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Safety Evaluation and Hepatoprotective Effects of *Sauromatum guttatum* and *Leonotis nepetaefolia* root

Jitendra Singh[1], Zulphikar Ali[1*], Ranjit Singh[1]

1-Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur-247341, Uttar Pradesh, India

*Corresponding author
Zulphikar Ali[1*]
Email-Zuphikar.ali@shobhituniversity.ac.in

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Abstract

The ethanol extract of *Sauromatum guttatum* and *Leonotis nepetaefolia* isolated from it were evaluated for hepatoprotective activity against Rifampicin, Ethanol, Paracetamol and carbon tetrachloride induced toxicity in Wistar rats. The parameters studied were estimation of liver function serum markers such as Serumglutamic Pyruvate transaminase, serum Glutamic Oxaloacetic Transaminase and Alkaline phosphatase activities. The extract of *Sauromatum guttatum* showed moresignificant hepatoprotective activity than *Leonotis nepetaefolia* extract. The histological profile of the liver tissue of the root extract of *Sauromatum guttatum* treated animal showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration as similar to the controls. The results when compared with thestandard drug silymarin, revealed that the hepatoprotective activity of the constituent extract of *Sauromatum guttatum* is significant as similar to the standarddrug.

Keywords: *Sauromatum guttatum*, *Leonotis nepetaefolia*, Hepatoprotective activity, Paracetamol, carbon tetrachloride

INTRODUCTION

The liver is one of the biggest organs in the human body and the chief site for strong metabolism and excretion. So, it has a surprising role in the maintenance, performance and regulating homoeostasis of the body. It is concerned with almost all the biochemical pathways to growth, fight against the disease,nutrient supply, energy provision, and reproduction [1]. The main functions of theliver are carbohydrate, protein and fat metabolism, detoxification, secretionof bile, and storage of vitamins. Therefore, to maintain a healthy liver is an essential factor for overall health and well-being. But it is continuously and variedly uncovered to environmental toxins, and abused by poor drug habits, alcohol,and prescribed and over the counter drugs which can eventually lead to different liver ailments such as hepatitis, cirrhosis, and alcoholic liver ailment [2, 3]. Thus, liver diseases are some of the lethal diseases in the world today.They pose a serious challenge to international public health.

Liver injury can be caused by different agents, such as viruses, chemicals, alcohol, and auto-immune diseases [4]. Rifampicin, Ethanol, Paracetamol and carbon tetrachloride are well-

established hepatotoxicant, it induces a diffuse type of liver injury closely resembling human viral hepatitis [5] and acute self-limiting hepatitis with necrosis, inflammation and regeneration, resembling drug- induced diseases in humans [6]. The toxicity of Rifampicin, Ethanol, Paracetamol and carbon tetrachloride is mainly related to the depletion of uridine pools that areassociated with limited ribonucleic acid (RNA) and protein synthesis, thus alteringhepatocellular function [7]. Modern drugs have little to offer for the alleviation of hepatic diseases and it is mainly the plant- b a s e d preparations which are employed for their treatment of liver disorders. World Health Organization (WHO) estimated that around 80% of the world's population depends on medicinal plants as their primary health care source. The WHO has reported around 21,000 plants are used for medicinal purpose. Of which 2500 species are in India, among these 150 species are used commercially on a fairly large scale [8].

The present research work aimed for detailed and systematic phytochemical and pharmacological investigation of some plants *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br for hepatoprotective activity. The plants selected for the present scientific evaluation were based on the evidence of their ethnopharmacological usage and availability.

Sauromatum guttatum (Wall.) & *Leonotis nepetifolia* (L.) R. Br root was selected because in Ayurveda the decoction of bark is used for washing and cleansing of wounds, ulitis and ulemorrhagia. Mimusops elengi fruits were selected because of its astringent action. Also, anti-tyrosinase activity of fruits was reported [9]. *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root was selectedbecause traditionally rose water has been used for moisturizing, hydrating and lightening of the skin, while having a general stimulant and antiseptic action. Traditionally, the petals are also used to repair broken capillaries, inflammation aswell as skin redness [10]. But still, no scientific investigation has so far been reported in the literature regarding its action on the liver. Therefore, the present investigation has been designed to study the possible mechanism of ethanol extract of *Sauromatum guttatum* and *Leonotis nepetaefolia* for hepatoprotective activity. In this study, we report the activity of *Sauromatum guttatum* and *Leonotis nepetaefolia* root in Rifampicin, Ethanol, Paracetamol and carbon tetrachloride induced toxicity in a rat model.

