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ANTI-INFLAMMATORY ACTIVITY OF *VIOLA ODORATA* ON LEAD ACETATE INDUCED HEPATOTOXICITY IN RATS

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Abstract

Introduction: Hepatotoxicity is injury to liver impairment caused due to exposure of xenobiotics pre oxidized fatty acids radioactive isotope. Liver is the major visceral organ for detoxification from harmful chemicals and chief area of metabolizing the drugs given for treatment. *Viola odorata*, a flowering plant native to Europe commonly called wood violet, is a herbal medicine, which has been in use for numerous respiration related diseases and disorders in some conditions associated with stress. The aim is to analyze the anti inflammatory activity of *V. odorata* on lead acetate induced hepatotoxicity in rats. and the objective was to determine the anti inflammatory activity of *V. odorata* on lead acetate induced hepatotoxicity in rats. **Materials and Methods:** Study setting is done in *in vivo* evaluation using animal models. Wistar rats were arbitrarily put into 3 groups with six animals in each group, as Group I - Control, Group II - Lead acetate induction and Group III - Lead acetate induction treated with *V. odorata.* Lead was given orally in drinking water at a dose of 10 milligram/kilogram of body weight for 60 days duration. *V. odorata* treatment was given for 30 days after lead induction. Comparison between experimental and treatment groups with respect to control. **Results:** Histopathological analysis depicted extreme liver tissue damage leading to hepatic steatosis, where the hepatocytes are degenerating ending with micro and macro vacuolization eventually filled with lipids in Group-II. Also there was a marked increase in the IL-1 mimicking increased inflammatory reaction in the tissue of Group-II and remarkable decrease in Group-III ($p<0.05$). All these pathophysiological scenarios were remarkably decreased in the Group-III with minimal damages. **Conclusion:** By this study we can conclude that hepatotoxicity is reduced by a wide spectrum of therapeutic properties of *V. odorata*. It is found to have a modulatory effect in reducing hepatotoxicity of rats upon lead acetate induction.

Keywords: Lead, Hepatotoxicity, *Viola Odorata*, Liver Function Test, Antioxidant Enzymes.

INTRODUCTION

Hepatotoxicity injury to the liver is caused by exposure to xenobiotics pre-oxidized fatty acids and radioactive isotopes. Liver is the metabolic warehouse of the body which functions continuously to filter chemical toxicants and other metabolites from the blood. Once the harmful toxicants and heavy metals settle in the liver on a long term basis it causes detrimental effects and damages the liver seriously. Plenty of heavy metals are found To treat a damaged liver through several drugs available in the medicinal field, to invoke less or no side effects, medicinal plants are utilized (1). *Viola odorata* (*V. odorata*) is a plant species of the flowering type of plant under the genus category “Viola”. This little plant perennial type of species is popularly referred to as ‘wood violet’ which was introduced in North America and Australia.

Many medicinal plants contain a great bio active component that is reported to have a prophylactic and protective function upon metal induced liver toxicity. Liver is the major visceral organ for detoxification from harmful chemicals and chief area of metabolizing the drugs given for treatment.

Many allopathic drugs showed extreme toxicity on almost all organs in the body like the liver, kidney, lungs, etc., causing organ disorders leading to free radicals to induce cell damage, tissue damage and finally organ damage by impairing biomolecules such as lipids at first, then proteins and ultimately destroying DNA (2).

A species of flowering plant of the viola genus that is native to Europe and Asia is called *V. odorata*. Numerous plants, such as the 19 species of the genus viola that make up the family violaceae, are widely known for their hepatoprotective properties. In Europe and Asia, respectively, *V. odorata* is referred to as "banafsha" or "sweet violet."

Flavonoids with anti-inflammatory and antibacterial properties have been found in *V. odorata*, and because of these properties, the plant has been shown to be hepatoprotective against hepatotoxicity caused by paracetamol. Liver injuries are now recognized as a serious health issue that are posing challenges to the domains of nutrition and toxicology. Indian natural plants have long been used as a medical solution for a wide range of illnesses that are affecting people (3,4).

V. odorata is one of the significant medicinal plants that is used to make pharmaceuticals.Due to its numerous pharmacological synthesises, it is categorized as a medication. The higher consumption of alcohol in developed nations is one of the main contributing factors. The causes that affect liver cells most frequently are malnutrition, blood shortage, infectious diseases, and availability of over-the-counter hepatotoxic medications (5).

