ORIGINAL ARTICLE

Anti-inflammatory and hepatoprotective activities of the roots of *Uraria picta*

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Abstract

Introduction: *Uraria picta* (Jacq.) commonly known as *Prishnaparni*, is one of the important ingredients of the 10 herb formulation called "*Dashmula*," used for the treatment of fever and inflammation. **Materials and Methods:** This study was conducted to investigate the anti-inflammatory and hepatoprotective activities of methanol extract of the roots of *U. picta* (UPME) at a dose of 100, 200 and 400 mg/kg, p.o. in experimental models of rats. Anti-inflammatory activity of methanol extract was performed by egg albumin-induced and formalin induced rats paw edema. Paracetamol (PCM)-induced liver injury model was used to explore the hepatoprotective activity of UPME. **Results:** Methanolic roots extract showed significant activity against both models of inflammation. UPME (400, 200, and 100 mg/kg, p.o.) reduced inflammation in egg albumin and formalin treated in dose-dependent manner. Administration of PCM 2000 mg/kg induced liver injury in rats, and therefore, increased the level of enzymes alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) in the blood. Administration of UPME 400, 200 and 100 decreased the level of enzymes ALT, ALP and AST significantly which were found comparable with the standard drug silymarin 100 mg/kg. **Conclusion:** This study demonstrated the ability of *U. picta* to exert anti-inflammatory and hepatoprotective effects.

Key words: Anti-inflammatory, hepatoprotective, *Uraria picta*

INTRODUCTION

Uraria picta (Jacq.) usually known as *Prishnaparni* belonging to the family Fabaceae, is one of the important ingredients of 10 herb formulation called *Dashmula*.[1] *U. picta* is reported to be a perennial erect woody herb[2-7] reaching about 1-2.5 m height. *U. picta*, though widely distributed throughout the India,[8] is increasingly becoming rare and endemic.[4] Apart from India, *U. picta* is also reported from different parts of Africa (Nigeria, Egypt, Ethiopia, Congo, and South Africa),[9] China, Japan, Bangladesh, Pakistan, Bhutan, Nepal and Australia.[10]

The herb *U. picta* is an ingredient of *Dashmula* used for the treatment of fever and inflammation.[11] The plant is used as an antidote for the snake venom at Bastar (Madhya Pradesh, India).[12] Its leaves are used as antiseptic. The fruits and pods are used for the treatment of oral sores in children. Roots of *U. picta* are used for the treatment of cough, chills, and fever.[5] This plant is also known for healing the fractures, as it has the property of accumulation of phosphorous and deposition of calcium. Dried leaf powder is used in the treatment of gonorrhea and for contractions of the uterus leading to abortion.[13] Alcoholic and aqueous extracts of the woody root contain an active aphrodisiac ingredient used in Nigeria and Guinea-Bissau.[14,15] In Ghana, the plant is employed for treating heart trouble. The roots and pods are employed in Bihar, India to treat prolapsed anus in infants. It is used for the treatment of urinary diseases, tumors, edema, burning sensation, and difficulty in breathing.[16] A decoction of leaves is prescribed in Malaysia for diarrhea. The species *Uraria* is increasingly becoming rare due to overexploitation by various pharmaceutical industries as well as local tribes for medicine and trade purpose coupled with poor seed viability.[4]

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Phytochemical investigation on *U. picta* led to the isolation of stigmasta-4, 22-diene-3-one, *β*-sitosterol,[17] lupeol,[18] 12-ole-anene-3b, 22b-diol[19] isoflavanones 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone, 5,7,4'-trihydroxy-2',3'-dimethoxyisoflavanone, 4',5,-dihydroxy-2',3'-dimethoxy-7-(5-hydroxyoxychromen-7yl)-isoflavanone, 4',5,7-trihydroxy-2'-methoxyisoflavanone (isoferreirin),[20] 2',4',5,7-tetrahydroxy-6-(3-methylbut-2-enyl) isoflavanone, and 2',4',5,7-tetrahydroxyisoflavanone.[21]

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g., bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair.[22] Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury.[23] The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection.[24] However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease.[25] Inflammation is a major component of the damage caused by autoimmune diseases and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever.[26,27] Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide, prostaglandin E2, and tumor necrosis factor α,[28,29] that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site.[30] In addition, it is broadly accepted that cytokines, produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors.[31] More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues."[32]

Since *U. picta* is an important ingredient of *Dashmula*, used for the treatment of inflammatory diseases and fever, this study was aimed to explore the anti-inflammatory and hepatoprotective[5] activities of methanolic root extract of *U. picta* (UPME).

