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In-vivo study of cedar wood oil on the protective effects of hepatotoxicity, renal toxicity and hyperlipidemia

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Abstract

Plants and plant-derived materials play an immensely vital role in disease management and health care programs. Essential oil from wood chips of Himalayan Cedar, *Cedrus deodara (Roxburgh)* family: *Pinaceae*), was obtained by hydrodistillation. The cedar wood oil at various test doses of 250mg/kg, 500mg/kg, and 1000mg/kg was evaluated for various activities like liver function tests, lipid profile, and renal function tests by using the in vivo mice model. The study's bioassay results revealed the potential of cedar oil for its use in health management and its efficacy in treating high levels of Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), ALP, AST, ALT, Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Urea (U) and Creatinine. In conclusion, Cedar wood oil can be an effective therapeutic agent against hepatotoxicity, renal toxicity, and hyperlipidemia. Furthermore, the detailed analysis of cedar wood oil will further explore the formulation of new medicinal plant-based drugs.

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Introduction

Medicinal and aromatic plants are extensively used as natural organic compounds and as medicines. Numerous efforts are made to explore the usage of the essential oil used as a treatment for various infectious diseases that are supernumerary to the pharmaceutical remedies. (Irshad *et al.*, 2020). Humans have greatly benefited from plants and their secondary metabolites.

During the plant-human coevolution, plants represented (and represent) a nearly unlimited source of food, feed for domesticated animals, fibers for clothes, and, not the least, medicaments. Among the vastness of plant products, essential oils deserve particular attention. These are complex mixtures of hydrocarbons and oxygenated hydrocarbons arising from the isoprenoid pathways, mainly consisting of monoterpenes and sesquiterpenes. Essential oils are produced and secreted by glandular trichomes, specialized secretory tissues diffused onto the surface of plant organs (Marcello, 2006).

Cedars are very popular ornamental trees and are mostly used in horticulture. True cedar trees are native to the northern and western mountains of the Middle East countries. The cedar wood oil is derived from cedar trees by distillation of their wood. Egyptians used the oil of cedar trees to protect themselves from the insects (repellent effect).

The Literature shows that cedar oil has been used as mosquito repellent responsible for causing dengue fever, chikungunya, yellow fever, filariasis, and other diseases. It has also got antifungal, anti-termite, anti-ulcer, antioxidant, anticancer, anticough, and antimicrobial activities (Hammer *et al.*, 1999, Matsunaga *et al.*, 2000, Ramadass and Thiagarajan, 2015). Cedarwood oil and its potential use as a therapeutic agent are key due to its high potency and compatibility compared to synthetic compounds.

The present study is designed and executed to evaluate the efficacy of cedar wood oil by focusing on the lipid profile, liver function tests, and renal function tests in experimental animals (rats).

Materials and methods

Materials, Chemicals, and apparatus

Tween 80 and kits for Lipid profile, LFT, and RFT were purchased from (Sigma-Aldrich, St. Louis, USA). Chemicals and solvents used in the current study were used of analytical grade. The plant was collected from different hilly areas of Pakistan. Oil was extracted by the hydrodistillation method (Liaqat *et al.*, 2018).

Collection of Cedar wood plants and Oil Extraction

Aged and rather reddish colored twigs were collected of cedar wood and crushed into small pieces then carefully put in the sandy pot having small holes (bored out) in the center and at the base. The pot was loaded up in such a manner that the base hole of the primary pot is precisely at the center of the mouth of the following (lower pot). These identical pots, one over another were sited in soil, keeping one-third of the lower pot dug in the soil. The upper pot containing wood pieces was covered with a lid and heated by burning wood from above; taking proper care that heat doesn't dissipate much to the lower pot. Heating burns the wood pieces whereby oil is released in the process which is collected in the flower pot kept inside the soil. The oil thus extracted, has multiple uses in agricultural allied areas. Tween 80 was used for dissolving the oil mixture in the ratio of 1:1 v/v in a vile.

Animals Husbandry and Experimental Setup

Albino rats of either sex, weighing 100-150g were used in this study were obtained from the Pharmacology section, Medicinal Botanical Center, Pakistan Council for Scientific and Industrial Research Labs Complex, Peshawar, and then were acclimatized to normal laboratory conditions for 1 week before the study and were supplied with a pellet diet and water ad libitum. The temperature of the facility was 25 ± 3°C and light/darkness alternated 12 hours apart. The animals were divided into 5 groups of 5 animals each. The study was conducted following the approval of the Institutional Animal Ethical Committee of PCSIR Labs Complex, Peshawar.

