragmentation of DNA of pancreatic cell is another target of these free radicals (Rohilla and Ali, 2012).

The outcomes derived from the oral glucose tolerance test (OGTT) delineate a discernible protective influence exerted upon elevated blood glucose levels subsequent to a glucose load, manifesting with distinct efficacy in response to two doses of the plant extract, administered at 100 mg/kg and 200 mg/kg, respectively. Furthermore, a noteworthy observation emerged, elucidating a dose-dependent relationship governing the impact of the plant extract on blood sugar levels, wherein higher doses thereof exhibited a heightened capacity to forestall the ascent of blood sugar levels. The comparative analysis of OGTT results involving the 100 mg/kg CE diet and the 200 mg/kg essential oil diet unveiled a parallelism, albeit one signifying the superior efficacy of the crude extract (CE) over its essential oil counterpart. In the realm of acute studies, a meticulous scrutiny of the findings unveiled an absence of any discernibly significant reduction in blood sugar levels resultant from the administration of both doses of essential oil (EsO). Conversely, the crude extract (CE) manifested a conspicuous and statistically significant effect, underscoring a dose-dependent modality, wherein an augmented dosage of CE correlated with a commensurately amplified reduction in blood glucose levels. The overarching context of clinical studies, characterized as abbreviated trials designed to assess the immediate and direct impacts of a given treatment or intervention, served as the backdrop against which the efficacy of essential oils and crude extracts was appraised vis-à-vis their effects on blood sugar levels. Poignantly, neither iteration of the essential oil diet evinced a noteworthy and statistically significant reduction in blood sugar levels within the confines of the tested doses, intimating a potential dearth in the expeditious blood sugar-lowering efficacy of the essential oil. In stark contrast, the crude extract, leveraging a dose-dependent trajectory, unequivocally demonstrated a consequential and meaningful effect on blood glucose levels. This means that as the dose of the crude extract increased, there was a corresponding increase in the reduction of blood glucose levels. The dose-dependent response suggests that higher doses of the crude extract may have a more pronounced effect in lowering blood glucose levels. In acute study for diabetic rats, CE at the dose of 100mg/kg and EsO at the dose of 200mg/kg showed comparable response at 2$^{nd}$ and 4$^{th}$ hour. Similarly in chronic study the effect of positive control in lowering blood glucose level was dominant with almost 42% reduction, effect of CE at both the doses (100mg/kg and 200mg/kg) was also significant. Body weight loss is directly affected by the blood glucose level.

A study indicates that higher blood sugar level is directly correlated with the lipolysis. Weight loss can be a common outcome in certain conditions or interventions that effectively reduce blood glucose levels, such as in the case of diabetes management or calorie-restricted diets. However, it seems that the reduction in blood glucose levels achieved with the treatments did not translate into significant changes in body weight. It appeared that the groups which demonstrated a significant reduction in blood glucose levels did not show a significant weight loss. This suggests that the observed effects on blood glucose levels were not accompanied by noticeable changes in body weight. EsO showed significant reduction in body weight at both the doses. The rats given with metformin show significant weight gain comparable to normal control group. The maximum weight loss was in diabetic control that was upto 14%.

As diabetes is directly related with the hyperlipidemia, lipid profile was also evaluated at the 14$^{th}$ day. Dyslipidemia is caused by the insulin resistant causing increase in level of triglyceride, and LDL while decrease in HDL level (Assmann

and Schulte, 1988). Insulin plays major role in lipid regulation and metabolism of lipids in body (Coelho et. al., 2011). In adipose tissues insulin inhibit lipolysis by inhibiting activity of lipase so it has anti lipolytic activity in adipose tissues (Schwartz and Brunzell et al., 1981). Insulin also enhances storage of triglycerides in adipose tissue and decreases the release of free fatty acid into blood (Ruotolo *et al*., 1994). It also activates the lipo protein lipases (LPL) which causes catabolism of lipoproteins of all densities (Williams et. al., 1992); insulin is also having direct positive effect on LPL gene and enhances its production. It also cause clearance of LDL and has direct effect on HDL metabolism. So both qualitative and quantitative lipids profile abnormalities are observed in diabetic patient due to deficiency of insulin (Verges, 2005). The crude extract causes significant reduction in LDL and cholesterol in dose dependent manner while essential oil do not show any significant reduction if LDL and cholesterol. HDL level was significant increased by metformin no other group show any significant effect HDL level. As diabetes also affects the hepatic profile so the hepatic enzymes were also evaluated in the study. Patients with DM have very high incidence of abnormal liver function test (LFT). The most common LFT include bilirubin test, aminotransferases such as ALT and AST, albumin and alkaline phosphatase (Nannipieri et al., 2005). Insulin resistant is associated with the increase in level of ALT and AST. Three time than normal raise in value of ALT and AST indicate the use of anti-diabetic agents for diabetic patient. ALT and AST actually measure the leaked intera cellular hepatic enzymes to the blood circulation so these enzymes are the good biomarker of hepatic injury; billirubin is biomarker of billary function.

