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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF OCIMUMCANUM LEAVES AGAINST CCL4– INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

This study was designed to investigate the hepatoprotective activity of aqueous extract of Ocimumcanumleaves against CCl4 – induced liver damage in rats. Hepatic damage was induced by CCl4. Thereafter, the levels of some serum biochemical parameters such as alanine trasaminase (ALT), aspartate transaminase (AST), alkalinephosphatase (ALP), albumin, total bilirubin (TBIL) and total protein (TP) were investigated. The activities of ALP, AST, ALT and histological changes in the liver of rats were also determined. Silymarin was used as the standard hepatoprotectivedrug. The pre – treatment of rats with aqueous extract of O. canum leaves caused a significant increase in theserum levels of TP and albumin. There was a significant decrease in the serum levels of ALP, AST, ALT and TBIL with acorresponding increase in the activities of ALP, AST and ALT in the liver of extract treated rats. The hepatoprotection was confirmed by histological examinations of liver sections of normal and treated rats. Furthermore, rats intoxicated with Ccl4 alone had their serum ALP, AST, ALT and TBIL levels significantly increased, while TP and albumin concentrationsdecreased when compared with the normal rats. The aqueous extract of Ocimum canum leaves at doses of 250and 500 mg /kg p.o. have significant hepatoprotective ability against – CCl4 induced hepatic damage in rats.

KEYWORDS: Ocimum canum, Hepatoprotective Activi, CCl4, Biochemical Parameters.

INTRODUCTION

The liver is the chemical factory which regulates, synthesizes, stores and secretes important macromolecules inthe body. It has a strategic anatomical location and large capacity for metabolic transformation of drugs and othertoxins entering from the gastrointestinal tract. As a result ofthis, the healthy functioning of the liver determines thehealth status of an individual.[1] Liver diseases are a globalproblem and the synthetic drugs available for the treatmentof liver disorders are believed to have serious adverseeffects on biological systems.[2] Due to these facts, attentionhas been given to finding suitable curative agents forthe treatment of liver diseases from natural product of plantorigin.[3] The aerial parts of the *Ocimum* species are considered as antispasmodic, stomachic and carminative in native medicine.[4] These species are extensively studied and explored for the antimicrobial, antimicrobial, adaptogenic, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radioprotective, immunomodulatory, neuro-protective, cardio-protective and mosquito repellent properties.[5-9] *O. canum*has been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough,malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite etc and in treatment of gastric, hepatic, cardiovascular & immunological disorders.[10] The extraction yields showed that the leaves of *O. canum*are four times richer in essential oil (0.44%) than those of *O. basilicum*(0.11%). Analysis by (GC) and (GC / MS) revealed that these oils are monoterpenic (83.4 to 92.4%). The oxygenated monoterpenes are predominant in the essential oil of *O. canum*(63, 3%), while the monoterpene hydrocarbons are mainly in essential oil of *O. basilicum*(56.2%). The major components identified in essential oil of *O. canum*are linalool (53.8%) and limonene (22.2%). The essential oil of *O. basilicum* is distinguished by the predominance of compounds such as linalool (18.9%), limonene (30.9%) and β-phellandrene (15.3%). Bioassay tests done by the World Health Organization (WHO) standard protocol revealed that these essential oils have remarkable adulticidal properties on *An. Funestus*ss.[11] It is also an excellent source of vitamin K and manganese; a very good source of copper, vitamin A and vitamin C, calcium, iron, folate, and omega-3 fatty acids as well.[12] *Ocimum canum*a common medicinal plant has a wide range oftherapeutic potentials. The present study was designed to validate the hepatoprotective effect of *Ocimum canum*Linn. on ccl4 induced hepatotoxicity.

MATERIALS AND METHODS

Collection of plant material

Leaves of Ocimum canum was collected from the hill forest of Chittoor District, Andhra Pradesh. The characters were confirmed by Dr. K. MadhavaChetty, Assistant professor, Department of botany, S.V. University, Titupathi.

Preparation of ethanolic extract

Leaves were separated from the plant and were dried under shade. The ethanolic extraction was done using soxhlet apparatus (Obromax, ATI-177). Further ethanolic extract was dried and was stored at 40°C for further use. The plant was cleaned and shade dried for 48 h before extraction.

