eISSN 2255-0569

ORIGINAL

Toxicity profiles of the hydroalocholic seed extract of *Psoralea Corylifolia* L Fabaceae in Wistar rats

Perfiles de toxicidad del extracto hidroalcohólico de semillas de Psoralea corylifolia L Fabaceae en ratas Wistar

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Received: 20 - III - 2022
Accepted: 2 - IV - 2022

doi: 10.3306/AJHS.2022.37.03.120

Abstract

Introduction: Ayurveda is well known system of medicine and practiced in Indian subcontinent since about 5000 years. Ayurveda offers holistic life style caring for the individual's mind and spirit as well as their body. *Psoralea corylifolia* Linn. (Fabaceae) is well known traditional plant used in Ayurveda and Chinese system of medicines and known for its beneficial actions in skin diseases such as psoriasis, alopecia, leprosy, leucoderma, and vitiligo etc.
Objective: The aim of present study to determine toxicity of hydroalcoholic extract of *P. corylifolia* in acute and sub-chronic toxicity assay models.
Methods: The albino mice were used in cute toxicity study for dose ranging from 10 to 1000 mg/kg, b.w.; and in sub-chronic toxicity assay, albino wistar rat used for dose ranging from 100 to 500 mg/kg, b.w. Daily body weight, food intake and different biochemical and haematological parameters were measured. Macroscopic observation of the vital organ of the test groups when compared to the control group.
Results: The results of toxicity study showed that there are no toxic symptoms produced in acute as well as in sub-chronic toxicity studies upto 1000 mg/kg, b.w. when tested in albino mice and rats orally. The overall behaviour of the animals was more active and healthy during the treatment period. It was found that there was no remarkable difference indicating the non-toxic property of the seed extract on acute toxicity and sub-acute repeated administration causes mild adverse effects.
Conclusion: The current study provides scientific data on safety of *P. corylifolia*.

Key words: Psoralea corylifolia, babchi, fabaceae, acute toxicity assay, sub-chronic toxicity assay.

Resumen

Introducción: El Ayurveda es un sistema de medicina muy conocido y practicado en el subcontinente indio desde hace unos 5000 años. El Ayurveda ofrece un estilo de vida holístico que cuida la mente y el espíritu del individuo, así como su cuerpo. La *Psoralea corylifolia* Linn. (Fabaceae) es una planta tradicional muy conocida que se utiliza en el sistema de medicina ayurvédica y china y es conocida por sus acciones beneficiosas en enfermedades de la piel como la psoriasis, la alopecia, la lepra, la leucodermia y el vitíligo, etc.
Objetivo: El objetivo del presente estudio es determinar la toxicidad del extracto hidroalcohólico de *P. corylifolia* en modelos de ensayo de toxicidad aguda y subcrónica.
Metodología: En el estudio de toxicidad aguda se utilizaron ratones albinos con dosis de 10 a 1000 mg/kg, p.c.; y en el ensayo de toxicidad subcrónica, ratas albinas wistar con dosis de 100 a 500 mg/kg, p.c. Se midieron el peso corporal diario, la ingesta de alimentos y diferentes parámetros bioquímicos y hematológicos. La observación macroscópica de los órganos vitales de los grupos de prueba se comparó con el grupo de control.
Resultados: Los resultados del estudio de toxicidad mostraron que no se produjeron síntomas tóxicos en los estudios de toxicidad aguda y subcrónica hasta 1000 mg/kg, b.w. cuando se probó en ratones y ratas albinos por vía oral. El comportamiento general de los animales fue más activo y saludable durante el período de tratamiento. Se comprobó que no había diferencias notables que indicaran la propiedad no tóxica del extracto de semillas en la toxicidad aguda y que la administración repetida subaguda causa efectos adversos leves.
Conclusión: El presente estudio aporta datos científicos sobre la seguridad de *P. corylifolia*.

Palabras clave: Psoralea corylifolia, babchi, fabaceae, ensayo de toxicidad aguda, ensayo de toxicidad subcrónica.