Due to the direct link of diabetes with abnormality of LFT the liver profile including ALT, AST and bilirubin were also evaluated after 14 days study. For all the enzymes the results were significant for crude extract in dose dependent manner. EsO at dose of 200mg/kg also show some results in reduction of these hepatic enzymes but these were less significant that CE. This might be due the reason that CE maintain the blood sugar level in normal range so no glycogenolysis happened and serum level of enzymes was also in normal range. The other reason is that *JE* itself has hepatoprotective claim in traditional medicine and has been used to low serum bilirubin.

AqEE of the plant showed more significant dose dependent effects then EsO. This might be due to the different phytochemistry of both EsO and CE. It has been reported that the EsO of barriers mainly contains alpha-pinene, beta-pinene and lemonine contributing 43%, 32% and 9% respectively while AqEE mainly contain diterpines, and sterols. It also contains other phytochemicals constituents such as alkaloid, phenols and tannins. The diterpenes might be responsible for better hypoglycemic response of crude extract because many studies indicate diterpenes as therapeutic phytochemical for diabetes e.g dehydroabietic acid which is diterpene and have antidiabetic activity (Kang *et al*., 2009). Icetexane diterpene, contain alpha glycosidase inhibitor activity, dihydrisotenshinon having PTPIB inhibitory activity are also diterpene. Similarly Saurufran A and Doiabellane diterpenes isolated from Nigella sativa also have antidiabetic potential due to PPAR activation pathway (Nagarajan and Brindha, 2012). So diterpens play a major role in lowering blood sugar level. The CE of berries of *Juniperus excelsa* contain ditterpens as major phytochemical such as 3a-acetoxy-labda-8(17), 13(16), 14-trien-19-oic acid, iso-communic acid, -ent! trans communic acid, isopimaric acid (Gulacti *et al*., 1999). So this might be a reason that CE has much significant blood glucose lowering effects than the EsO.

Finally it has been concluded that *JE* contain come active ingredients

responsible for hypoglycemic activity. Although natural products are beneficial for many diseases and are used traditionally but they have numerous problems regarding oral absorption, bioavailability and pharmacokinetics (Antony *et al.*, 2008). Based on the current study it is suggested that further studies are required to isolate the active ingredient and elucidate its mechanism of action.

## 5. Statements and Declarations

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***Author Contributions:*** AY supervise the whole study and proof read the final manuscript, RH and NM perform experiments and wrote manuscript.

***Conflict of Interest:*** All the authors have no conflict to disclose.

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# Hepatitis C Virus Infection and Its Correlation with Multiple Risk Factors in Local Population of Gujranwala Pakistan

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## Abstract

Hepatitis C virus is a single-stranded RNA virus belonging to the family of Flaviviridae that affects 71 million people in the world. In Pakistan, the occurrence of HCV is 10 million and national efforts are required to identify people. The current study was therefore planned to assess the occurrence of HCV relative to risk factors among the local population of Gujranwala, Pakistan. The viral infection was analyzed qualitatively by using PCR / third-generation ELISA, detailed history, and clinical examination. Data entered in already prepared Performa. Data analysis was done as mean ± standard deviation for the quantity's variable, whereas to assess the association between different group p-value as a level of significance, ANOVA was applied. Descriptive statistics mean ± standard deviation was used. LFTs have been performed. In the present study, a total of 320 patients (183 females and 137 males) were included. There were 172 (97 females and 75 males) patients diagnosed with HCV while 148 (85 females and 63 males) patients were non-infected. HCV-infected patients were 53.75% and non-infected were 46.25%. There were some additional diseases, 84 patients with HCV patients have diabetes mellitus, 34 patients have smoking addiction and 93 have hypertension. When the ratio between LFTs was observed for non-HCV and HCV patients it is revealed that it was greater in HCV patients. Numerous complications observed like ascites, diabetes mellitus, and splenomegaly. Numerous risk factors were observed in this trial, the most dangerous are the dentist procedures followed by frequent vaccinations, surgery, nose/ear piercing, shaving by barber, blood transfusion, and Jaundice. The result of this study showed that HCV patients have a significantly short life span.