Animal maintenance

The animal experiment and all procedures were carried out in accordance with guidelines for care and use of laboratory animals of institutional animal ethical committee (IAEC), Avanthiinstitute of pharmaceutical sciences, hyderabad, Telangana, India (Registration number: 994/a/ GO/06/CPCSEA/23/oct/2006). Male adult albino rats (Wister strain) weighing 150 ± 10 g of approximately same age were procured from Mahaveer Enterprises, Ghatkesar, India. They were housed in polypropylene cages with proper bedding, feeding and water ad libitum. After an adaptation period of two weeks rats were randomly divided into following experimental groups.[13]

Experimental design

The rats were divided into 5 groups, consisting of six rats each.

Group 1: Untreated animals and served as Normal control(Normal saline 0.5ml/kg b.w/p.o).

Group 2: Disease control (ccl4 is administered orally at a dosage of 2ml / kg body weight)

Group 3: Standard (Sylimarin 100ml/kg b.w/p.o).

Group 4: ethanolic extract of *Ocimumcanum*250mg/kg b.w/p.ofor 14 days.

Group 5: ethanolic extract of of*Ocimumcanum*500mg/kg b.w/p.ofor 14 days.

animals were sacrificedunder light ether anesthesia 24 h after their respective doses.Blood samples were collected into EDTA bottles by cardiacpuncture while the liver was quickly removed into ice cold0.25 M sucrose solution. Thereafter, the liver was blotted with clean tissue paper and homogenized in Tris Hcl buffer(0.1 M pH 7.4 1: 10 w/v). The homogenates were kept fro-zen overnight before being used for various enzyme assays[14]

PARAMETERS EVALUATED

Gravimetric analysis: Livers were removed and adherent fat was removed. The weight of livers were noted to observe the changes in weight on and after the dosage of ethanolic extract of leaves of *O. canum* in order to compare it with the control.

BIOCHEMICAL PARAMETERS STUDIED

Aspartate transaminase

The assay mixture containing1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37ºC. To the control tubes serum was added after the reaction was arrested by the addition of 1 ml of DNPH.The tubes were kept at room temperature for 30 min. Added 0.5 ml of Na OH and the color developed was read at 540 nm. The activity of AST was expressed as µmoles of pyruvate formed /min/mg of protein.

Alanine transaminase

The assay mixture containing1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37ºC. Added 1 ml of DNPH and kept at room temperature for 20 min. Serum was added to the control tubes after the reaction was arrested by the addition of 1 ml of DNPH. Added 5 ml of Na OH and the color developed was read at 540 nm.

The activity of ALT was expressed as µmoles of pyruvate formed /min/mg of protein.

Alkaline phosphatase

The reaction mixture containing 1.5 ml carbonate buffer, 1 ml Di sodium phenyl phosphate, 0.1 ml Magnesium Chloride and 0.1 ml of serum was incubated at 37 ºC for 15 min. The reaction was arrested by the addition of Folin's phenol reagent. Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's phenol reagent. Added 1ml of Sodium Carbonate. The color developed was read after 10 min at 640 nm.

The activity of ALP was expressed as µmoles of phenol liberated /min/mg of protein.[15]

Serum bilirubin

For the determination of total bilirubin 0.2 ml of serum was taken and made up to 2 ml with water. Then added 0.5 ml of diazo reagent, 2.5 ml of methanol. To the blank 0.2 ml serum was added and made up to 2 ml with water and added 0.5 ml of diazo blank and 2.5 ml methanol. The color developed was read at 540nm. The values were expressed as mg/dl.[16]

Total protein

Aliquots of the suitably diluted serum(0.1ml to 10ml by two serial dilutions) was made up to 1.0 ml with water and 4.5 ml of alkaline copper reagent was added to all the tubes including blank, containing 1.0ml water and standards containing aliquots of standard BSA and made up to 1ml with water. The tubes were incubated for 10 min at room temperature. 0.5 ml was added to all the tubes and incubated for 20 min at room temperature. The blue color developed was read at 640nm. The protein content was expressed as g/dl.[17]

Albumin

The serum albumin was estimated by the method given by Corcoran and Durnan (1977) using albumin test kit