The production of free radicals upon oxidative stress culminating with lipid peroxidation generates superoxide ions, hydroxyl radicals, etc., which will damage the tissues upon liver toxicity. Antioxidant enzymes including glutathione, catalase, and superoxide dismutase are produced by the body's natural antioxidant system in reaction to these occurrences. These enzymes work to counteract oxidative stress (6,7).

The inability of the host tissue to fight against the stress is assisted by means of therapeutic treatment using *V. odorata*. The medicinal plants have the ability and property to resolve and neutralize the free radicals causing cellular injury (8). The antioxidant properties of many medicinal plants have been studied extensively by many research scientists and have proved their therapeutic roles in the development of a disease prognosis (9,10).

In addition, a variety of substances, including alcohol, heavy metals, and organic toxicants, can cause liver damage. Moreover, liver damage can develop into malignancies, cirrhosis, hepatitis, fatty liver, fibrosis, and liver failure (11). As a result, liver damage is now considered a serious health issue, causing issues with nutrition and toxicity. It is used to treat a variety of inflammatory bowel conditions, including Crohn's disease and ulcerative colitis, psoriasis, psoriatic arthritis, rheumatoid arthritis in both younger and older individuals, acute lymphoblastic leukemia, and ectopic pregnancy (12,13). Thus the aim is to compare the efficacy of *V. odorata* on lead acetate induced hepatotoxicity in rats.

MATERIALS AND METHODS

Adult female wistar albino rats weighing 250 gm ± 20g weight were randomly divided into 3 groups without any bias. Group I - Control, Group II - Lead acetate induction, Group III - Lead acetate induction and *V. odorata* treatment. Lead acetate was induced in rats at a box of 10 mg/kg body weight per day. The body weighs and the lead acetate dissolved in water with regular drinking water for 60 days.

After 60 days of induction, *V. odorata* was given, leaf extract orally at a dose of 200 mg/kg body weight for 30 days. At the end of the experimental period of 90 days, the rats were anesthetized using isoflurane and blood was collected, centrifuged and the serum was separated which was used for biochemical analysis. The animals were sacrificed using CO_2, the liver tissues were collected and fixed in 10% neutral buffered formalin, then processed for histopathological analysis.

The study setting is done *in vivo* evaluation in an animal model. Comparison between experimental and treatment groups with respect to control. The advantages of this study are unbiased random sampling, statistically significant sample size, experimental investigation in animal models before pre clinical studies for validation.

Institutional animal ethics committee approved study. Case sheet verification includes animals procured from the CCCSEA approved breeder. Unbiased random sampling from total available population. Sex of the rats were checked based on study specificity, weight of the rats correlated for age, health status of rats verified thoroughly.

All the data were statistically analyzed using SPSS version 23.0.The ANOVA test for various analysis was performed followed by least significant difference test for multiple comparison to examine differences among the group.Statistical significance was defined with the value p is less than 0.05. Tukeys HSD post-hoc test was done for inter group differences.

RESULTS

Histopathological analysis depicted extreme liver tissue damage leading to hepatic steatosis, where the hepatocytes are degenerating ending with micro and macro vacuolization eventually filled with lipids in Group-II. Numerous additional histological alterations are visible, such as the liver's altered lymphocytes around the major vein and the hypertrophy and multiplication of kupffer cells.

Furthermore, there were several granulomatous lesions observed in the liver parenchyma, and the liver parenchyma exhibits big, circular, transparent fatty vacuolate formations that resemble cysts. All these histopathological manifestations were comparatively reduced in the *V. odorata* treated lead induced rats, Group-III.

Also there was a marked increase in the inflammatory marker, IL-1 mimicking increased inflammatory reaction in the liver tissue of Group-II when compared to Group-I. To the surprise, the inflammatory marker IL-1, showed evidently less in Group-III after *V. odorata* treatment ($p<0.05$) indicating statistically significant decrease in IL-1 when compared to Group-II.