Introduction

Traditional medicine has a high impact on the prevention, control and treatment of diseases. In this regard, several medicinal plants and essential oils have been developed for treatment and control of diseases[1]. *Psoralea corylifolia* Linn. (Babchi) is an erect annual herb belonging to Fabaceae family. Medicinal values of *P. corylifolia* have been described in Ayurveda and Chinese Traditional system of medicine[2]. *P. corylifolia* has been reported to possess several bioactivities like laxative, stimulant, aphrodisiac, leprosy, leucoderma, vitiligo, and psoriasis etc[2,3]. *P. corylifo* seeds mainly rich in furocoumarins (e.g. psoralens and 8-methoxypsoralen)[4]. *P. corylifolia* has been reported by several authors as potent antibacterial[5], anti-inflammatory[6,7], anticancer[8-10], antipsoriatic[11], hepatoprotective agent, antifungal, antioxidant, estrogenic, immunomodulatory activity[12,13], and also causes vasodilation. This seed mainly used in the treatment of leucoderma (vitiligo), menopausal symptoms, depression, impotence and leprosy[14].

Due to wide application and utilization of *P. corylifolia* plant as medicinal agent, it becomes necessity of the day to examine its toxicity in in-vivo model. Toxicity assays are performed in short term and long term basis; short term assays are known as acute toxicity assay while long term study refers as sub-chronic toxicity assay[15]. Acute toxicity refers to those adverse effects occurring within a short time but in the case of sub-acute toxicity tests are intended to evaluate the toxicity of the plant samples after repeated administration[16,17]. These methods should be based on the OECD guidelines and examination should include the behavioural responses, mortality, food intake and determination of ALP, ACP, SGPT, SGOT and creatinine level along with histopathological parameters[18,19]. Hence the objective of present study to perform acute, sub-chronic toxicity assays including haematological examination to determine its safety and efficacy as medicinal agent[20]. The seed extract is safe on short term acute study and long term chronic toxicity by repeated oral administration causes mild adverse effects.

Materials and methods

Seed collection and Identification

Dried seeds of *Psoralea corylifolia* (500 g) were collected from Tampcol herbal at Chennai, and it was authenticated by the Pharmacognosist at Captain Srinivasamurthy Drug Research Institute (CSMDRI), Anna Hospital Arumbakkam, Chennai, Tamil Nadu India and confirmed by RAPINAT herbarium, St. Joseph College Trichy.

Chemicals and Equipment

Weighing balance, desiccators, empty bottles, EDTA bottles, capillary tubes, water bath, evaporating dish, haematocrit centrifuge, and Haematology meter. Other materials include methanol, chloroform, distilled water and 10 % formalin.

Preparation of the Hydroalcoholic seed extract

Dried seeds of *P. corylifolia* were shade dried for a week. After drying the seeds (100 g) were coarsely powdered using a pulverizer. Size reduced seeds were extracted by cold percolation method using water and ethanol (1:4) as solvent (200 ml). The extract was concentrated by separating the solvent from the extract and drying it in a water bath. The extracts were weighed and kept in the refrigerator at 4°C until needed.

Experimental Animals

The toxicity study utilized healthy Wistar albino rats of either sex and of about the same age, weighing about 170-250 g, and healthy albino mice of either six and of about the same age, weighing around 20 to 35 g. The Institutional Animal Ethical Committee reviewed the experimental protocol and gave it their approval before it could begin. 817 / 04 / ac / CPCSEA is the CPCSEA registration number. The animals were kept in polypropylene cages with standard pellet feed (Tamil Nadu Veterinary University Animal House, Chennai) and water ad libitum, and were kept in regular conditions (12 hr light / 12 hr dark cycles, 20 ºC to 27º C, 36-60 % humidity).