(Span Diagnostics Ltd.). 3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

Blood urea nitrogen (BUN) content: The serum blood urea nitrogen was estimated by Enzymatic Urease (Berthelot) method (Fawcett and Scott, 1960) using Urea Berthelot test kit (Span Diagnostics Ltd.).1.5 ml Solution I was added to clean test tubes. 0.01 ml serum was added for the test and 0.01 ml Reagent III was added for the standard. It was then mixed well and incubated at 37ºC for 3 min; then 1.5 ml of Solution II was added. It was then mixed well and incubated at 37ºC for 5 min. The absorbance was read at 578 nm against reagent blank.

catalase activity: The catalase was colorimetrically assayed as described by Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of phosphate buffer (10 mM, pH 7.0), 0.1 ml of liver homogenate and the reaction was started by addition of 0.4 ml of H2O2 (2000 mM). The reaction mixture was incubated for 3 min at room temperature. The reaction was stopped by addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio), was incubated at 100°C for 2 min. The absorbance was measured at 620 nm.

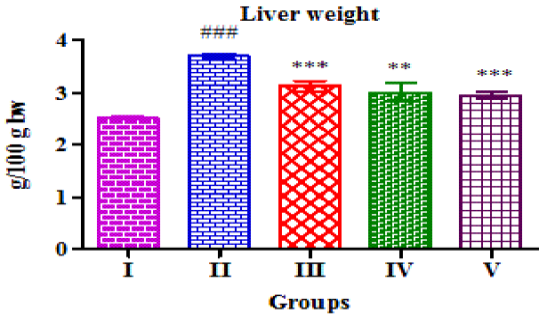
Histopathological studies

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and Sections of 5μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.[14]

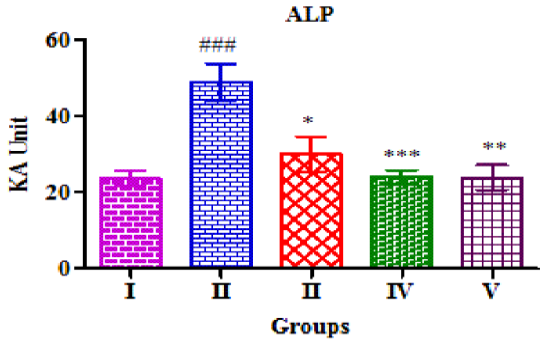
RESULTS

Toxicity study

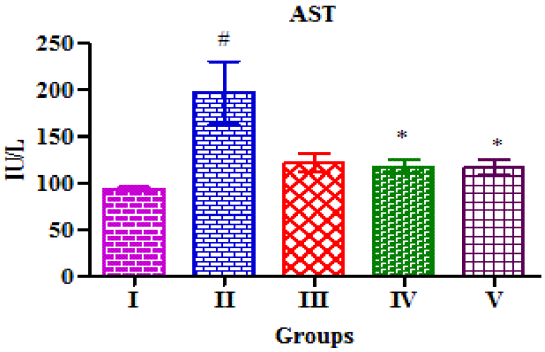
In toxicity study (limit test) the ethanolic extract Ocimumcanumwas shown no signs and symptoms, morbidity and mortality on *Wistar* rats. Based on the acute toxicity studies carried out the low and high dose of extract is chosen (EEOC of 250 mg/kg and 500mg/kg body weight).



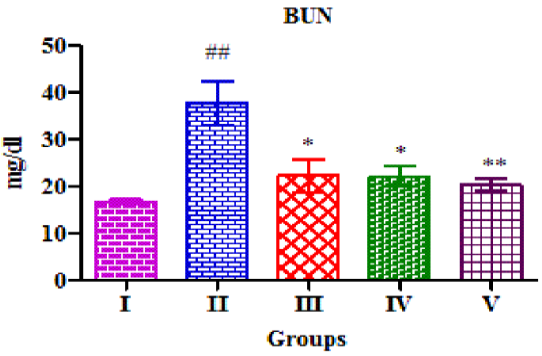
ALT: The administration of CCl_4 markedly increased serum ALT levels which were significant as compared to normal control group ($P < 0.05$, $P < 0.01$ respectively). The groups that received the pre-treatment of EEOC at dose levels of 250 and 500 mg/kg body weight significantly controlled the change in the biochemical parameters.