Experimental design and protocol

Animals were divided into four groups, containing five rats in each group. Tween 80 was used for dissolving oil. a Group 1 was treated with water as a control

(negative). Animals of groups II to IV were treated with cedar wood oil (CWO) at dose levels of 250, 500, and 1000mg/kg body weight orally by using a feeding tube, and the treatment was continued for 14 days.

Blood Collection and Preservation

Within 24 hours of the experiment, animals were anesthetized by using chloroform vapors before dissection. Blood was collected through the cardiac puncture into plasma separator tubes containing anticoagulants. The blood was refrigerated for 1 hour and centrifuged at 3000 rpm for 15 minutes. Then serum was separated and stored at - 20°C.

Biochemical analysis

The biochemical perimeters like lipid profile and Renal Function Test were screened from collected blood samples. The parameters in the lipid profile were Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Very Low-Density Lipoprotein (VLDL). The parameters in Liver Function Tests (LFT) were Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The parameters in Renal Function Tests (RFT) were Urea (U) and Creatinine. All these parameters were assessed using the kits (Sigma-Aldrich, St. Louis, USA) purchased from the market with the help of Chemistry Analyzer (Micro lab 300, Merck, NJ, USA).

Statistical analysis

All the values obtained were represented as a mean ± SEM for six animals in each group (n=6). Graph Pad Prism 06 version (San Diego, CA, USA) was used for statistical analysis of data. One-way analysis of variance (ANOVA), followed by Dunnett 's test for comparison was used for statistically significant differences between the mean values. The value of $p < 0.05$ was considered to be statistically significant in all cases.

Results

Lipid Profile

It can be observed that there is a decrease in TC, TG, LDL, and VLDL while an increase in HDL plasma level in the test groups when compared with the normal control group. It can be further noticed that there was a dose-dependent effect. The reduction of TC at dose levels of 250, 500and 1000mg/kg body weight was 13.79, 20.68, and 28.44% respectively. The decrease in the TG at dose levels of 250, 500and 1000mg/kg body weight was 15.12, 23.08, and 32.67% respectively. The effect of the cedar wood oil on the level of plasma lipid parameters is shown in Fig. 1.

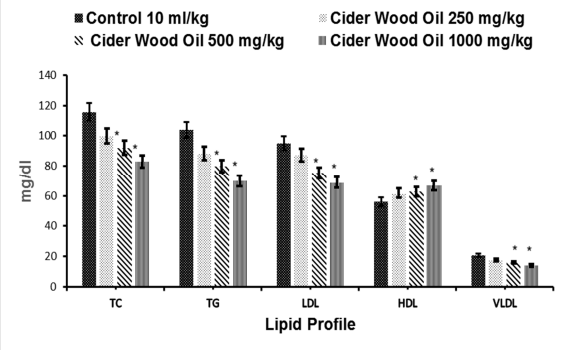

Fig. 1. Data are shown as mean ± SEM. * represents $p < 0.05$ Vs control.

The decline in the level of LDL at the above-mentioned doses was 8.22, 20.72, and 27.37% respectively. The decrease in the values of VLDL at dose levels of 250mg/kg, 500mg/kg and 1000mg/kg body weight was 15.12%, 23.08% and 32.67% respectively. The HDL values were increased and found to be 10.17, 12.23, and 19.07 at the dose levels of 250mg/kg, 500mg/kg, and 1000mg/kg body weight respectively.

Liver Function Tests

It can be seen that there is a decline in the values of bilirubin, ALT, AST, and ALP in the test groups, as compared to the control group. A dose-dependent effect was observed. The percent effect of the test oil on bilirubin at dose levels of 250mg/kg, 500mg/kg, and 1000mg/kg body weight is 11.51%, 25.43%, and 35.72% respectively. The activity of the cedar wood oil on the LFTs is given in fig. 2. The decrease in the ALT at dose levels of 250, 500and 1000mg/kg body weight was found to be 12.47, 27.24, and 37.42% respectively. The percent effect of the test oil on AST at the above-mentioned doses was 11.10, 25.80, and 39.69% respectively. The effect on ALP was calculated to be 7.07, 15.34, and 25.90% respectively at the above-stated doses.

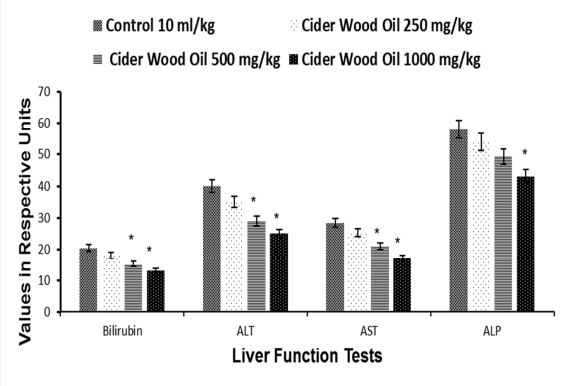

Fig. 2. Data are shown as mean ± SEM. * represents $p < 0.05$ Vs control.