**Keywords:** Hepatitis C virus, correlation, population, LFT, AST.

## 1. Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the family of Flaviviridae that affects an estimated 71 million people in the whole world and in 2015, 1.75 million new infections were reported (WHO, 2019). Chronic Hepatitis C is a major source of chronic liver disease (C.L.D.) globally with an estimated 71 million people diseased and a 20-year risk for developing cirrhosis of up to 30% (WHO, 2017). Leading to 400,000 deaths yearly from hepatocellular carcinoma (H.C.C.) and end-stage liver disease (WHO, 2017; EASL, 2018). Well-tolerated treatments

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for Chronic Hepatitis C with cure rates above 95% are now available (Hezode, 2018). Treatment resulting in sustained virological response (SVR) is associated with a lowering in liver stiffness (Singh et al., 2018; Lledo et al., 2018). Decrease in the risk of developing cirrhosis, H.C.C., and end-stage liver disease (Konjeti and John, 2018; Su et al., 2018).

HCV infection often evolves asymptomatically for 20–30 years, and most deaths from HCV result from liver cirrhosis or hepatocellular carcinoma (HCC) years after the incident of HCV infection (Alter, 2007; WHO, 2017). HCV results in an estimated 27% of Liver cirrhosis and 25% of HCC cases globally (Perz and Alter, 2006). The entry of HCV virus in humans is through different routes and the most common is direct transfusion by blood. Which include needle sharing, organ transplantation, mother-to-child, and sexually transmitted is also noted. The major route which is under consideration is injection, blood transfusion, sex with intravenous drug users, piercing, and scarification (Peleg, 2017).

About 7 to 9 million people in Pakistan are living with HBV infection and 10 million people are suffering from HCV infection, with increased morbidity and mortality (Umar and Manzoor, 2014). Pakistan is fronting the Hepatitis C epidemic in the nation. A maximum percentage of people do not know their health status because the infection has no symptoms in its initial phase. The main factors to spread the disease are the usage of syringes, unsafe blood transfusions, unsafe practices of surgery and medical procedures, ear or nose piercing, and shaving at barbers. Pakistan is an average-earning country and has inadequate resources. Therefore, we should emphasize more on planning and executing the operative precautionary policies to decrease the disease load of HCV (Mahmood and Raja, 2017).

Many people did research to assess the causes of the spread of hepatitis C. One particular study in Pakistan (1994) established, that family members who had more than four injections per year have a greater chance (9-11 times) of being diseased with Hepatitis C. The study found that the use of unsterilized injections and shaving at barbers' shops are major noteworthy causes of spread (Bari et al., 2001; Shah et al., 2002). The earlier study found that injections by untrained medical professionals, blood transfusions, surgical procedures, shaving at barbers' shops, and dental procedures were common causes of HCV (Rehman et al., 2002). It was presented that para-medical staff are at increased risk of developing disease from hepatitis C (Akhtar et al., 2004). It was noted that people who have a history of hospitalization or those who have been injected with unsterilized injections are at increased risk of developing the disease (Arash et al., 1993).

The occurrence of HCV infection is higher in Pakistan and national efforts are required to identify persons who may have been diseased with HCV and HBV. The current study was therefore planned to evaluate the prevalence of the Hepatitis C virus (HCV) in relation to risk factors among the local human population of Gujranwala, Punjab, Pakistan.