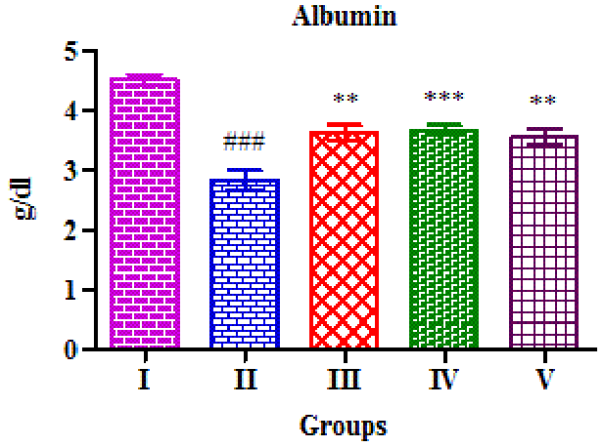
ALP: The ALP levels increased significantly ($P < 0.01$, $P < 0.001$ respectively) in the group treated with CCl_4.The ALP level also significantly decreased in EEOC-250 ($P < 0.05$) as well as in EEOC- 500 group ($P < 0.001$).



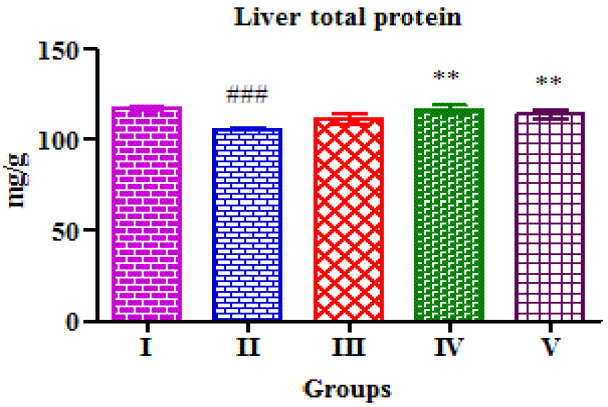
AST: The administration of CCl_4 markedly increased serum AST levels which were significant as compared to normal control group ($P < 0.05$, $P < 0.01$ respectively). The groups that received the pre-treatment of EEOC at dose levels of 250 and 500 mg/kg body weight significantly controlled the change in the biochemical parameters.



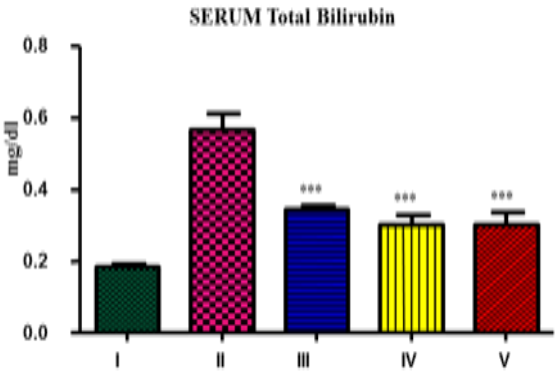
BUN: The BUN levels increased significantly ($P < 0.01$, $P < 0.001$ respectively) in the group treated with CCl_4.The BUN level decreased in both the dose groups significantly ($P < 0.05$) as compared to toxin control group.



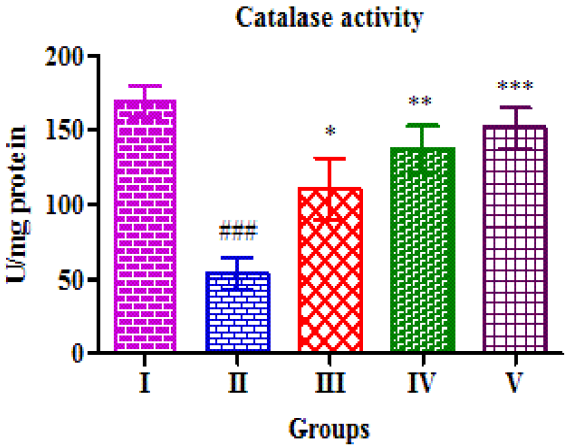
Albumin: The albumin level in lower as well as in higher dose group increased significantly ($P < 0.01$, $P < 0.001$ respectively) as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin.