Chemical and Reagents

Paracetamol (PCM) 500 mg I.P. tab (GlaxoSmithkline, India), silymarin tab (Ranbaxy, India), serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase (ALP) were procured from Ayush Diagnostic Centre, Varanasi, Uttar Pradesh, India.

MATERIALS AND METHODS

Collection of Plant Materials

Roots of *U. picta* were collected in the month of September 2015 from herbal garden of Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh. Taxonomical identification and authentication of the roots of *U. picta* were done by Professor A. K. Singh, Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University. A voucher specimen (APRIL/HERB/15/16/32) of the roots of *U. picta* has been placed in Ayurvedic Pharmacy Research Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh.

Preparation of Extract

Dried roots were pulverized in the form of coarse powder with the help of mechanical grinder and passed through sieve 20#. The grounded powder (1 kg) was exhaustively extracted with 3.0 L methanol in a soxhlet apparatus for 72 h. The extract was filtered and the filtrate obtained was evaporated under reduced pressure to obtain the dried UPME (80 g) which was finally stored in a desiccator to dryness.

Animals

Healthy adult Charles foster albino rats (160 ± 20 g) of either sex were used for this study. Animals were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in large polypropylene cages in a temperature controlled room (22 ± 2°C). Humidity (55 ± 10%) was maintained properly and 12 h light and 12 h dark cycle was also followed. The animal was provided with standardized pelleted feed (Amrut Pvt. Ltd.) and clean drinking water. Rats were acclimatized to the standard laboratory condition for at least 1 week before using the experiment. Body weight of rats measured regularly. Principle of Laboratory Animal Care Guidelines (NIH publication number 85-23, revised 1923) was followed. The study has got the clearance from the Institutional Animal Ethical Committee (IAEC) for the Purpose of Control and Supervision of Experiments on Animals (Dean/2015/CAEC/1430).

Acute Toxicity Study

The acute oral toxicity study of UPME was performed according to the Organization for Economic Cooperation

and Development - 425 guidelines. Single dose of UPME 2000 mg/kg, p.o., was administered in 24 h fasted rats ($n = 5$) and rats were observed at 0, 30, 60, 120, 180, and 240 min and then once a day for the next 14 days for any signs or symptoms of toxicity or abnormalities. The number of rats that survived at the end of the study period was recorded.[33]

Anti-inflammatory Activity

Egg albumin-induced hind paw edema

Adult rats were divided into six groups of six animals in each group. Test and standard drugs were suspended in 0.5% carboxy methyl cellulose.

- Group 1 (Normal group) - Received vehicle only.
- Group 2 (Negative control treated group, NC) - Egg albumin 0.1 ml injected in sub planter region.
- Group 3 (Standard drug treated group - indomethacin [IND]) – Administered with IND (10 mg/kg, p.o.).
- Group 4 (UPME - 400) - Administered with UPME (400 mg/kg, p.o. body weight) 30 min before the administration of egg albumin 0.1 mL injected in sub planter region.
- Group 5 (UPME - 200) - Administered with UPME (200 mg/kg, p.o. body weight) 30 min before the administration of egg albumin 0.1 ml injected in sub planter region.
- Group 6 (UPME - 100) - Administered with UPME (100 mg/kg, p.o. body weight) 30 min before the administration of egg albumin 0.1 ml injected in sub planter region.

Paw volume was measured plethysmometer before and at 30, 60, 90, 120, 150, 180, 210, 240 min after induction of inflammation. The level of inhibition of edema was calculated using the formula:[34,35]

Inhibition (%) = $100(1-(a-x/b-y))$

Where, a = Mean paw volume of treated animals after egg albumin injection,
x = Mean paw volume of treated animals before egg albumin injection,
b = Mean paw volume of control animals after egg albumin injection,
y = Mean paw volume of control animals before egg albumin injection.

Formalin Induced Hind Paw Edema

Adult rats were divided into six groups of six animals in each group. Test and standard drugs were suspended in 0.5% carboxy methyl cellulose.

