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Original article

# Hepatoprotective activity on *Commiphora* species. And its polyherbal formulation

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ABSTRACT

To study the hepatoprotective effect on ethnaolic extracts of *Commiphora* species and its formulation on $CCl_4$ induced severe liver damage in Wistar Albino Rats. *Commiphora berryi*, C. *caudata, C. pubescens* are indigenous species in South India has wide distribution. The activity was assessed by monitoring the various liver biomarkers viz., (AST/SGPT), (ALT/SGOT), (ALP), (ACP) and total bilirubin and liver was reported by histopathological sections. The Commiphora species recruited the normal value by declined the $CCl_4$ intoxication of hepatic damage, hyperplasia and fatty changes. From the results, formulation showed significant ($p<0.001$) activity and given the scientific evidences in ethanolic extracts and its formulation.

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## 1. Introduction

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way medicinal plants play significant role of an economy of a country. Phytoconstituents are the main effective compounds of medicinal plants. They have various pharmacological activity used for the treatment of various diseases. At present thousands of phytoconstituents is being successfully used in the treatment of variety of diseases. According to an estimate 80% of the world's population relies upon plant for their medication [1]. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparation, for example vincristine (an anti-tumor drug), digitalis (a heart regulator) and ephedrine (A bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. Phytoconstituents have been used as drugs for millennia. For example; Hippocrates in 400BC used to prescribe willow tree and later synthetically produced to become the staple over the counter drug called aspirin. Number of chemical tests was available for the identification and quantification of phytoconstituents. The plant material is subjected to preliminary phytochemical screening for the detection of various plant constituents. The present study was conducted to evaluate the hepatoprotective activity of the ethanolic extract of the *Commiphora* species by using $CCl_4$-induced hepatic injury in rats.

## 2.Materials and Methods

### *2.1.Selection of plants*

Based on the traditional uses reported on the literature review, the following plants of the same genus were selected for the study.

*Commiphora berryi* (Arn) Engl (Fam: Burceraceae)
*Commiphora caudata* (Wight & Arn) Engl (Fam: Burceraceae)
*Commiphora pubescens* (Wight & Arn) Engl (Fam: Burceraceae)

### *2.2.Plant Collections and Authentications*

The bark of *Commiphora berryi* which has been predominantly grown in arid places was procured from Tirunelveli and Pudhukottai district of Tamil Nadu; leaves *Commiphora caudata* was grown in arid places were procured from Tuticorin and Perambalur district of Tamil Nadu; leaves of *Commiphora pubescens* was grown in arid places were procured from Pudukottai district in Tamil Nadu. The freshly collected plants were then authenticated by the botanists of Botanical Survey of India, Coimbatore, Tamil Nadu, India and a voucher specimen of all the plants were kept in our laboratory, Department of Pharmaceutical Technology, Anna university Tiruchirappalli, Tiruchirappalli, Tamil Nadu. The specimens voucher number: *C. berryi*-BSI/SC/5/23/07-08/Tech.669., *C. caudata*-BSI/SC/5/23/06-07/Tech.1821., and *C. pubescens*-BSI/SC/5/23/06-07/Tech.1664.

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*2.3.Garbling process*

Garbling refers to the separation of particular portion of the plant to be used from other parts of the plants, like dirt and other extraneous matter. This step is often done during the collection process. Usually it is performed by hand. After removing all such unwanted adhered materials; the required plant parts were then spread over trays and dried under shade, with regular sifting of collected plant materials everyday in order to avoid fungal growth. Such shade dried bark/leaves/aerial portions of plant were ground to powder and then it was subjected for extraction.

*2.4. Preparation of Plant Extracts*

The collected plant was dried under shade and the dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (1 kg) was extracted with petroleum ether thrice to remove the fatty material and further plant material was extracted thrice with ethanol (99.9%, v/v). The extracts were filtered, pooled and concentrated at reduced temperature (5ºC) on a rotary evaporator (Buchi, USA) and then freeze dried (Freezone 4.5, Labconco, USA) at high vacuum and at temperature 40±2ºC (yield 6.12%, w/w). Preliminary qualitative phytochemical screening of EECB, EECC and EECP has given the positive testes for, alkaloids, glycosides, flavonoids, saponins, carbohydrates, protein, amino acids, lipids, steroids, and tannins [2]. The above mentioned extracts and its formulation were then screened for their pharmacological activities.

