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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

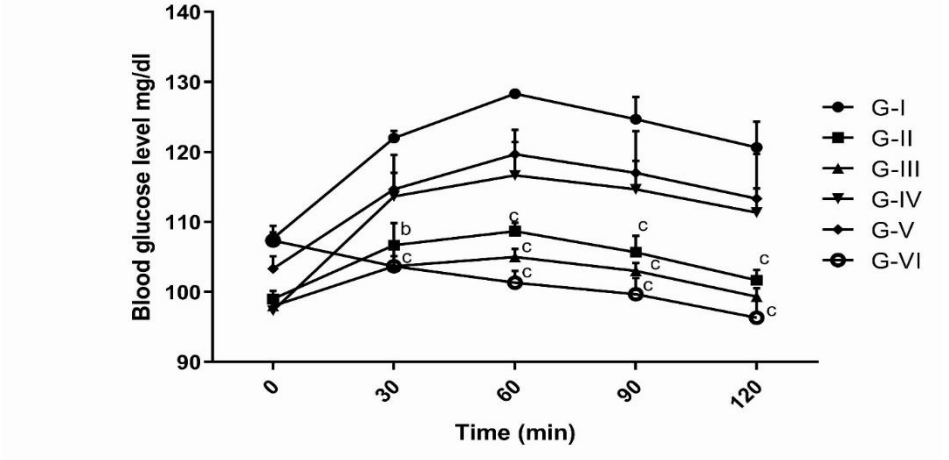

Figure 1 Effect of different groups on oral glucose tolerance test in normoglycemic rats. Values are expressed as mean ± SEM, where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups. G-1= normal control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg),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

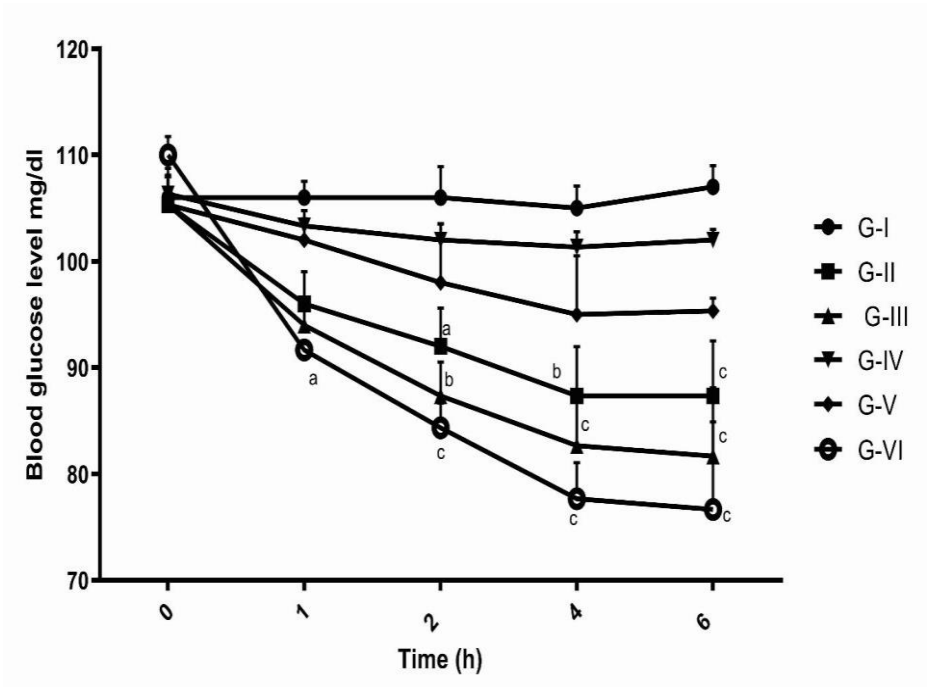

Figure 2 Variations in glucose levels in normoglycemic rats. Values are expressed as mean ± SEM, where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups G-1= normal control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg), G-V= EsO (200 mg/kg), G-VI= metformin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