- Group 1 (Normal group) - Received vehicle.
- Group 2 (Negative control group, NC) - 0.2 ml of 1% (w/v) formaldehyde injected in sub planter region.
- Group 3 (Standard drug treated group, IND) - Administered with standard drug IND (10 mg/kg, p.o.).
- Group 4 (UPME - 400) - Administered with UPME (400 mg/kg, p.o. body weight) 30 min before the administration of 0.2 ml of 1% (w/v) formalin injected in sub planter region.
- Group 5 (UPME - 200) - Administered with UPME (200 mg/kg, p.o. body weight) 30 min before the administration of 0.2 ml of 1% (w/v) formalin injected in sub planter region.
- Group 6 (UPME - 100) - Administered with UPME (100 mg/kg, p.o. body weight) 30 min before the administration of 0.2 ml of 1% (w/v) formalin injected in sub planter region.

Paw volume was measured before and at 30, 60, 90, 120, 150, 180, 210 and 240 min after induction of inflammation. The level of inhibition of edema was calculated for each extract using the relation.

Inhibition (%) = $100(1-(a-x/b-y))$.

Where, a = Mean paw volume of treated animals after formalin injection,
x = Mean paw volume of treated animals before formalin injection,
b = Mean paw volume of control animals after formalin injection,
y = Mean paw volume of control animals before formalin injection.

Hepatoprotective Activity

Adult rats were divided into six groups of six animals in each group. Test and standard drugs were suspended in 0.5% carboxy methyl cellulose.

- Group 1 (Normal group) - Received vehicle only for 7 consecutive days.
- Group 2 (Negative control group, PCM) - Received PCM (2000 mg/kg, p.o.) on 5th, 6th, 7th day.
- Group 3 (Standard drug treated group) - Administered with silymarin (100 mg/kg body weight p.o.) for 7 consecutive days and PCM (2000 mg/kg body weight, p.o) on 5th, 6th and 7th days.
- Group 4 (UPME - 400) - Administered with UPME (400 mg/kg, p.o.) for 7 consecutive days and PCM (2000 mg/kg body weight, p.o) on 5th, 6th and 7th days.
- Group 5 (UPME - 200) - Administered with UPME (400 mg/kg, p.o.) for 7 consecutive days and PCM (2000 mg/kg body weight, p.o) on 5th, 6th and 7th days.
- Group 6 (UPME - 100) - Administered with UPME (100 mg/kg, p.o.) for 7 consecutive days and PCM (2000 mg/kg body weight, p.o) on 5th, 6th and 7th days.

After 7 days, all the animals were sacrificed and blood samples as well as liver collected.[36]

Collection of Serum and Tissue Samples

After 24 h of the administration of the last dosage, the rats were anesthetized using ether and blood sample were collected by cardiac puncture method in a heparinized 1 ml tuberculin syringe and allowed to clot at 37°C for 30 min and serum separated by centrifugation at 2500 rpm for 10 min[37,38] for the estimation of biochemical parameters. Liver tissue samples were preserved in formalin solution for histopathological analysis.

Statistical Analysis

All results are expressed as mean ± standard error of mean (n = 6). Statistical comparison was determined by one-way ANOVA followed by the Tukey's multiple comparison tests for anti-inflammatory activity and Dunett's multiple comparison test using graph prism software version 5.01.

RESULTS

UPME at the dose of 2000 mg/kg, p.o., did not exhibit any behavioral changes or symptoms of toxicity. Hence, the drug was found to be safe up to the tested dose of 2000 mg/kg, p.o.

UPME showed significant activity against both model of inflammation [Tables 1 and 2]. UPME (400, 200 and 100 mg/kg, p.o.) reduced inflammation in egg albumin and formalin treated animals in dose-dependent manner. The paw volume in egg albumin induced paw edema of standard, UPME - 400, UPME - 200 and UPME - 100 at 210 min was found to be 0.105 ± 0.012, 0.100 ± 0.012, 0.150 ± 0.012 and 0.225 ± 0.012 ml, respectively. Paw volume in formalin induced animal model for standard, UPME - 400, UPME - 200 and UPME - 100 at 210 min was found to be 0.105 ± 0.012, 0.100 ± 0.012, 0.151 ± 0.012 and 0.225 ± 0.012 ml, respectively. The significant reduction in paw volume of UPME (400, 200, 100 mg/kg) treated animals indicates that the *U. picta* has excellent contribution as an anti-inflammatory agent.