*2.5. Polyherbal capsule formulation presented in Table 1.*

Table 1. Ingredients of polyherbal formulation

| Ingredients | Weight taken in mg | Concentration (%) |
|---|---|---|
| *Commiphora Caudata* | 91.25 | 25 |
| *Commiphora Berryi* | 91.25 | 25 |
| *Commiphora Pubescens* | 91.25 | 25 |
| Lactose | 73.0 | 20 |
| Magnesium Stearate | 18.25 | 5 |
| Total | 365.0 | 100% |

*2.6. Preparation of granules*

Extracts were dried and made a solid form; ground to a sticky powder. The powder weighed and mixed with sufficient quantity of lactose (diluent) for increasing the bulk of the preparation. The bulk powder material passed through sieve (6-12) for coarse screening to form granules. These granules were dried and screening a suitable sieve (14-20) to form fine granules. The granules were mixed with disintegrating agent (Magnesium stearate). Finally the granules were filled in the capsules as 300 mg/capsule.

*2.7. Phytochemical Screening*

The ethanolic extracts and its formulation of *Commiphora* species of the leaves of the plant was subjected to various chemical tests in order to determine the secondary plant constituents presents by employing the use of various methods as follows:

*2.8.Test for Reducing Sugars*

To 2 ml of the extract, 5ml of a mixture (1:1) of Fehling's solution IA and Fehling's solution II (B) was added and the mixture boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars [3].

*2.9. Test for the presence of anthraquinones*

0.5g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10 percent ammonia solution added to the filtrate. The mixture was shaken; the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones [4].

*2.10.Test for Saponins*

0.5g of the extract was dissolved in a 10 ml of distilled water in a test-tube, the test tube was stopperred with a cork and shaken vigorously for 30 seconds and then allowed to stand for 45 minutes. The appearance of frothing which persists on warming indicated the presence of saponins [4].

*2.11.Test for Flavonoids*

To a portion of the dissolved extract, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus [3].

*2.12.Test for Steroids/Terpenes*

0.5g of the extract was dissolved and 2 ml of acetic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A color change from violet to blue to green indicated the presence of a steroidal nucleus [4].

*2.13.Test for Tannins*

0.5 g of the extract was dissolved in 5 ml of water followed by a few drops of 10 % ferric chloride. A blue-black, green, or blue-green precipitate would indicate the presence of tannins [4].

*2.14.Test for Alkaloids*

0.5g of ethanol extract was stirred with 5ml of 1 percent aqueous hydrochloric acid on a stem bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitation with either of these reagents would indicate the presence of alkaloids in the extracts [4].

*2.15.Test for resins*

10ml of petroleum ether extract was obtained in a test-tube, the same amount of cupper acetate solution was added and the mixture was shaken vigorously and allowed to separate, a green colour indicates the presence of resin [5].

*2.16. Experimental animals*

Healthy young adult animals of commonly used laboratory strains should be employed. Females (180-200g) should be non-pregnant; at the commencement of its dosing, each animal should be between 8 and 12 weeks old and its weight should fall in an interval within ±20% of the mean initial weight of any previously dosed animals.

The temperature in the experimental animal room should be 22ºC (±3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. The animals are housed individually, for feeding, conventional rodent laboratory diets may be used with an unlimited supply of drinking water.

Wistar rats were obtained from the approved breeder of the animal house of Laboratory animal medicine, Veterinary University, Madhavaram, Chennai and King Institute, Guindy, Chennai, Tamil Nadu, India, with the proof of the CPSCEA acknowledgement.

2.17. CCl4 induced hepatotoxic activity on *Commiphora* species and its capsule formulation

Rats were divided into six groups (n=6). Group I (control) animals were administered a single dose of water (1 mL/kg/p.o.) daily for 7 days and received liquid paraffin (1 mL/kg/i.p.,) on day 2 and 3. Group II ($CCl_4$) received water (1 mL/kg body weight, i.p.,) once daily for 7 days and received CCl4: liquid paraffin (1:1, 2 mL/kg body weight, i.p.) on day 2 and 3. Group III received standard drug silymarin (50 mg/kg/i.p.,) once daily for 7 days. Test groups of (Group IV, V, VI, VII, VIII & IX) were administered orally as *C. berryi, C. caudata* & *C. pubescens* at the dose of (100 & 200 mg/kg/p.o.,) respectively once daily. The X group was administered orally a dose of 300mg/kg of polyherbal capsule formulation, in the form of suspension with NaCl once daily. The Groups IIX animals were administered simultaneously $CCl_4$: liquid paraffin (1:1, 2 mL/kg body weight, i.p.) on day 2 and 3 after 30 min of administration of the silymarin, formulation and extracts. Animals were sacrificed 24 h after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out further the liver was dissected out and used for histopathological studies [6].