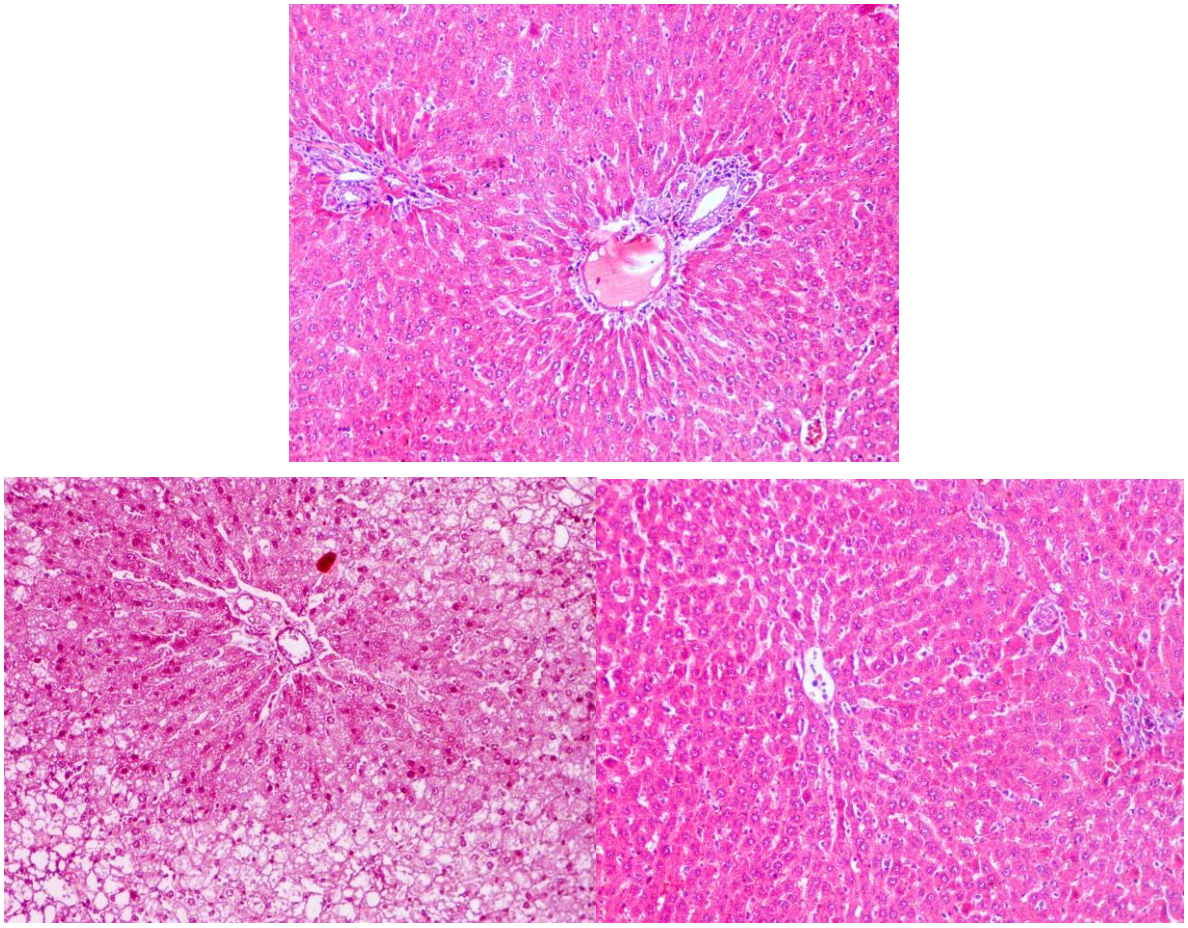

Figure 1: Histopathological Images of Liver Tissues Stained with H&E at 10X Magnification