STUDY PLAN

Procurement of animals

About 180-200 g weighed Male or female Albino rats were used for this study. These experimental rats were kept in the animal cages following a cycle of 12-h light/12-h dark. This experimental protocol was approved by Institutional Animal Ethics Committee (1122/PO/Re/S/2007/CPCSEA) by using the national guidelines of laboratory animals.

Chemicals and Drugs

The gift sample of acetaminophen was obtained from Primus pharmaceuticalLimited, Simour, Himanchal Pradesh, India. Standard drug Sylibon-140 (Silymarin) purchased from market manufactured by Microlab Limited solon, Himanchal Pradesh india. The biochemical analytical kits for alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) from Transasia biochemical Limited Solon, Himanchal Pradesh, India. All other reagents used in the experiment were of analytical grade. DMSO (dimethyl sulfoxide) was purchased from Sigma Aldrich (India). The detection of malondialdehyde used by thiobarbituric acid reactive substance, kit was purchased from sigma Aldrich (India) were

purchased from orchev pharma pvt ltd. All of the glassware and lab equipment utilised in the research was of the highest quality and came from a reputable source.

Acute oral toxicity study (OECD, 2000)

Intense danger study was completed according to rules set by Organization for Economic Co-operation & Development (OECD), overhauled draft 423 rules; get from Committee with end goal of Control & Supervisions of Experiments on Animals (CPCSEA), Ministry of Social Justice & Empowerment, & Government of India. Intense oral poisonous quality review was completed by utilizing Albino rats (150-200 gm). Tempture in test room was around 25˚C. Lighting was regular arrangement that is, being 12 hours obscurity, 12 hrs light. Traditional research facility eating regimen was sustained with satisfactory supply of drinking water. Animals were haphazardly chosen, set apart to allow singular distinguishing proof& kept in propylene confines for one-week preceding dosing to permit their acclimatization to lab condition. Important depended on stepwise method with utilization of base number of creatures per venture to acquire adequate data on intense poisonous quality of test substance to empower its grouping. Substance wastried utilizing stepwise strategy, every progression utilizing five creatures of everydosage. Strategy empowered judgment regarding grouping test substance to one ofarrangement of poisonous quality works of art characterized by settled LD50 cut of qualities All concentrates were set up as suspension by triturating with water & 1%tween 60. Albino rats (160-200 g) were utilized for intense poisonous quality reviewto decide LD50 of concentrates of Solanum melongena. Content substance was controlled in solitary measurements by gage into stomach tube. Before dosing, animal was kept for 12 h of fasting. At that point c animal were weighed & test substances were controlled. Every concentrate of *Sauromatum guttatum* (Wall.) &*Leonotis nepetifolia* (L.) R. Br, at various measurements in expanding request. In initial step every dosage was tried on single rodent & afterward managed to other four rats. Perception was made amid initial four hours after medication organizationto notice change in skin & hide, eye, bodily fluid film, hyperactivity, preparing, writings, sedation, hypothermia, tremor, salivation, trance like state.

1/tenth deadly measurements were taken as compelling dosage (remedial measurements) LD50 cut off esteem is 200 & 1/fifth 400 were chosen to assess measurements subordinate activity for assessment of calming & hostile to ligamentmovement [11].

i) Principle of test

Guideline of technique relies on organization of deadly measurements & dosages which cause checked torment & trouble ought to be stayed away from. Technique empowers judgment concerning arranging test substances to one of arrangement of poisonous quality classes characterized by settled LD50 cut-off qualities.

ii) Housing & encouraging condition

Temp. inside animal room was kept at 25±30C. Put animal in 12 h light & 12 h obscurity. For nourishing, convential research facility eating routine was utilized with water not obligatory. Same conditions were taken after for animal utilized forappraisal of hostile to ligament action.