*2.18. Histopathological studies*

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H-E) dye for photo microscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration.

*2.19. Statistical analysis*

The data are expressed as mean±SD. The difference among means has been analyzed by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant.

## 3.Results

## 4.Discussion

In the present study ethanolic extracts of *Commiphora berryi, C. caudata* and *C. pubescens* and its formulation were evaluated for the hepatoprotective activity by using hepatotoxic induced $CCl_4$ in Wistar Albino rat model and find out the therapeutically better efficacious extracts and its formulation. This study given the scientific evidences on effect of ethanolic extracts and its formulation constituting chemical compounds. The phytochemical screening showed (Table 2.) that the presence of alkaloids, amino acids, flavonoids, glycosides, proteins, reducing sugars, starch, steroids, tannins, terpenoids.

Liver damage was assessed by biochemical parameters (SGPT, SGOT, ALP, ACP and Total bilirubin) and by Histopathological examinations in liver. $CCl_4$ produces an experimental damage that histologically resembles viral hepatitis [7]. Administration of $CCl_4$ led to increase the serum enzymes level by 2-3 folds as compared to control group. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [8]. CCl4 induces fatty liver and cell necrosis [9], being cytoplasmic in location [10]. The damage marker enzymes SGOT, SGPT, ALP, ACP and Total bilirubin are released in serum [11].

Table 2. Phytochemical screening on EECB, EECC and EECP and its formulated capsule

| Ingredients | ECCB | EECC | EECP | Capsule formulation |
|---|---|---|---|---|
| Alkaloids | - | - | + | + |
| Amino Acids | - | + | - | + |
| Anthraquinones | - | - | - | - |
| Flavonoids | + | + | - | + |
| Glycosides | + | + | + | + |
| Gums and Mucilage | - | - | - | - |
| Proteins | - | + | - | + |
| Reducing Sugars | + | + | – | + |
| Saponins | - | - | - | - |
| Starch | + | + | + | + |
| Steroids | + | + | + | + |
| Tannins | + | + | - | + |
| Terpenoids | + | + | - | + |

Table 3. Biochemical changes on hepatotoxic activity treated upon Silymarin, EECB, EECC, EECP, Formulated capsule

| Groups | Total bilirubin (mg/dL) | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | ACP (IU/L) |
|---|---|---|---|---|---|
| Negative control | 0.28±0.11 | 49.33±4.76 | 38.05±2.95 | 134.97±8.59 | 1.23±0.04 |
| Positive control | 1.81±0.24ns | $185.16±9.66^{ns}$ | $129.5±5.75^{ns}$ | $252.49±2.62^{ns}$ | $3.90±0.61^{ns}$ |
| Silymarin (50 mg/kg) | $1.26±0.16^{b}$ | $58.83±4.02^{b}$ | $49.46±2.16^{b}$ | $148.18±2.39^{c}$ | $2.43±0.38^{a}$ |
| EECB (100 mg/kg) | $0.53±0.10^{a}$ | $62.50±4.13^{a}$ | $57.11±3.09^{b}$ | $137.64±1.82^{a}$ | $1.41±0.05^{c}$ |
| EECB (200 mg/kg) | $0.39±0.22^{a}$ | $52.66±4.50^{a}$ | $41.25±1.27^{a}$ | $151.04±4.08^{a}$ | $1.29±0.03^{b}$ |
| EECC (100 mg/kg) | $0.95±0.24^{b}$ | $128.08±8.42^{b}$ | $82.1±1.52^{c}$ | $143.12±4.96^{b}$ | $2.12±0.05^{d}$ |
| EECC (200 mg/kg) | $0.71±0.17^{c}$ | $89.36±6.40^{b}$ | $64.35±1.13^{b}$ | $168.68±6.20^{a}$ | $1.85±0.10^{c}$ |
| EECP (100 mg/kg) | $1.73±0.38^{e}$ | $146.31±6.72^{d}$ | $106.48±1.10^{ns}$ | $173.81±2.48^{e}$ | $2.57±0.19^{ns}$ |
| EECP (200 mg/kg) | $1.28±0.23^{c}$ | $113.10±7.24^{c}$ | $92.61±1.15^{d}$ | $159.38±4.56^{d}$ | $2.38±0.10^{e}$ |
| Formulated capsule (300 mg/kg) | $0.25±0.19^{a}$ | $55.18±5.30^{a}$ | $46.33±1.57^{a}$ | $141.25±2.48^{b}$ | $1.32±0.03^{a}$ |