## 2. Materials and Methods

The current study was conducted from 2018 to 2019. A total of 320 (Males: 138, 43.13%; females: 182, 56.87%) samples from the Medical Unit of Chaudhary Hospital and Civil Hospital, Gujranwala were collected. Patients who were admitted either with the diagnosis of liver cirrhosis or were diagnosed as such during their stay in the hospital. The patients above 15 years of age (both males and females) were in this study while those with acute liver failures were excluded. Demographic characteristics were recorded. After a detailed history, a clinical examination was performed to look for the prevalence of the HCV virus. This study was approved by the ethical committee of the Institute of Molecular Biology and Biotechnology, University of

**Table 1** Demographic and biochemical features of HCV & Non-HCV (Control) Patients

| | **HCV (N=172)** | | **Non-HCV (N= 148)** | | **P-Value** |
|---|---|---|---|---|---|
| | **Mean** | **Standard Deviation** | **Mean** | **Standard Deviation** | |
| Age (years) | 49.1162790 | 13.84324828 | 47.25 | 14.72555047 | 0.2439 |
| Weight (kg) | 71.9651162 | 14.68376011 | 74.4121 | 16.58206927 | 0.16250 |
| WBC | 8.21831395 | 7.977684157 | 8.43087 | 3.48527123 | 0.76409 |
| HB | 13.1052325 | 5.618570551 | 21.8655 | 106.368704 | 0.28156 |
| Platelets | 202.069186 | 102.0194107 | 266.723 | 113.7184027 | 0.002 |
| Albumin | 4.04029069 | 4.24069817 | 4.08162 | 4.42127972 | 0.193 |
| Billirubin | 2.30318604 | 10.59834918 | 1.19439 | 1.414310835 | 0.451 |
| AST (U/L) | 97.4563953 | 189.3606557 | 54.6081 | 61.7980731 | 0.041 |
| ALT (U/L) | 96.3604651 | 169.9604297 | 52.6351 | 66.68457059 | 0.0391 |
| ALP | 164.568604 | 103.2866117 | 149.560 | 126.8355959 | 0.3013 |
| Urea | 29.2447674 | 31.74235432 | 28.4799 | 27.19427715 | 0.5814 |
| Creatinine | 1.42215116 | 5.314107179 | 2.56770 | 10.6595725 | 0.4145 |
| Na | 138.337209 | 4.426423754 | 137.902 | 5.461572244 | 0.5144 |
| K | 4.10930232 | 0.551643564 | 5.49101 | 11.654292 | 0.5213 |
| Blood Sugar | 128.296511 | 61.52866863 | 136.058 | 83.65828626 | 0.4294 |
| INR | 1.28313953 | 0.705762847 | 1.31878 | 0.869841572 | 0.0451 |
| AST/ALT Ratio | 1.11689926 | 0.608759683 | 1.36030 | 1.17925134 | 0.0389 |

Lahore and Lahore Clinical Laboratory and Research Centre, Lahore, Pakistan.

The patients were divided into two groups. Group 1 was designated as the HCV group and Group 2 as Non-HCV

(Control) group. Among 320 patients, 172 were affected with HCV, and 148 were Non-HCV (Control) patients. The patients with severe HCV or liver infection and those having severe co-infection with other diseases or illnesses were not included in this study. The designated patients were examined by skin specialists specifically oral cavities, nails, and hairs to diagnose the cutaneous condition.

A conversational survey, studied and accepted in earlier studies was used to gather information on individual data, past and present health, socioeconomic features, and risk sources for the spread of HCV infection such as dentist procedures, surgical operations history, transfusion of blood, schistosomiasis treatment, contaminated needles or syringes, hospitalization, share toothbrushes, share shaving razors, circumcision, use of a condom, use of drug oral or with common syringes, smoking, multiple sexual partners, tattooing and nose or ear piercing. At the same time, blood samples from the people were collected to examine the HCV-RNA virus by the qualitative real-time PCR (polymerase chain reaction).