iii) Preparation of animal

Animals are arbitrarily chosen & kept in pens for at least 5 days before dosing to take into account acclimatization to lab conditions.

iv) Preparation & organization of Doses

The dried roots (air-dried for 5 days) of *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br were pulverized into a fine powder and were mixed with ethanol at a ratio of 1:3 (w/v, 100g powder in 300 ml ethanol). After 24 hours, the mixtures were filtered; filtrate was

collected and the residue was again mixed withethanol at a ratio of 1:2 (w/v, 100g powder in 200 ml ethanol) for 24 hrs. After filtration, filtrates were combined and evaporated to dryness (approximate yield 10.1%) using rotary evaporator. Extracts were suspended in 1% Tween 80 in water prior to administration. In all cases fixations were set up as indicated by 1 ml/100gof body weight.

Standard substances are controlled in single dosage by gavages utilizing stomach tube. In wake of fasting for 3 to 4 hours, rats are weighed & test substance managed.1 to 2 hours in rats after organization of measurements.

i. Number of animal & dosage levels.

ii. In every means three animals were utilized. Since there was no data on substancesto be tried i.e. removes, beginning dosage was chosen to be 300 mg/kg body weight.Accompanying LD50 qualities were gotten for different concentrates/divisions.

iii. 1/10th of this lethal dose was taken as effective dose (therapeutic dose) forhepatoprotective study.

Observations

Animal are viewed at first in wake of dosing at any rate once in midst of beginning30 minutes, discontinuously in midst of underlying 24 hours. In all cases end was seen inside beginning 24 hours. Additional recognitions like changes in skin, eyes & mucous layers & besides respiratory, circulatory, autonomic & central tactile frameworks & somatomotor & direct pattern. Attention was also given to impression of tremors & shakings.

Experiment design and treatment

Rifampicin induced hepatotoxicity [12]

After the adaptation period, the rats were randomly divided into four groups of sixanimals:

Normal group: Rats received normal saline orally (NaCl, 0.9%) daily, throughout the experimental period.

Disease group: Each rat received Rifampicin (100 mg/kg body weight, p.o.) for 21 days.

Treatment group I: Each rat received Rifampicin (100 mg/kg body weight, p.o.)for 21 days + Extract of *Sauromatum guttatum* 200 mg/kg (orally) for 15 days.

Treatment group II: Each rat received Rifampicin (100 mg/kg body weight, p.o.)for 21 days + Extract of Leonotis nepetaefolia 200 mg/kg (orally) for 15 days.

Standard group: Each rat received Rifampicin (100 mg/kg body weight, p.o.) andSilymarin 25 mg/kg body weight p.o for 21 days.

Ethanol induced hepatotoxicity [13]

Induction of experimental hepatotoxicity Rats were treated with four-hundredthplant product (2 ml/100 g, orally) for 21 days to study the effect of ethanolic which was used as a standard drug in this study. Animals were divided into fiveteams of six animals every.

Normal group: Rats received distilled water for 21 days.

Disease group: Each rat received Ethanol 2ml/100g body weight, p.o for 21 days.

Treatment group I: Each rat received Extract of *Sauromatum guttatum* 200 mg/kgbody weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Treatment group II: Each rat received Extract of Leonotis nepetaefolia 200 mg/kgbody weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Standard group: Each rat received Silymarin 25 mg/kg body weight p.o + Ethanol2ml/100g body weight, p.o for 21 days.

Paracetamol induced hepatotoxicity [14]

Normal group: received only distilled water and fed with a normal diet, p.o.Disease group:

received the acetaminophen at dose 1 g/kg b.w., p.o.
Standard group: received both silymarin (100 mg/kg b.w., p.o) and acetaminophen.
Treatment group I: received Extract of *Sauromatum guttatum* 200 mg/kg body weight p.o and acetaminophen.
Treatment group II: received Extract of Leonotis nepetaefolia 200 mg/kg body weight p.o and acetaminophen.