Values are means±SD of the six numbers of animals. Rats were used for study with EECB, EECC, EECP (100 & 200 mg/kg/p.o.) and its formulation (300 mg/kg) of drug administration.
[a]p< 0.001, [b]p< 0.01, [c, d, e]p< 0.05, [ns]p<0.1 vs. control group with one way analysis i.e., DMRT

As shown in (Table. 3) activities of serum GPT, GOT, ACP, ALP and total bilirubin markedly elevated while CCl4 treated animals comparable to normal control rats. Administration of different plant ethanolic extracts of C. berryi, C. caudata and C. pubescens at dose of (100 and 200 mg/kg), Silymarin (50 mg/kg) and formulation (300 mg/kg) prevented $CCl_4$ intoxication of elevated biochemical parameters. The ethanolic extracts and its formulation decreased as revert the intoxication of the serum GOT, GPT, ALP, ACP and total bilirubin by value of significance at p<0.001, 0.01, 0.05, respectively; in dose dependent manner. Since extracts of *Commiphora* species and its formulation do not produce any gross behavioral changes or mortality even at a dose of 200 mg/kg. P.O. in rats as also reported in the literature [12]. In case on the other hand, the percentage of hepatoprotectivity of *Commiphora* species and its formulation deliberately having significant activity in different proportions. It has been, therefore, found that the different extracts of *Commiphora* species have varied degrees of antihepatotoxic activity.

*3. Histopathological observations*

Histopathology of the liver sections showed in (Figure. 1), Group I as negative control group (Figure. 1.1.) seems to the normal hepatic cells with well preserved cytoplasm, prominent nucleus and visible central veins.

Figure 1. Histopathological sections of Liver treated upon Silymarin, EECB, EECC, EECP, Formulated capsule

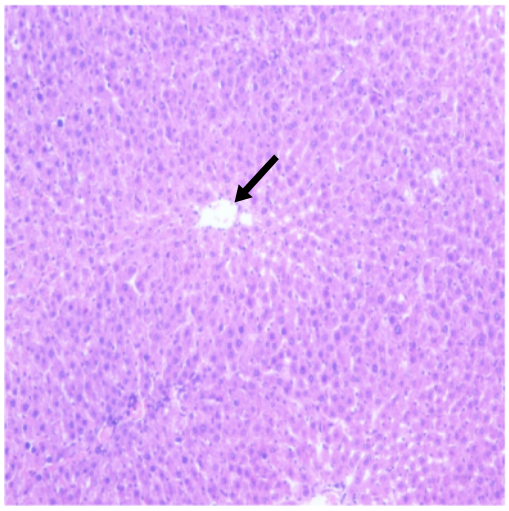

Figure. 1.1. Negative Control (Sections shows liver tissue. There is dilations of sinusoids, kupffer cell hyperplasia and mild periportal inflammation. (Hematoxycylin and Eosin 10X))

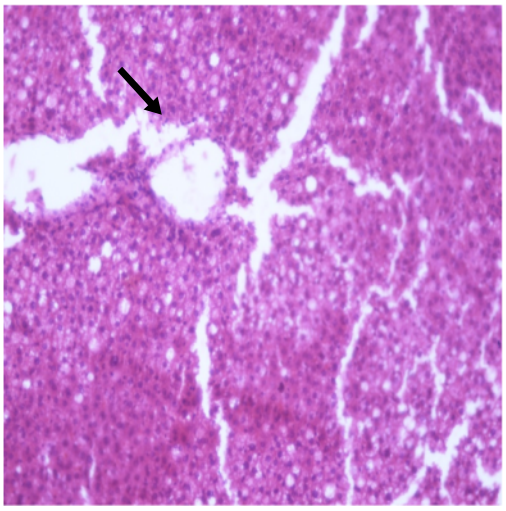

Figure. 1.2. Positive Control (Sections shows liver tissue with altered architecture. There is loss of hepatic plate formation, with replacement by macro vesicular fatty vacuoles. The sinusoids showed that congestion and perivascular inflammation (Hematoxycylin and Eosin 10X))