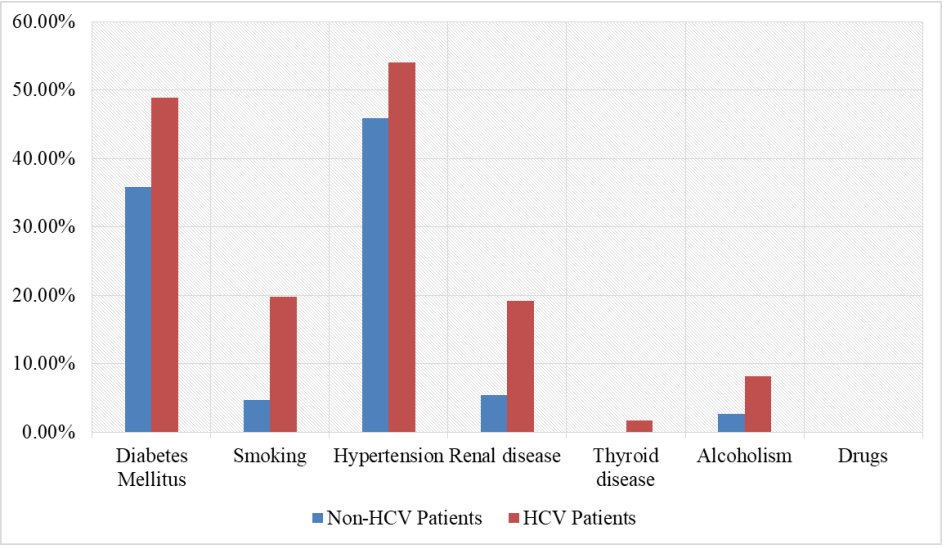


**Figure 1** Distribution of co-morbidities in non-HCV and HCV patients

After HCV PCR and genotyping, different clinical tests including Liver Function Test (LFT), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphate (ALP), Albumin and Bilirubin Total were conducted following the standard protocols (Afzal, 2017).

## 3. Statistical Analysis

Data analysis was done by using SPSS (Scientific Package for Serial Sciences, version 21) as mean ± standard deviation for the quantities variable, whereas to assess the association between different group p-value as a level of significance, ANOVA was applied.

## 4. Results

The demographic and biological features of the HCV group and control group have been given in (Table 1) in which the mean value, standard deviation, and p-values for both groups have been provided.

In the participants of this study, there were some additional morbidities found which means that they were suffering from some additional diseases. Figure 1 represents the graphical form of co-

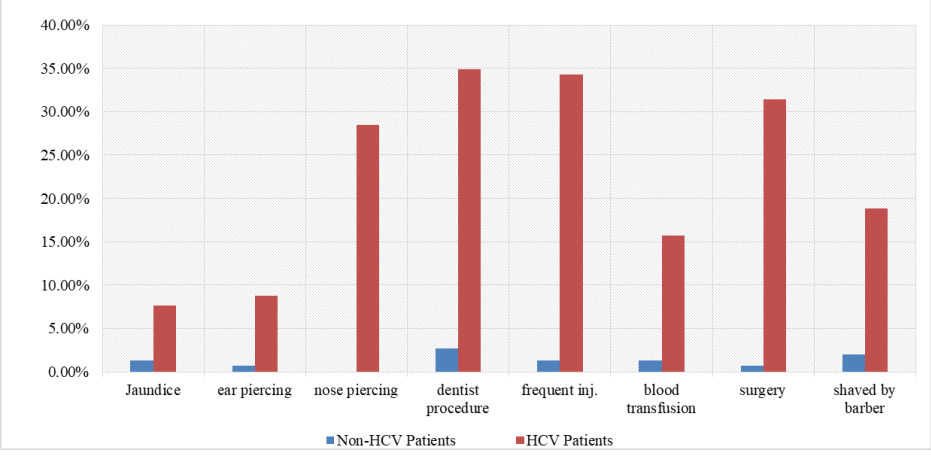


**Figure 2** Prevalence of different Risk factors in non-HCV and HCV patients

**Table 2** Distribution of disease burden in non-HCV and HCV patients

| Disease Burden | Non-HCV (N= 148) | HCV (N=172) | p-value |
|---|---|---|---|
| Ascites | 26 (17.57%) | 29 (16.86%) | 0.8231 |
| Portal Hypertension | 0 (0%) | 2 (1.16%) | 0.4213 |
| Cholelithiasis | 12 (8.11%) | 24 (13.95%) | 0.2169 |
| Hepato-renal syndrome | 8 (5.41%) | 0 (0%) | 0.8134 |
| Hepato-cellular Carcinoma | 4 (2.70%) | 19 (11.05%) | 0.0913 |
| Umbilical Hernia | 3 (2.03%) | 1 (0.58%) | 0.5213 |
| Splenomegaly | 2 (1.35%) | 19 (11.05%) | 0.0452 |

morbidities in both groups in which the proportions of study participants having respective additional diseases have been plotted on the graph. It can be seen in Figure 1 that the chances of having additional diseases in Hepatitis C patients are greater than non-Hepatitis C patients.