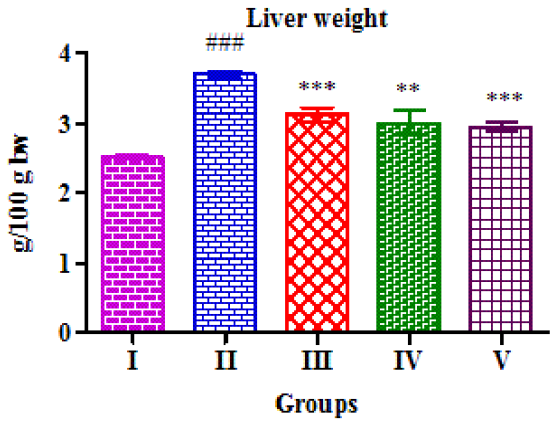
Liver Total Protein: The level of total protein and albumin depleted in the group treated with CCl_4 (toxin control) and were significantly decreased ($P < 0.001$) when compared with the normal control group. The extract at dose levels of 250 and 500m**.**



Total Bilurubin: Total bilirubinin serum was estimated by using commercial Erba total bilirubin kit and the results were shown. Rats induced with CCl_4 in toxic control group (G-II) showed a significant increase in total bilirubin,when compared to normal group (G-I). The groups (IV and V) receiving EEOC(250 mg/kg and 500 mg/kg) showed a significant ($p<0.001$) decrease in total bilirubin, when compared to control group (G-II).



Catlase activity: The catalase (CAT) activity in the toxin control group was also significantly ($P < 0.001$, $P < 0.05$ respectively) depleted as compared to the normal control group.



Liver Weight: The mean relative liver weight decreased significantly in EVT-250 ($P < 0.001$) and EEVT-500 ($P < 0.01$) treated group as compared to the toxin control group. The result of the higher dose group was comparable to the standard drug treated group ($P < 0.001$).

Histopathology: The hepatoprotective potential of O. canum leaf extract was confirmed by histological examination of normal and treated rats. The histological profile of normal rats showed normal cellular architecture (Fig GR 1). In CCL_4 intoxicated animals, there were drastic alterations in the histological architecture of the liver. Histological examination showed distende hepatocytes, fatty degeneration and area of necrosis (Fig GR 2). The administration of O.canum extract and silymarin brought about significant recovery. There was less degeneration of hepatocytes as a result of marked regeneration activity. (Fig GR 3-5-).

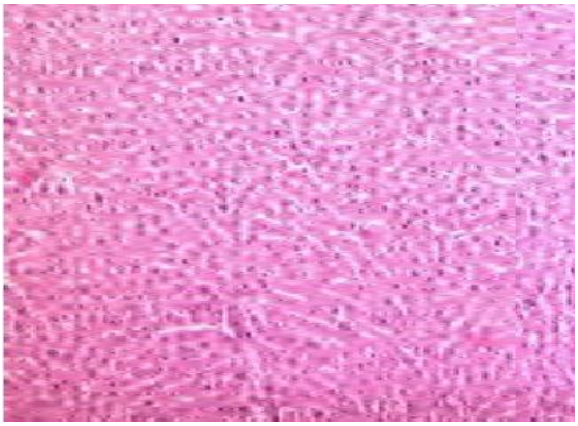


Figure 1.

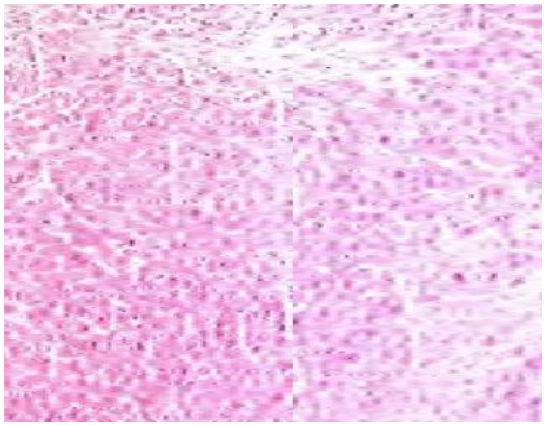


Figure 2.