CCl4 induced hepatotoxicity [14]

The acclimatized rats were divided into five groups as follows:Normal group: Rats were received only the vehicle (olive oil).
Disease group: Rats were received a single dose of CCl4 (1 ml/kg bw) dissolved in olive oil (1:1 volume) in the 1st and 4th day every week of the experiment through anintraperitoneal (IP) injection.
Standard group: Rats were treated with 0.05 g/kg of silymarin for 30 days. Treatment group I: Rats were treated with Extract of *Sauromatum guttatum* 200mg/kg body weight p.o using oral gavage for 30 days.
Treatment group II: Rats were treated with Extract of Leonotis nepetaefolia 200mg/kg body weight p.o using oral gavage for 30 days.

Assessment of Liver Function

At the end of the study, blood sample was taken by retro-orbital sinus. Serum waspartitioned from blood by centrifugation at 3000 g for 15 min and kept at -20 ºC until analysis. The estimation of activity of the Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) using commercially available test kits was done.

Histopathological Studies

The rats were sacrificed and liver was rapidly excised followed by fixing it for 48h in 10% formalin, and was dehydrated by passing successively in different mixtures of ethyl alcohol–water (50%, 80%, and 95%) and finally in absolute alcohol, cleared in xylene and embedded in paraffin. Thick sections (4–5 mm) wasprepared and then stained with hematoxylin and eosin dye for microscopicobservation of cell necrosis, fatty change. [15-16].

Statistical Analysis

All data was expressed as mean± standard error of mean (SEM). The significance data was analysed by one-way analysis of variance followed by Tukey-Kramer multiple comparison post-hoc test. The value of probability $P < 0.05$ was consideredas significant.

Acute Toxicity Studies

In the skin irritation test, no erythema or oedema was seen in both the control animals and the test animals after 3 days of experimentation. In determining the dermal toxic effect of both extract in acute dermal toxicity bioassay, no significantclinical change was observed in any of the treated rat groups except for the initialreaction within the first 30 mins of patch attachment when the rats tried to tear the patch off. The behavioral patterns and general appearance of the rats in the control and test groups were recorded after one hour and twelve hours post-application of test substances. No change meant that the manner in which the animals behaved after acclimatization did not alter when the skin was shaved andtest substances were applied. No erythema or oedema was observed over the 14-day study period in both the control and both extract-treated animals.

Body Weight

There was no mortality or morbidity observed in animals through the 14- day period following single oral administration at all selected dose levels of the aqueous extract of the coded drug. There was no toxicologically significant difference in bodyweights between control and treatment groups (Table 1 and 2).

Effect of plant on tissue damage markers

After 45 days of treatment with *Sauromatum guttatum and Leonotis nepetifolia* at200 mg/kg rats, tissue markers returned to normal compared to the treatment rats. The control rats and both extract showed similar effect on AST, ALT, ACP, ALP,and LDH in Table 3

Assessment of Liver Function

Rifampicin induced hepatotoxicity

Administration of rifampicin (100 mg/kg body weight, p.o.) for 21 days significant($P<0.05$) increased the level of ALP, ALT and AST as compared to normal group. Treatment of Silymarin 25 mg/kg body weight p.o for 21 days significant ($P<0.05$)decreased the level of ALP, ALT and AST as compared to diseased group. However, treatment of extract of *Sauromatum guttatum* 200 mg/kg, orally and extract of *Leonotis nepetaefolia* 200 mg/kg, orally for 21 days significant ($P<0.05$)decreased the level of ALP, ALT and AST as compared to diseased group (Figure 1).