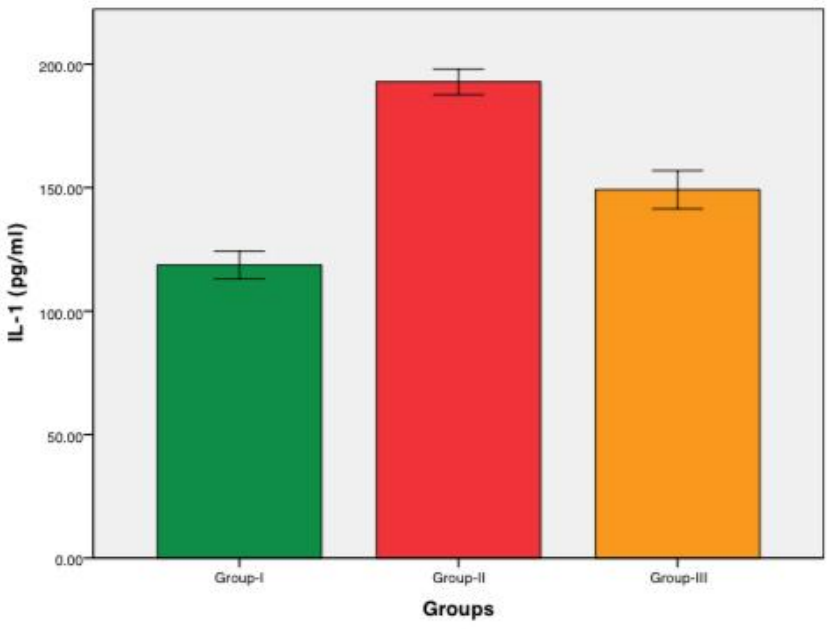

Figure 2: Bar Chart Shows the Level of Interleukin 1 (IL-1) in Control and Experimental Groups

The x-axis represents the animal grouping and Y-axis represents the IL-1 level in pg/ml. All the values are expressed as Mean ± Standard Deviation. Between the groups, Group-III showed p= 0.027 ($p<0.05$) indicating statistically significant decrease in IL-1 when compared to Group-II.

DISCUSSION

The liver has experienced increased exposure to harmful substances and drug side effects, which has led to the production of free radicals, which are partly to blame for liver deterioration. According to a study by Kariot et al., *V. odorata* contains flavonoids, glycosides, alkaloids, saponins, and vitamin C. All of these compounds represent antioxidant action, and flavonoids have hepatoprotective effects. It was also evident that treating mice with *V. odorata* was significantly more effective than giving them MTX for seven days (14).

These results are consistent with other reports and also show an increase in free radical, hydrogen peroxidase, and lipid peroxidation fatty acids. They were also responsible for the toxicity of lead by impairing the antioxidant system and preventing the conversion of folic to folinic acid. It is hypothesized that these modifications result in damage to the hepatic parenchymal plasma membrane, which releases AST and ALT from cells into serum. Thus, the increased presence of AST and ALT in the bloodstream indicates liver structural degradation and cell death (15,16).

Administration to the V. odorata group represents the best choice to use it as an antioxidant because it contains flavonoids, which represent as hydrogen donors that reduce oxidative stress, but not enough mechanisms to explain the significant elevation of serum levels of liver function biomarker enzymes in HTC that the current study found. The anti-inflammatory and hepatoprotective role of *V. odorata* on the level of ALP, AST, and ALT was evaluated significantly by lead acetate given group.

According to Patel et al., the primary lesion in the current histological results is characterized by hazy edema of the hepatocytes, significant mononuclear cell aggregation, especially of lymphocytes surrounding the central vein, and proliferation and hypertrophy of kupffer cells. These results corroborate those of previous researchers who have determined that hydronic blood vessels with aggregation surrounding the bile duct are hazy and swollen due to lead acetate. Other explanations for *V. odorata's* hepatoprotective properties include its capacity to stabilize membranes and scavenge free radicals. (17). It is suggested that the organs and the human body as a whole should be protected and kept healthy for a better quality of life (18, 19). Also the people working in mines, industrial sectors, manufacturing units, are advised to avoid direct exposure to heavy metals and to take proper precautions to avoid ingestion or infestation on a lifelong basis (20, 21, 22).

CONCLUSION

This investigation leads us to the conclusion that V. odorata has a therapeutic impact on hepatotoxicity, which can be caused by alterations in hemodynamics, direct tissue and cell damage, inflammatory tissue injury, and obstruction of bile excretion. Environmental contaminants and a wide range of medicinal medications can often cause hepatotoxicity. Because of its antioxidant activity, *V. odorata* demonstrated protective properties against the hepatotoxic effects of hydro alcoholic extract.

Therefore, it can be said to be effective against oxidative stress in liver tissue caused by long-term exposure to lead acetate.

Author Contributions

Author 1: Vantipalli Raga Sai Harshitha, carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and the preparation of the manuscript.

Author 2: Raj Kumar. D, assisted in carrying out the study by collecting data and did necessary statistical analysis.

Author 3: Karthik Ganesh Mohanraj, aided in conception of the topic, designing the study, supervision of the study, correction, verification of the data for validity and final approval of the manuscript.

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Conflicts of Interest

There is no conflict of interest to declare.

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