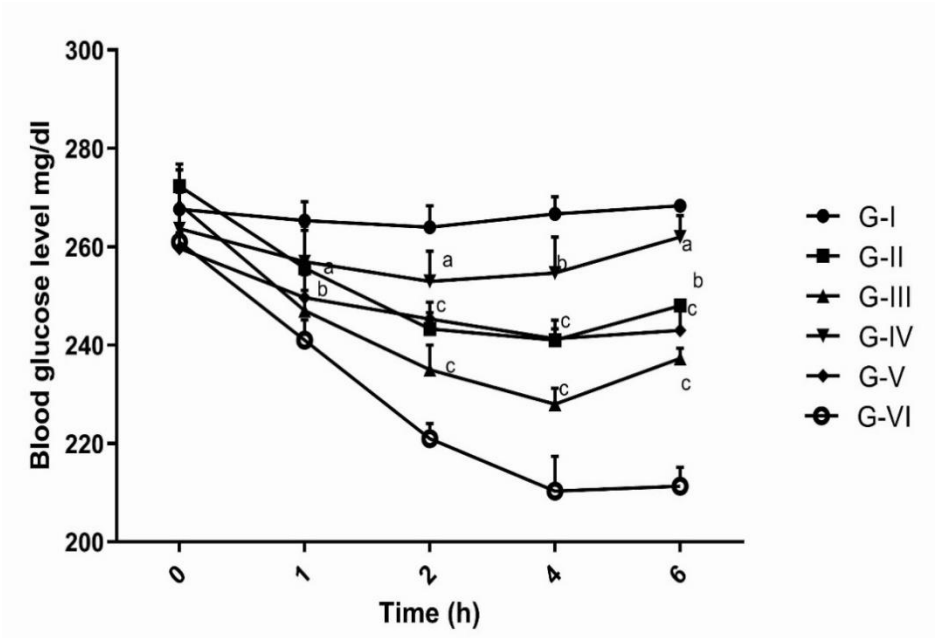

Figure 3 Variations in glucose level in diabetic rats. Values are expressed as mean + SEM,where (a) = P < 0.05. (b) = P<0.01, (c) = P<0.001 between the control and other groups. G-1= diabetic control, G-II= CE (100 mg/kg), G-III=CE (200 mg/kg), G-IV= EsO (100 mg/kg), G-V= EsO (200 mg/kg), G-VI= metformin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

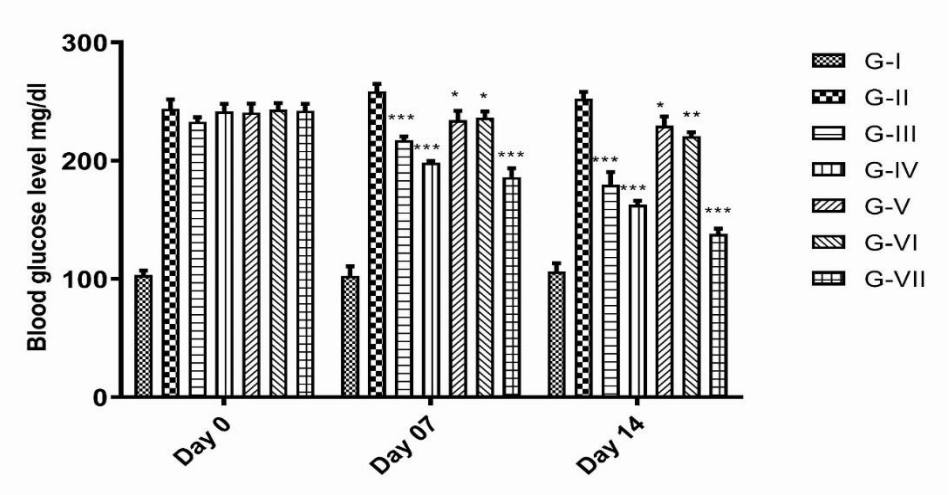

Figure 4 Effect of different groups on blood glucose level (mg/dl) in alloxan-induced diabetic rats in 14 days study. Values are expressed as mean ± SEM, where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, G-II= Diabetic control, G-III= CE (100 mg/kg),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