Administration of PCM 2000 mg/kg induced liver injury in rats and therefore increased the level of enzymes alanine transaminase (ALT), ALP, and aspartate aminotransferase (AST) in the blood. Administration of UPME - 400, UPME - 200 and UPME - 100 decreased the level of enzymes ALT, ALP and AST significantly which were found comparable with the standard drug silymarin 100 mg/kg. The levels of AST, ALT, and ALP of UPME - 400 treated animals were found to be 404.17 ±27.9, 151.50 ±21.65, and 57.170 ± 8.04 IU/L, respectively, which was found comparable to the standard drug silymarin (100 mg/kg). An obvious significant enhancement in the activities of ALT, AST and ALP enzymes was recorded in UPME (100-200 mg/kg p.o.) groups [Figures 1 and 2].

Table 1: Anti-inflammatory activity of the UPME and IND (standard drug) on egg albumin induced paw edema in albino rats

Treatment	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
NC	0.361±0.013	0.390±0.006	0.390±0.006	0.428±0.011	0.42±0.022	0.46±0.017	0.475±0.012	0.489±0.012
IND	0.130±0.009[a]	0.160±0.012[a]	0.160±0.012[a]	0.160±0.012[a]	0.21±0.015[a]	0.107±0.013[a]	0.105±0.01[a]	0.125±0.012[a]
UPME - 400	0.116±0.008[ab]	0.150±0.004[ab]	0.150±0.004[ab]	0.140±0.0125[ab]	0.19±0.015[ab]	0.102±0.008[ab]	0.100±0.01[ab]	0.110±0.012[ab]
UPME - 200	0.172±0.008[ab]	0.190±0.012[ab]	0.190±0.012[ab]	0.190±0.0128[ab]	0.260±0.014[ab]	0.155±0.011[ab]	0.150±0.01[ab]	0.160±0.012[ab]
UPME - 100	0.291±0.290[ac]	0.290±0.014[ac]	0.290±0.014[ac]	0.320±0.011[ac]	0.330±0.014[ac]	0.22±0.009[ac]	0.225±0.01[ac]	0.222±0.011[ac]

Values are expressed as mean±SEM (n=6). Statistical comparison was analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. [a]P<0.05, statistically significant as compared to negative control; [b]P<0.05, statistically significant as compared to UPME (100 mg/kg, p.o.). [c]P<0.05, statistically significant as compared to IND (10 mg/kg, i.p.). SEM: Standard error of mean, UPME: Methanol extract of the roots of *Uraria picta*, IND: Indomethacin

Table 2: Anti-inflammatory activity of the UPME and IND (standard drug) on formalin induced paw edema in albino rats

Treatment	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
NC	0.275±0.008	0.36±0.012	0.387±0.011	0.420±0.011	0.41±0.017	0.4610±0.013	0.475±0.012	0.489±0.012
IND	0.163±0.007[a]	0.13±0.012[a]	0.160±0.012[a]	0.140±0.013[a]	0.21±0.013[a]	0.107±0.012[a]	0.105±0.012[a]	0.125±0.012[a]
UPME - 400	0.161±0.009[ab]	0.112±0.013[ab]	0.140±0.012[ab]	0.125±0.012[ab]	0.20±0.011[ab]	0.102±0.008[ab]	0.100±0.012[ab]	0.110±0.012[ab]
UPME - 200	0.179±0.008[ab]	0.170±0.01[ab]	0.197±0.011[ab]	0.190±0.013[ab]	0.254±0.009[ab]	0.155±0.011[ab]	0.151±0.012[ab]	0.160±0.012[ab]
UPME - 100	0.247±0.0131[ac]	0.29±0.014[ac]	0.320±0.019[ac]	0.360±0.012[ac]	0.341±0.009[ac]	0.220±0.009[ac]	0.225±0.012[ac]	0.222±0.01[ac]

Values are expressed as mean±SEM (n=6). Statistical comparison was analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. [a]P<0.05, statistically significant as compared to negative control; [b]P<0.05, statistically significant as compared to UPME (100 mg/kg, p.o.). [c]P<0.05, statistically significant as compared to IND (10 mg/kg, i.p.). SEM: Standard error of mean, UPME: Methanol extract of the roots of *Uraria picta*, IND: Indomethacin