The risk factors have been assessed in terms of their occurrence in HCV and non-HCV groups of patients. The graphical representation of risk factors is showing that surgery was found as a risk factor in a large number of HCV patients while it was identified as a risk factor in 0.68 percent of non-HCV patients only. It means that surgery can really be a serious risk factor among patients with HCV. Similar results have been shown by ear piercing, jaundice, dentist procedure, frequent injection, and blood transfusion because they were found as risk factors in 8.73%, 7.65%, 34.88%, 34.30%, and 15.70% HCV patients respectively, while they were identified as risk factors only in 0.68%, 1.35%, 2.70%, 1.35%, and 1.35% patients of non-HCV groups respectively (Figure 2). The Ascites, Portal hypertension, Cholelithiasis, Hepato-renal syndrome, Hepato-cellular Carcinoma, Umbilical Hernia, and splenomegaly were analyzed in non-HCV and HCV patients to know the percentages of patients in both groups in

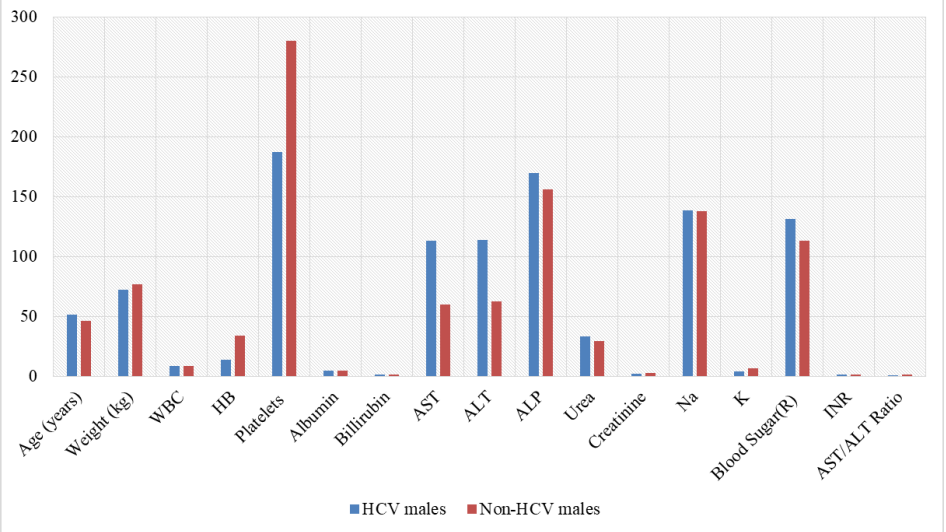


**Figure 3** Demographic and biological features of non-HCV and HCV male patients

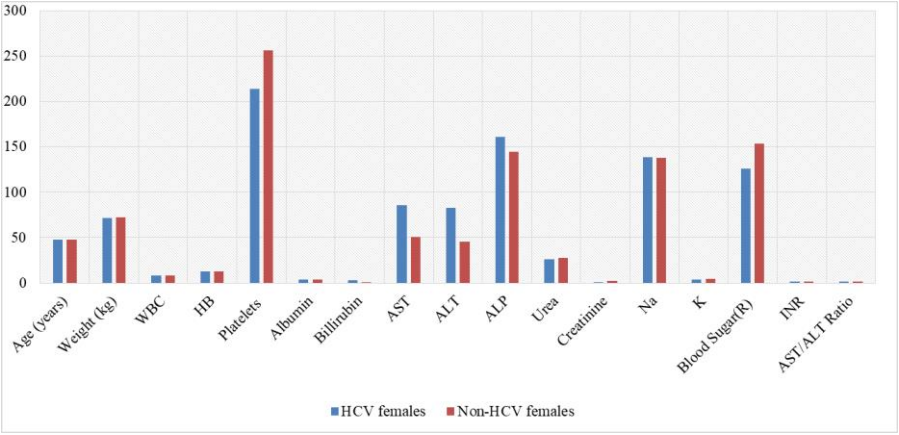


**Figure 4** Demographic and biological features of non-HCV and HCV female patients

which these disease burdens were found (Table 2).

Demographic and biological features of male and female non-HCV and HCV patients is represented in Figure 3 & 4, respectively. It is clear from the graphs that there is no significant difference between the average age and average weight of male HCV patients and male non-HCV patients. It means that demographic characteristics do not vary between male patients of HCV groups and non-HCV groups.