Renal Function Tests

Creatinine levels were decreased by 1.05%, 12.63%, and 29.4% at the test doses of 250mg/kg, 500mg/kg, and 1000mg/kg body weight of the cedar oil, respectively. The effect of Cedar wood oil on the renal function tests is shown in fig. 3. The Urea levels also increased by 16.73%, 19.50%, and 35.73% at the aforementioned doses, respectively.

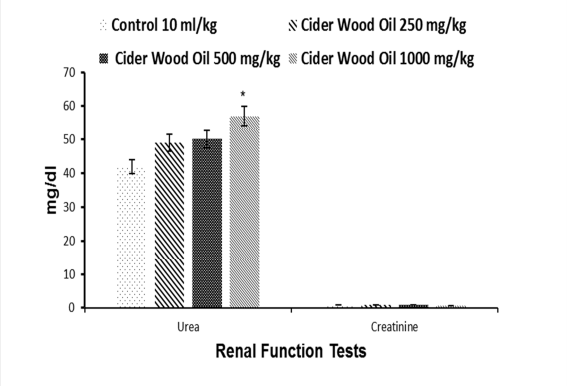

Fig. 3. Data are shown as mean ± SEM. * represents $p < 0.05$ Vs control.

Discussion

Lipid profile helps evaluate cardiovascular health by analyzing total cholesterol, triglycerides, HDL, LDL, and VLDL in the blood. This test is mostly used to identify hyperlipidemia that can cause blockage of the blood vessels and arteries, damaging them and heightening the risk of problems like heart disease, stroke, and heart attack. In this study, cedar wood oil was found to be effective in decreasing total cholesterol levels in the experimental animals as compared to the control group.

Triglycerides were also decreased by the test oil. High Triglycerides are directly related to coronary heart disease (Ganda *et al.*, 2018, Laufs *et al.*, 2020), and most antihypercholesterolaemic drugs do not decrease triglyceride levels but plant extracts do. These findings indicate the beneficial effects of cedar wood oil instead of its toxicological potential.

HDL, commonly known as good cholesterol, has an important function of transporting other lipids like cholesterol and triglycerides in the bloodstream. High-density lipoprotein (HDL) exerts multiple actions on metabolic homeostasis and thereby beneficially impacts atherosclerosis, thrombosis, inflammation, and glucose homeostasis (Assmann and Gotto Jr, 2004). Importantly, HDL removes excess cholesterol from peripheral tissues and cells. These cells include macrophage foam cells, which are directly involved in atherosclerotic plaque formation. HDL then transports this cholesterol to the liver for disposal in the bile. This process of peripheral cholesterol efflux to HDL and delivery to the liver is termed reverse cholesterol transport (RCT) and is important for the anti-atherogenic properties of HDL. Thus, high plasma HDL cholesterol levels are generally associated with a reduced risk for cardiovascular diseases (Nofer *et al.*, 2002, Röhrl and Stangl, 2013).

LDL is also called “bad” cholesterol because it blocks blood vessels and increases the risk of heart disease. VLDL cholesterol is a type of blood fat. It's considered one of the "bad" forms of cholesterol, along with LDL cholesterol and triglycerides. This is because high levels of cholesterol can clog arteries and lead to myocardial infarction.

In general, it can be concluded that hypercholesterolemia is a risk factor for cardiovascular diseases (CVD) such as atherosclerosis and heart attack, which is a common cause of mortality and morbidity(Wald and Law, 1995, Adaramoye *et al.*, 2008). From our studies, it can be inferred that cedar wood oil is useful in treating hypercholesterolemia. TC, TG, LDL, and VLDL values have been decreased in the experimental animals while HDL is elevated.

A bilirubin test measures the amount of bilirubin in the blood. It's used to help find the cause of health conditions like jaundice, anemia, and liver disease. Bilirubin is a byproduct (orange-yellow pigment) of the routine destruction of red blood cells occurring in the liver (Berk and Noyer, 1994). It is normally released as bile in the feces. Elevation of the bilirubin can suggest liver dysfunction. However, other conditions with increased destruction of red blood cells also can cause elevated bilirubin levels despite normal liver function (Fevery and Blanckaert, 1986). If bilirubin levels are higher than normal, it's a sign that either the red blood cells are breaking down at an unusual rate or that the liver isn't breaking down waste properly and clearing the bilirubin from your blood (Green and Flamm, 2002).