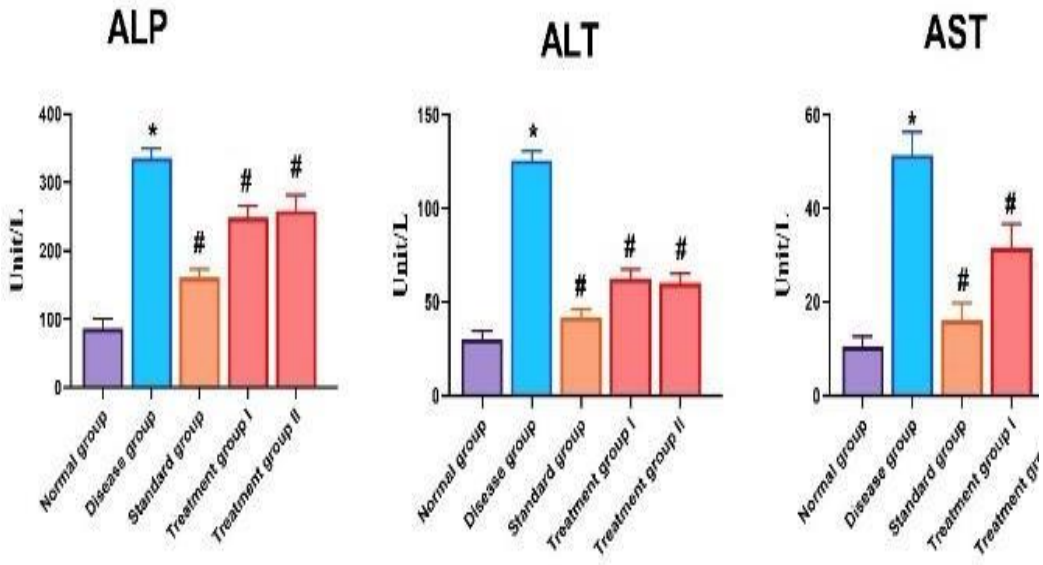

Figure. Biochemical parameter of extract of *Sauromatum guttatum* and *Leonotis nepetifolia* against rifampicin induced hepatotoxicity. Treatment group I= Extract of *Sauromatum guttatum*, Treatment group II= Extract of *Leonotis nepetifolia.*

*$P<0.05$ vs Normal group, #$P<0.05$ vs Disease group.

Ethanol induced hepatotoxicity

Administration of ethanol 2ml/100g body weight, p.o for 21 days significant ($P<0.05$) increased the level of ALP, ALT and AST as compared to normal group. Treatment of Silymarin 25 mg/kg body weight p.o for 21 days significant ($P<0.05$)decreased the level of ALP, ALT and AST as compared to diseased group. However, treatment of extract of *Sauromatum guttatum* 200 mg/kg, orally and extract of *Leonotis nepetaefolia* 200 mg/kg, orally for 21 days significant ($P<0.05$)decreased the level of ALP, ALT and AST as compared to diseased group (Figure 1).

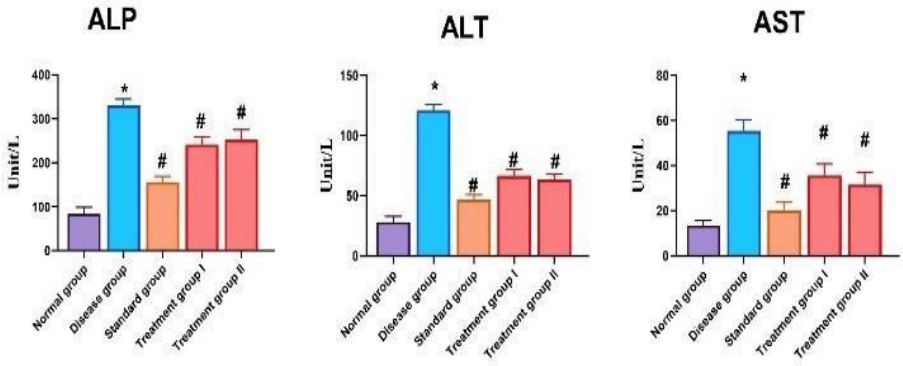

Figure. Biochemical parameter of extract of *Sauromatum guttatum* and *Leonotis nepetifolia* against ethanol induced hepatotoxicity. Treatment group I= Extract of *Sauromatum guttatum*, Treatment group II= Extract of *Leonotis nepetifolia.*
*P<0.05 vs Normal group**,** #P<0.05 vs Disease group**.**

Paracetamol induced hepatotoxicity

Administration of acetaminophen at dose 1 g/kg b.w., p.o for 21 days significant (P<0.05) increased the level of ALP, ALT and AST as compared to normal group. Treatment of Silymarin 25 mg/kg body weight p.o for 21 days significant (P<0.05)
decreased the level of ALP, ALT and AST as compared to diseased group.However, treatment of extract of *Sauromatum guttatum* 200 mg/kg, orally and extract of *Leonotis nepetaefolia* 200 mg/kg, orally for 21 days significant (P<0.05)decreased the level of ALP, ALT and AST as compared to diseased group (Figure 1).