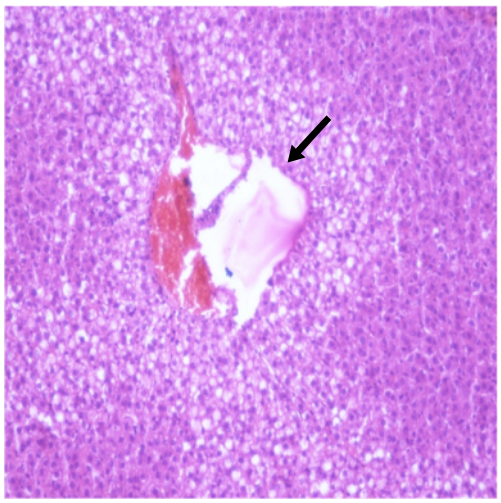

Figure. 1.3. Silymarin 50 mg/kg (Sections shows that liver tissue with fatty change in the periportal foci, dilatations of sinusoids and kupffer cell hyperplasia. (Hematoxycylin and Eosin 20X))

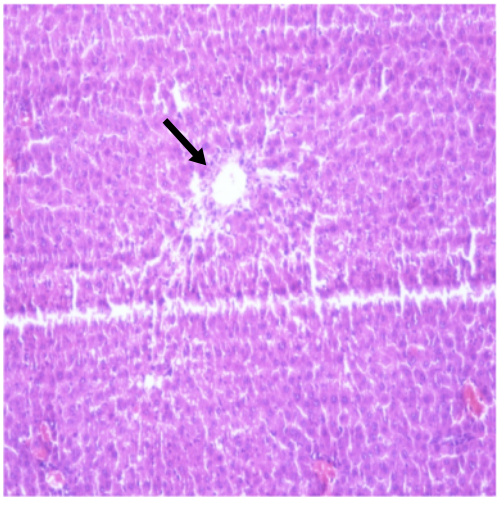

Figure. 1.4. EECB 100 mg/kg (Sections showed that early fatty change, with fatty vacuoles in the subcapsular and perivascular areas. There is mild congestion. (Hematoxycylin and Eosin 10X))

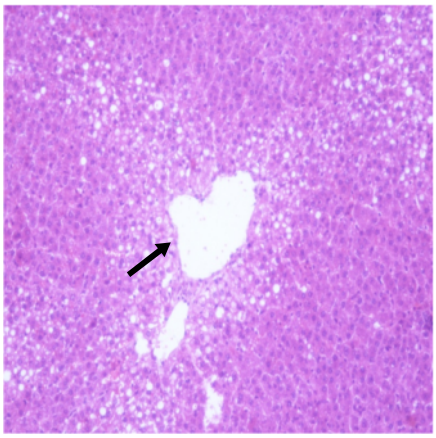

Figure. 1.5. EECB 200 mg/kg (Sections showed that liver tissue within normal limits and showed that normal hepatic vein and sinusoids. There was no dilation was not found. (Hematoxycylin

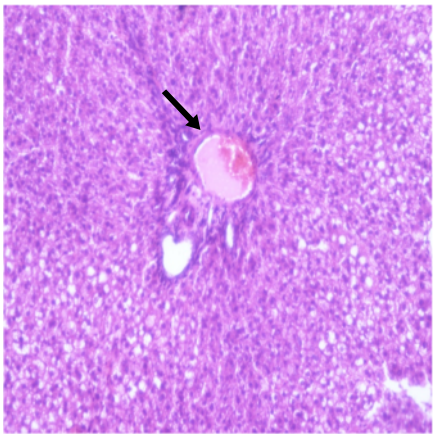

Figure. 1.6. EECC 100 mg/kg (Sections showed that liver tissue with micro vesicular fatty change, periportal inflammation and sinusoidal congestion(Hematoxycylin and Eosin 10X))