In children and adults, clinicians use it to diagnose and monitor liver and bile duct diseases. These include cirrhosis, hepatitis, and gallstones. It also helps to determine sickle cell disease or other conditions that cause hemolytic anemia. That's a disorder where red blood cells are destroyed faster than they are made. High levels of bilirubin can cause a yellowing of skin and eyes, a condition doctors call jaundice. In our experimental work, it can be viewed that bilirubin levels in the test groups have been decreased than the normal control group in a dose-dependent manner, which indicates that cedar wood oil is effective in treating the bilirubin levels in liver ailments.

Enzymes (proteins) help the liver break down other proteins so the body can absorb them more easily. ALT is one of these enzymes. It plays a crucial role in metabolism, the process that turns food into energy (Giannini *et al.*, 2005). ALT is normally found inside liver cells. However, when the liver is damaged or inflamed, ALT can be released into the bloodstream. This causes serum ALT levels to rise. Many times an increase in ALT is the first sign of a problem and is elevated before other symptoms start to appear. Measuring the level of ALT in the blood can help evaluate liver function or determine the underlying cause of a liver problem. The ALT test is often part of an initial screening for liver disease. An ALT test is also known as a serum glutamic-pyruvic transaminase (SGPT) test or an alanine transaminase test. Higher-than-normal levels of ALT can indicate liver damage. Increased levels of ALT may be a result of hepatitis, cirrhosis, and cancer in the liver.

In the above study, it is evident that ALT levels in the test groups of animals have been reduced as compared to the control group. So, it may be concluded that Cedar wood oil is efficacious in the management of higher ALT levels in the blood. AST is an enzyme that is secreted by the liver. Other organs, like the heart, kidneys, brain, and muscles, also secrete smaller amounts (Wróblewski, 1958). AST is also called SGOT (serum glutamic-oxaloacetic transaminase). Normally, AST levels in the blood are low. When the liver is damaged, it releases more AST into the blood, and the levels rise. A high AST level is a sign of liver damage, but it can also damage other organs like the heart or kidneys. High levels of AST in the blood may indicate hepatitis, cirrhosis, mononucleosis, or other liver diseases.

High AST levels also indicate heart problems or pancreatitis. From this research work, it can be assumed that Cedar wood oil has an important role in maintaining normal blood levels of AST in different diseases. ALP is an enzyme found in the bloodstream. It helps break down proteins in the body and exists in different forms, depending on where it originates. The liver is one of the main sources of ALP, but some are also made in your bones, intestines, pancreas, and kidneys. In pregnant women, ALP is made in the placenta (Fishman, 1990, Dufour *et al.*, 2000).

Abnormal levels of ALP in blood most often indicate a problem with the liver, gallbladder, or bones. However, they may also indicate malnutrition, kidney cancer tumors, intestinal issues, a pancreas problem, or a serious infection. The normal range of ALP varies from person to person and depends on age, blood type, gender, etc. (Sharma *et al.*, 2014). Fig. 2 shows that the level of ALP is decreased in test animals as compared to the control group. Therefore, it can be deduced that our test sample is beneficial in treating and managing high blood ALP levels.

Renal function tests help us diagnose the functional status of the kidney. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is a waste product in the blood that comes from muscle activity.

It is normally removed from the blood by the kidneys, but when kidney function slows down, the creatinine level rises (Clark and Kruse, 1990, Walker *et al.*, 1990). In this research study, it can be observed that blood urea level has been increased in the test groups. This may account for epithelial necrosis of the renal tubules. On the other hand, blood creatinine levels decreased in all the test groups.

According to different research worldwide, whenever there is an elevation in urea level and a reduction in creatinine level, there is no chance of kidney damage (Abarikwu *et al.*, 2018). *Nisha et al.,* has reported in their scientific work that renal injury is caused only when urea and creatinine levels are raised simultaneously (Nisha *et al.*, 2017)

Conclusion

This study reflects that reduction in TG, TC, LDL, VLDL, Bilirubin, ALT, AST, and ALP is considered beneficial, and a slight increase in urea and decrease in creatinine is not considered a toxic output of any tested material. It can be further concluded that Cedar wood oil is a gift of nature, which can be used in various ailments to protect the body from hazardous effects of hypercholesterolemia, liver disorders, renal abnormalities, etc. Hence more comprehensive studies are warranted concerning these studies for their effective use in the areas of pharmacology and toxicology. This scientific work supports the authenticity of the utilization of Cedar wood oil.

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