DISCUSSION

This study was conducted to evaluate the anti-inflammatory and hepatoprotective activities of UPME at a dose of 100, 200 and 400 mg/kg, p.o. in experimental rat models. Anti-inflammatory activity of methanol extract was performed by egg albumin-induced[34] and formalin induced rats paw edema.[39] Methanolic roots extract showed significant activity against both models of inflammation. UPME (400, 200 and 100 mg/kg, p.o.) reduced inflammation in egg albumin and formalin treated in dose-dependent manner. Egg albumin used as a phlogistic agent causes edema in rats hind paw. Egg albumin induced hind paw edema methods are suitable for screening agents for anti-inflammatory activity which are frequently used to assess the anti-edematous effect of natural product.[40,41] Several inflammatory mediators such as histamine, prostaglandins, kinin and pro inflammatory cytokinins have been suggested to play a role in the mechanism of inflammation.[42,43] Egg albumin act significantly on the mast cells. Edema induced by it appears to be mediated by release of histamine and serotonin. The paw volume in egg albumin induced paw edema in animals treated with standard drug, UPME - 400, UPME - 200 and UPME - 100 at 210 min was found to be 0.105 ± 0.012, 0.100 ± 0.012, 0.150 ± 0.012 and 0.225 ± 0.012 ml, respectively. Paw volume in formalin induced model treated with standard, UPME - 400, UPME - 200 and UPME - 100 at 210 min was found to be 0.105 ± 0.012, 0.100 ± 0.012, 0.151 ± 0.012 and 0.225 ± 0.012 ml, respectively. The reduction of paw volume for UPME (400, 200, 100 mg/kg) indicates that the *U. picta* has excellent contribution as an anti-inflammatory agent.

PCM-induced liver injury model is a conventional model used to explore the hepatoprotective activity of extracts.[44] Administration of PCM (200 mg/kg) to the animals increased the level of enzymes ALT, ALP and AST in the blood. Histopathological observation of PCM-treated liver tissues revealed severe destruction of the liver normal architecture accompanied by reduction in the number of viable cells. Various mechanisms may be linked to the damage of the liver by different toxins. For example, PCM, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinoneimine, a toxic metabolite, by cytochrome P_{450}2E1. N-acetyl-p-benzoquinoneimine is usually inactivated by hepatic glutathione, but when binds to centriobular hepatic proteins formed excessively, covalently, contributing to hepatic toxicity.[45] In the estimation of liver damage by PCM, the determination of enzyme activities such as ALT and AST is largely used. In this study, the increase in serum activities of ALT, AST and ALP and in PCM treated rats had been credited to the damaged structural integrity of the liver, because these are usually located in the cytoplasm, mitochondria or microsomes and are released into the circulation after cellular damage[46] or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases.[47] These results were in

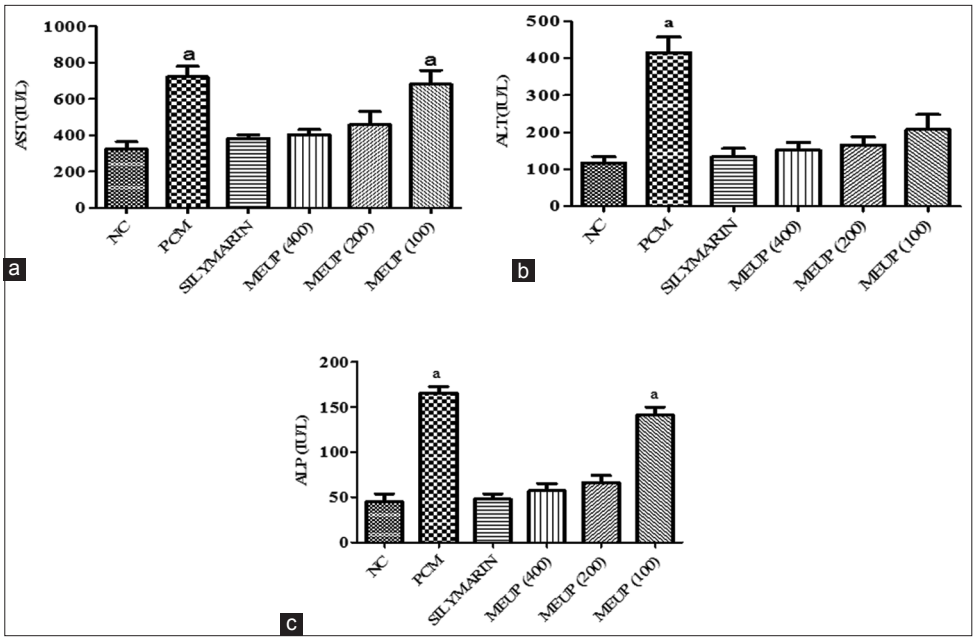

Figure 1: (a-c) Hepatoprotective activity of the methanol extract of the roots of *Uraria picta* and silymarin (standard drug) in albino rats