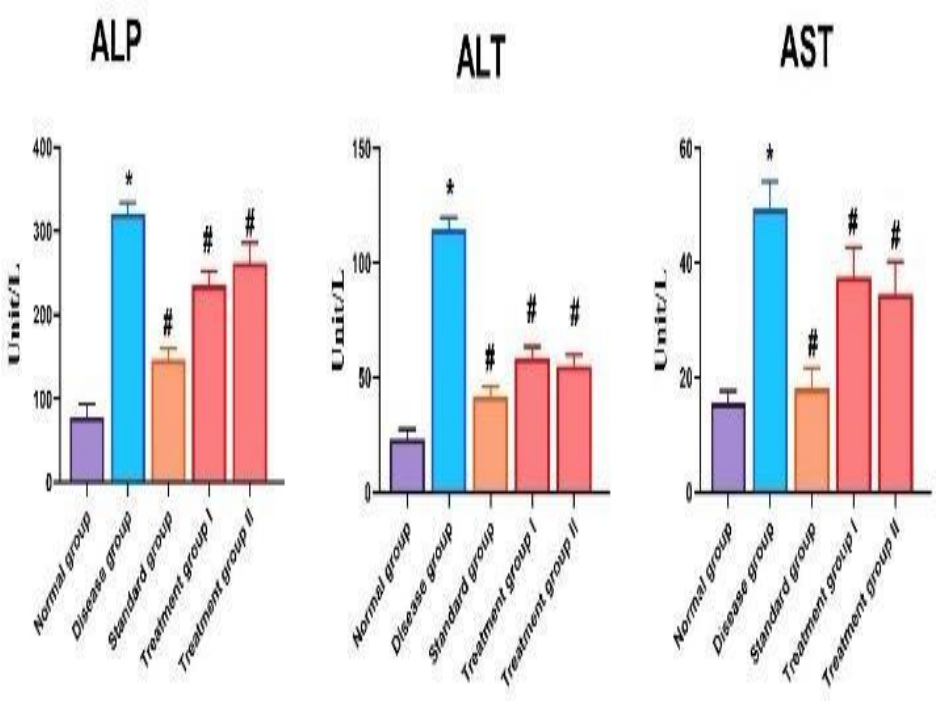

Figure. Biochemical parameter of extract of *Sauromatum guttatum* and *Leonotis*

nepetifolia against paracetamol induced hepatotoxicity. Treatment group I= Extract of *Sauromatum guttatum*, Treatment group II= Extract of *Leonotis nepetifolia.*

*P<0.05 vs Normal group**,** #P<0.05 vs Disease group**.**

CCl4 induced hepatotoxicity

Administration of a single dose of CCl4 (1 ml/kg bw) dissolved in olive oil (1:1 volume) in the 1st and 4th day every week significant (P<0.05) increased the levelof ALP, ALT and AST as compared to normal group. Treatment of Silymarin 25 mg/kg body weight p.o for 21 days significant (P<0.05) decreased the level of ALP,ALT and AST as compared to diseased group. However, treatment of extract of *Sauromatum guttatum* 200 mg/kg, orally and extract of *Leonotis nepetaefolia* 200 mg/kg, orally for 21 days significant (P<0.05) decreased the level of ALP, ALT andAST as compared to diseased group (Figure 1).