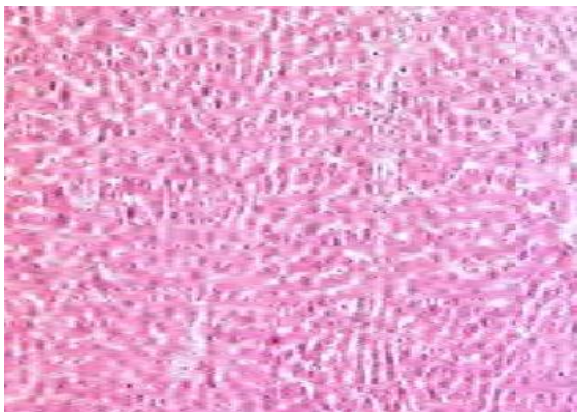


Figure 3.

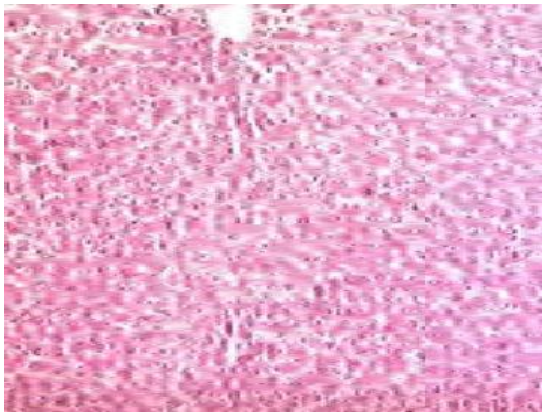


Figure 4.

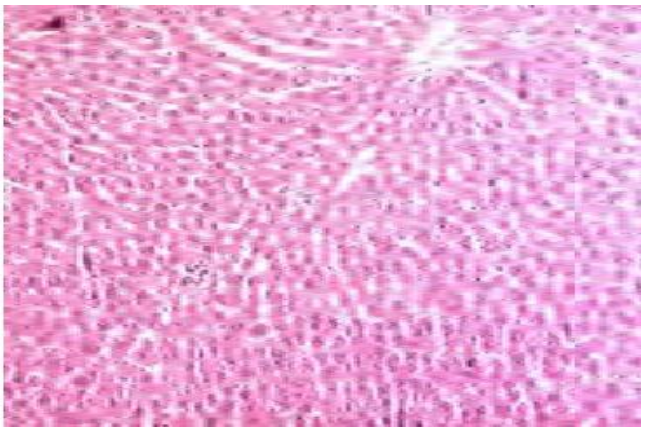


Figure 5.

Figure 5.2: Photographs of liver sections of CCl_4 (2 ml/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (GRP-1) Normal control, (GRP-2) Toxin control (CCl_4), (GRP-3) Silymarin-100 + CCl_4, (GRP-4) EEOC-250 mg+ CCl_4, (G-5) EEOC-500mg + CCl_4.

DISCUSSION

Hyperbilirubinemia seen in liver injury can result from impaired hepatic uptake of unconjugated bilirubin. From above results it is evident that the elevated serum Bilirubin levels due to Ccl4 induced toxicity was restored within the normal levels on treatment with the ethanolic extract of *Ocimumcanum* Linn. The restoration of the bilirubin levels indicates regeneration of the hepatic tissues and improved hepatic efficiencyand changesin theweight of liveralso controlled by EEOC.

Serum protein level is a gross measure of protein status and reflects major changes in the liver functions. Liver is the site of synthesis and storage of many proteins. Hepatotoxins impair the capacity of liver to synthesize protein. Liver is the major site of protein metabolism and a healthy functioning liver is required for the synthesis of the serum proteins except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. The biochemical studies of blood samples of Ccl4 induced animals

showed a significant decrease in total protein level reflecting liver injury, while the blood samples treated with aqueous extract of *Ocimumcanum* Linn showed an increase in total protein content.

AST, ALT and ALP are located in the cell cytoplasm under normal circumstances. Due to Ccl4 induced hepatotoxicity the membranes of hepatocytes become damaged releasing the enzymes into circulation causing elevation. It is noteworthy, that the ethanolic extract of *Ocimumcanum* Linn. has a profound effect in restoration of AST, ALT and ALP levels towards their respective normal values.

CONCLUSION

The study suggests that the ethanolic extract of *ocimumcanum* linn. controls the damage caused by CCL4 on hepatocytes membrane and provides a prognostic value with hepatoprotection.

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