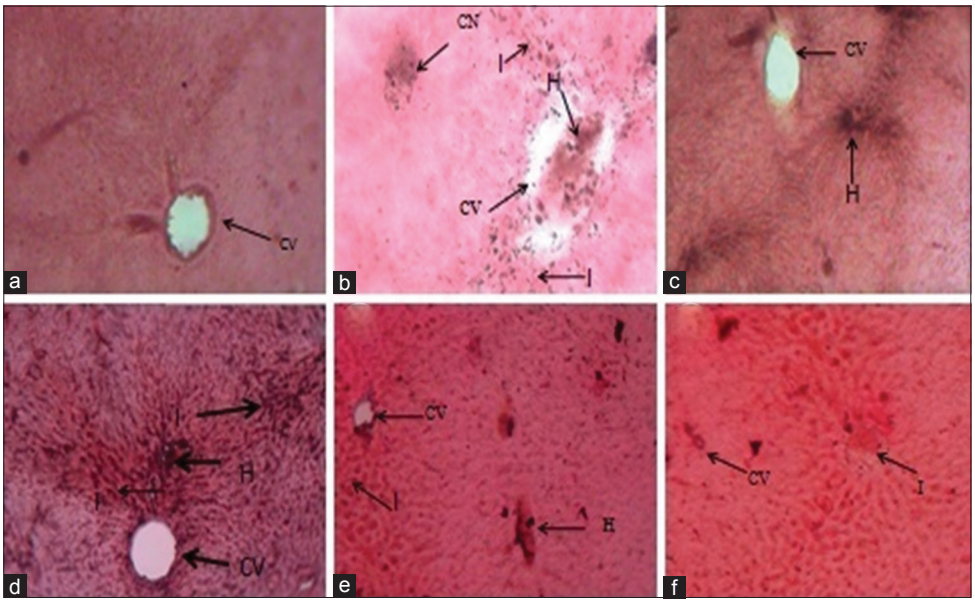

Figure 2: Histopathological examination of eosin-stained liver section of normal and experimental rats (a) - Vehicle treated control group. (b) Paracetamol (PCM) treated group, (c) PCM + silymarin treated group, (d) PCM + methanol roots extract (100 mg/kg) treated group, (e) PCM + methanol roots extract (200 mg/kg) treated group, (f) PCM + methanol roots extract (400 mg/kg) treated group. CV: Centrilobular, CN: Coagulative necrosis, I: Inflammation, H: Hemorrhage

accordance with those of Kuvandik *et al*.[48] who found that the serum levels of both ALT and AST were elevated almost fourfold in PCM treated group in comparison with the control normal group. Furthermore, Kanchana and Sadiq[49] mentioned that oral administration of 400 mg/kg PCM in rats increased serum activities of ALT, AST and ALP. In addition, histological findings revealed that PCM administration to rats exposed a remarkable centrilobular (Zone III) necrosis, cytoplasmic changes, and sinusoidal narrowing around the central vein and it has also been reported in some other studies that PCM intoxication can result in severe hepatic damage characterized by hemorrhagic centrilobular necrosis in both humans and animals.[50,51] Furthermore, Abdel-Monem *et al*. (2013)[52] mentioned that acute PCM toxicity induced extraordinary increase in plasma ALT, AST, ALP activities and significant decline in plasma level of total protein and albumin of rats. The AST, ALT, ALP values for UPME - 400 were found to be 404.17 ± 27.9, 151.50 ± 21.65 and 57.170 ± 8.04, respectively, which is comparable to the standard drug. An obvious significant enhancement in the

activities of ALT, AST and ALP enzymes was recorded in UPME (100-200 mg/kg p.o.) groups.

This study demonstrated the ability of *U. picta* to exert hepatoprotective activity and supported our earlier hypothesis that the extract possessed anti-inflammatory. The hepatoprotective potential of *U. picta* supported by the ability of the extract to reduce the serum liver enzymes (ALT and AST) level and the histopathological findings.

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