## 5. Discussion

The main objective of the current study was to assess the occurrence of Hepatitis C virus antibodies in Gujranwala, Pakistan. The relationship between possible causes and HCV seropositivity was evaluated for the whole nation and the population-attributable risk for many causes was evaluated by case-control method. This is the first population-based Hepatitis C virus prevalence study based on the PCR and genotyping and conducted in a characteristic sample of the region of Gujranwala with widespread levels higher than 2.5%. However, a similar study from China showed that 5.2% of co-infected HBV/HCV patients (Zhou et al., 2012). In contrast to another study in Burmese (Myanmar), the results showed an HBV/HCV co-infection value of 19.5% (Zhou et al., 2011).

In our study, the mean age of HCV patients was 49 and the people with non-HCV had a mean age of 47. The maximum weight of HCV patients was observed at a mean age of 74 while in the control group, it was observed at the age of 71. In 2017, a similar study was conducted in Pakistan having patient samples between ages 18-55 (Afzal,2017).

According to the findings of the current study, the mean age of the control group (47.25%) was less than the HCV group (49.17%). The results of white blood cells proposed that the mean value of the control group (8.43) was higher when compared to the HCV group (8.21). The mean values of blood sugar showed a decrease in the HCV group (128) when compared to the control group (136). The AST/ALT ratio is used to distinguish patients with liver cirrhosis due to chronic Hepatitis C infection from non-cirrhotic patients and to compare the ratio with the stage and grade of hepatitis and other biochemical indices. The mean AST/ALT ratio in the HCV patient was 1.11 which is relatively less than the non-HCV patients 1.36. A ratio less than 1 had 100% specificity and positive predictive value in differentiating cirrhotic from non-cirrhotic patients. In a study conducted in the U.S.A. showed that AST/ALT ratio in cirrhotic patients was 1.06 and in non-cirrhotic patients was 0.60 (Waheed et al.,

2017). From this AST/ALT ratio, the liver fibrosis stage was predicted.

Another study suggested a relationship between fibrosis and cirrhosis on the basis of the AST/ALT ratio they suggested that if the ratio is greater than 1 has a 100% positive prediction for cirrhosis from non-cirrhotic patients (Table 1; Figure 3) (Sheth et al., 1998). In non-HCV patients, the factor of hypertension was 45.95%, and in HCV patients that percentage was 54.07%. Similar results of hypertension (42%) were found in another study (Park et al., 2001). The risk of frequent injection in the non-HCV patient is very less than compared to patients with HCV, and the major risk of blood transfusion, is in non-HCV patients was less percentage as associated with HCV patients.

In this study the factor of surgery which may cause risk in the patients of HCV that proportion was 31.40%. Some earlier studies have shown that dental procedures were the main cause of HCV (39.7%) and surgical procedures (16.6%) (Shimotohno, 2000). Shaved by a barber is a type of risk for the HCV patient having a percentage of 16.86% and in the non-HCV patients that same risk was at 2.03%. The research has also explained the disease burden and how many burdens they face while bothering from HCV or also for patients non-HCV. Similar results were reported in a previous study (Mohammed et al., 2009).

The splenomegaly was also a burden in HCV patients. In the present study, different blood-related parameters (WBC, Hb, Platelets, Blood sugar, Albumin, Bilirubin, and AST/ALT etc) were also evaluated and the values were found higher in HCV patients when compared to non-HCV patients (Control group). In some earlier studies, similar results of blood transfusion and screening of blood were recognized as the major source of HCV transmission (Alashek et al., 2012; Sy and Jamal, 2006).

In the current study, mean values of ALP were found less in the control group as compared to the HCV male patients, and the avg. weight of female non-HCV patients was found higher than the HCV group. The Ascites, Portal hypertension, Cholelithiasis, Hepato-renal syndrome, Hepato-cellular Carcinoma, Umbilical Hernia, and splenomegaly were analyzed in non-HCV and HCV patients to know the percentages of patients in both groups in which these disease burdens were found (El-Serag and Mason, 2000).

## 6. Statements and Declarations

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***Author contributions:*** Iram Amjad conceived, collected, and analyzed data and wrote the manuscript. Mubasher Hassan and Syed Zeeshan Haidar supervised the study design, and read, edited, and approved the final manuscript. Dilawar Hussain: Prepared the edited and suggested version after review.

***Ethical approval:*** The data presented in this study was obtained from two hospitals in Gujranwala under the policy of the ethical approval committee of the University of Lahore (Letter no.15/2018).

***Conflicts of Interest:*** The authors declare no conflict of interest.

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