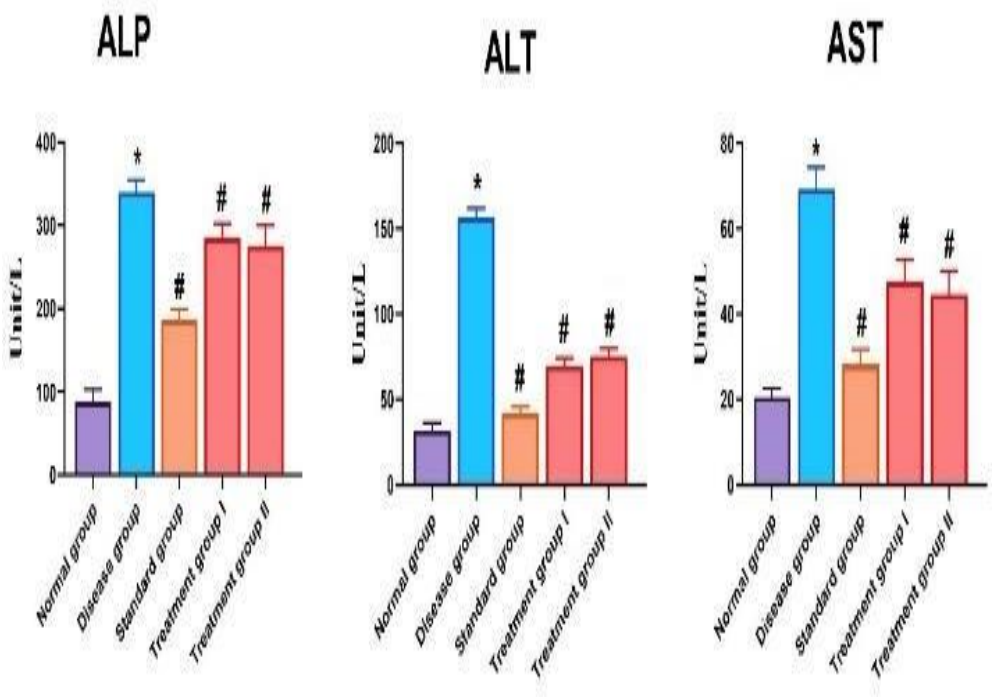

Figure. Biochemical parameter of extract of *Sauromatum guttatum* and *Leonotis nepetifolia* against CCl4 induced hepatotoxicity. Treatment group I= Extract of *Sauromatum guttatum*, Treatment group II= Extract of *Leonotis nepetifolia.*

*P<0.05 vs Normal group**,** #P<0.05 vs Disease group**.**

Histopathology

Histological evaluation of both extract-treated liver tissues. The tissues after the study period of 14 days were excised and evaluated histologically by staining the tissues with haematoxylin and eosin. Neovascularization, epithelial regeneration, and collagen deposition were looked out for in the evaluation. Tissue slides were read at a magnification of 40x and represented in Figure 4. Normal group showedNo regeneration of epithelial layer, inflammatory cells, mild increase in fibrous tissue**.** Disease group showed Complete and thick re epithelization and highnumber of fibroblasts**.** Standard group showed Well organized epithelization of thick layer with pre dominant collagen with few fibroblasts**.** Treatment I and II showed Regeneration of well-defined epithelial layer and well-formed granulation tissue and very few number of inflammatory cells.