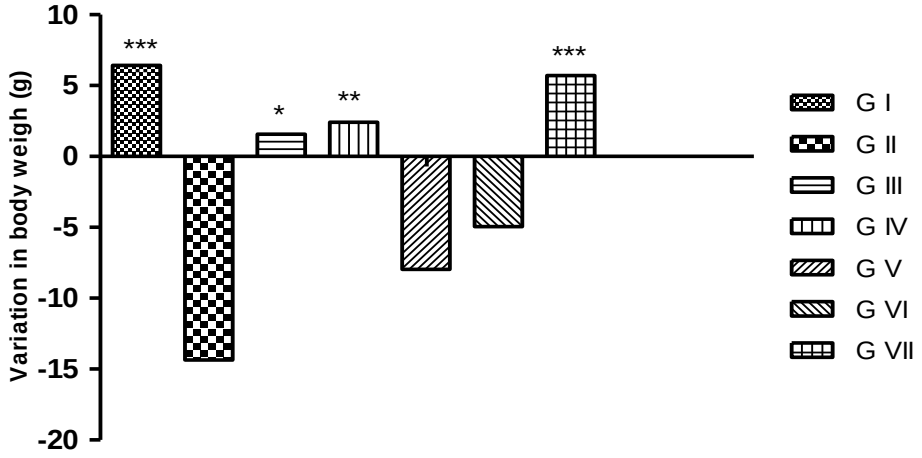

Figure 5: The effect of 14-days treatment with various groups on body weight (g) after alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the diabetic control (G-II) and other groups. G-1= normal control, G-II=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

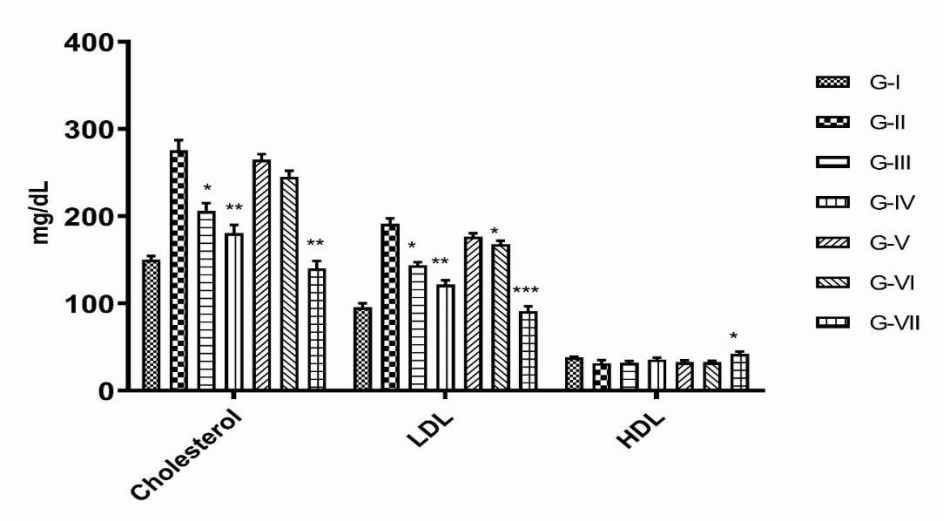

Figure 6 The effect of 14-days treatment with various groups on total lipid profile in alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, G-II= Diabetic control,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

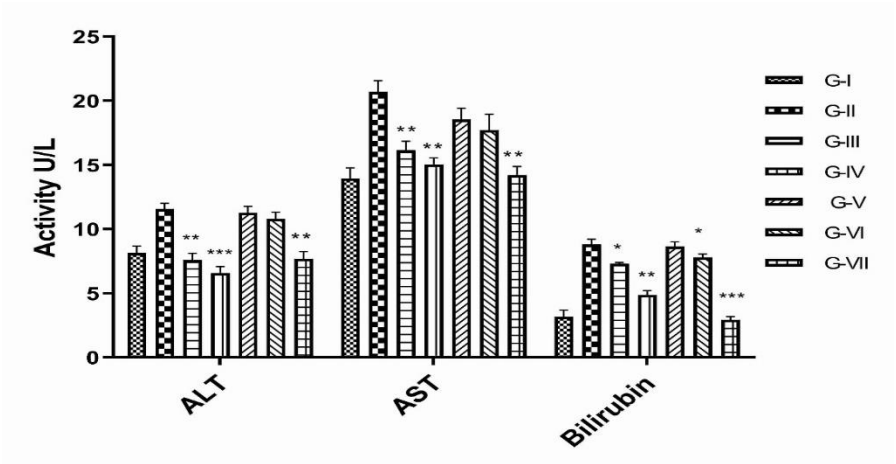

Figure 7 The effect of 14-days treatment with various groups on Hepatic profile (ALT, AST and bilirubin) after alloxan (150 mg/kg i.p.) induced diabetes in rats. Values are expressed as mean ± SEM. where * = P < 0.05, ** = P<0.01, *** = P<0.001 between the control and other groups. G-1= normal control, 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 2nd and 4th 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 14th 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.

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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.

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