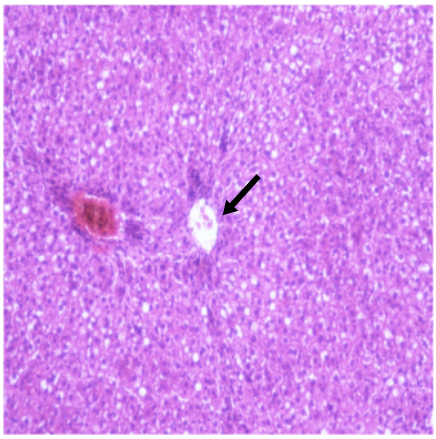

Figure. 1.7. EECC 200 mg/kg (Sections showed that liver tissue with micro vesicular fatty change, dilation of sinusoids and central vein and mild kupffer cell hyperplasia (Hematoxycylin and Eosin

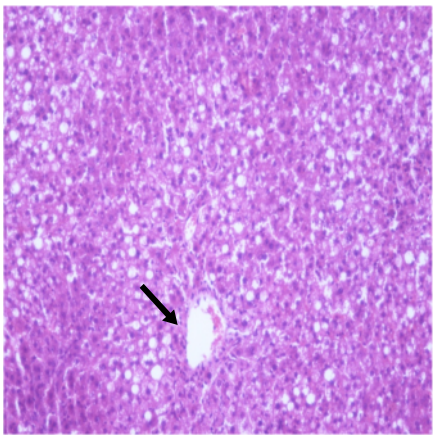

Figure. 1.8. EECP 100 mg/kg (Sections deliberately produced the action in liver tissue with micro vesicular fatty change. There is kupffer cells hyperplasia, periportal inflammation, dilatation of sinusoids and central veins and congestion. (Hematoxycylin and Eosin 10X))

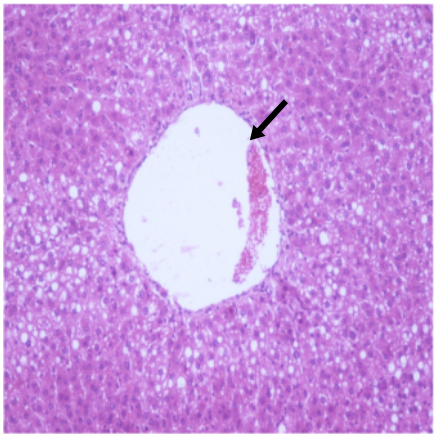

Figure. 1.9. EECP 200 mg/kg (Sections showed that liver tissue with micro vesicular fatty change foci of sinusoidal and central venular dilatation, periportal inflammation and cholestasis in the

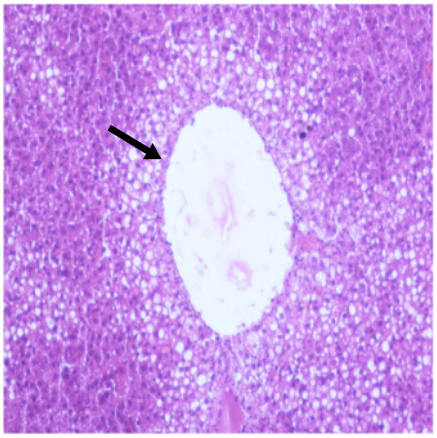

Figure. 1.10. Formulated Capsule 300 mg/kg (Sections showed that liver tissue with fatty change of periportal zones, dilation of sinusoids and congestion. (Hematoxycylin and Eosin 20X))

The liver sections of $CCl_4$ (Figure. 1.2) intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration, tissue cell damage, broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological architecture of the EECB (100 & 200 mg/kg), EECC (100 & 200 mg/kg), EECP (100 & 200 mg/kg) treated liver sections of the rats showed in (Figure.1.4.-1.9), more or less normal lobular pattern with a mild degree of fatty change, no necrosis almost comparable to the negative control and Silymarin (50 mg/kg) treated groups. In scientific the ethanolic extracts of the liver was recruited from the liver damage of $CCl_4$ intoxicated, further the formulated capsule (Figure. 1.10) exhaustively recover the liver damage from the $CCl_4$ intoxication. In conclusion, extracts of the Commiphora species and its formulation showed in the studies preserved that the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in $CCl_4$ treated rats.

## 5.Conclusion

The above observations have shown that the different extracts of the *Commiphora* species and its formulation contain some active principles effect on hepatotoxicity. Mean while, the test drug as *Commiphora* species and its formulation very in close proximity to to standard drug silymarin avail in market; showed that the hepatoprotectivity. The isolation and testing of constituents likely to be responsible for the hepatoprotective activity of *Commiphora* species and its formulation is in progress in the laboratory. It could be added to the normal flora of the Indian pharmacopeia by its pharmacological actions.

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