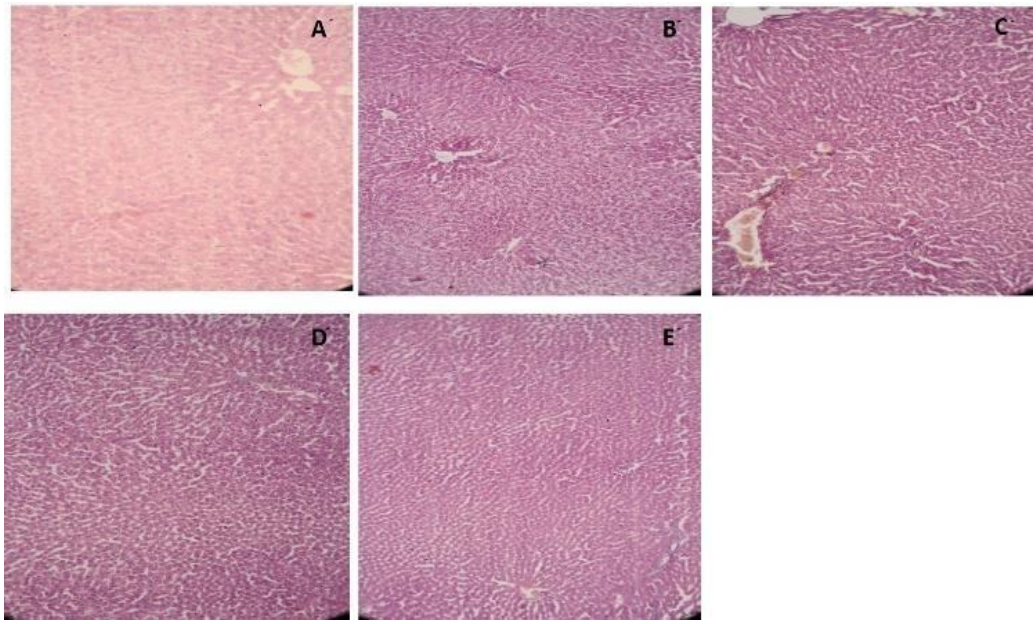

Figure. Effect of extract on tissue of rat. A= Normal group, B= Disease group, C=Standard group, D= Treatment I and E= Treatment II**.**

Discussion

The toxicity of acetaminophen is one of the most extensive drug-induced hepatic damage in the condition of acetaminophen overdose worldwide [17]. Therapeutic doses of the acetaminophen are chiefly metabolized by sulfate and glucuronide conjugation. The metabolites are excreted from the body and detoxified by glutathione. Overdoses of acetaminophen, glutathione are depreciated due to the elevated concentration of the acetaminophen metabolites in hepatic and other cells [18]. This ultimately causes apoptosis and necrosis by disrupting their cellular function. The reason behind this includes that the metabolites combine with the range of proteins [19].

The mechanism of *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root has excellent activity against toxicity of acetaminophen than the other antioxidant but it requires further investigation. In this experiment, oxidative profile of acetaminophen was ($p<0.05$) significantly prevent by the receiving of *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root. For determination of lipid peroxidation, we used malondialdehyde as an oxidative marker in the cells. *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root stuck the rise of malondialdehyde by the acetaminophen mediated in the liver in a dose-dependent manner, provide that it downward activation of lipid peroxidases [20].

The liver enzyme AST, ALT, ALP, TBL, HDL, RBS, SCL, ST are often used as the marker of liver function and their increase and liver enzyme DBL, TP, ALB are also often used as marker of liver function and their decrease in blood indicates hepatotoxicity induced by acetaminophen. The major activity in *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root at 400 mg/kg dose was justified by the ($p<0.05$) similarly elevated level of all liver enzyme and restoration to normal level in the animals [21].

The histological condition of tissue injury induced by acetaminophen in hepatic cell confirmed

prior findings, which cause significant focal hepatitis and mild focalhepatitis in lobules in the portal spaces [22]. From above results, *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root at 200 mg/kg was as potent as ($p<0.05$) Silymarin (100 mg/kg) as well as in control these histological changes in the liver.

The present research work aimed for detailed and systematic phytochemical and pharmacological investigation of some plants *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br for hepatoprotective activity. The plants selected for the present scientific evaluation were based on the evidence of their ethnopharmacological usage and availability.

Sauromatum guttatum (Wall.) & *Leonotis nepetifolia* (L.) R. Br root was selected because in Ayurveda the decoction of bark is used for washing and cleansing of toxicity, ulitis and ulemorrhagia. Mimusops elengi fruits were selected because ofits astringent action. Also, anti-tyrosinase activity of fruits was reported [23-29]. *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root was selected because traditionally rose water has been used for moisturizing, hydrating and lightening of the skin, while having a general stimulant and antiseptic action. Traditionally, the petals are also used to repair broken capillaries, inflammation aswell as skin redness [20].

Conclusion

This obtained result showed pharmacological evidence for the ethnomedical claim the *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root.as the hepatoprotective. *Sauromatum guttatum* (Wall.) & *Leonotis nepetifolia* (L.) R. Br root. might be a potential hopeful of biomedical centrality and to be used in combination therapy with standard and significantly decreases the therapeutic dose of standard as well as the side effect.

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