Acute Toxicity studies

The extract's oral median lethal dose (LD50) was measured in rats using the (20) Dixon and Mood method (1948). Healthy adult albino mice of either sex were separated into six groups (n= 6) and orally fed escalating doses of hydroalcoholic extract (10, 50, 100, 250, 500, and 1000 mg/kg b.w.). When given orally in doses up to 1000 mg/kg body weight, the whole hydroalcoholic extract did not cause any toxicity or mortality in mice when observed up to 24 hours after delivery. Clinical Observations (from the time of injection to 14 days later, clinical signs and symptoms). There were no unusual symptoms in any of the three groups. Below is a summary of the general clinical examination report.

Sub-chronic toxicity studies

Healthy adult albino rats of either sex were separated into five groups (n=5) and orally fed escalating dosages (100, 250, 3500, and 500 mg/kg b. w.) of hydroalcoholic extract, while the control group received 1 percent DMSO vehicle for 90 days, according to OECD-425 (21) recommendations. The treatment was done once daily by orally for 90 days. The rats were fasted for 18 hrs at the end of the 90th day, they were sacrificed after giving due anaesthesia. The blood was collected from the jugular vein and used for the determination of body weight changes, (at 9 am once weekly using a sensitive balance before the commencement of dosing), haematological parameters, biochemical parameters like ALP, ACP, SGPT and SGOT, serum urea and serum creatinine. The haematological parameters

were measured by retro-orbital method. Finally, the histopathological studies also carried out.

At the end of the 90-day observation period, all of the animals were necropsied for pathological abnormalities. The brain, heart, liver, kidney, lungs, spleen, GIT tract, and uterus were removed, weighed, and kept in 10% formalin until utilized for histological research. Each group had a portion of organ tissue preserved in 10% formalin and processed for histopathology. Serial slices of 5 µ thicknesses were made after paraffin embedding and block construction. They were inspected under a microscope after being stained with Haematoxylin and Eosin. Photomicrographs of a few sample specimens were also obtained.

Statistical Analysis

The 't' test was used to examine the results reported as mean ±SD, P<0.05 values were deemed statistically significant[21,22].

Results

Acute toxicity test

Toxicity experiments are carried out to find out the pharmacological level (Lethal dose) but they do not produce any toxic symptoms in the animals. Till the dose of 2000 mg/kg, it is found that all the groups have no mortality at all (**Table I**). However, all the animals are unusually behaved immediately after drug administration, but recovered after 24 hrs. Unusual symptoms of mild writhing and stretching of hind limbs are observed half an hour after drug administration but all the animals are recovered completely after 3 hrs.

Table I: Symptoms observed on treatment.

Dose (mg/kg)	Recovery/ Death After 24 hrs	No. of animals died/ animals treated	Toxicity Observed
10	Recovery	0/6	Nil
50	,,	0/6	F
100	,,	0/6	F
250	,,	0/6	F
500	,,	0/6	F
1000	,,	0/6	F

F = abnormal gait

At higher doses i.e., at the dose of 3000 mg/kg and 4000 mg/kg, it is seen that 80 % of the animals are dead. The survived ones have exhibited severe toxic symptoms, but recovered after 24 hrs (**Table II** and **Figure 1**).

From the above data, it may be concluded that the doses of 500 mg/kg and 1000 mg/kg (LD50) might be the appropriate safe doses to be administered to the mice in order to assess the pharmacological effects.

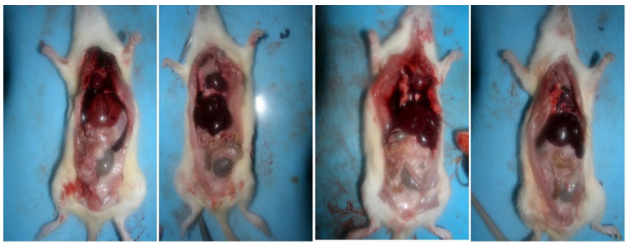
The body weight recorded has given a gradual increase even at higher dosages. The haematological observations for the parameters such as haemoglobin content and RBCs, have recorded decrease in their values in both of the sexes after the treatment with seed extract. But there is no significant reduction in the values. The values of MCV, MCH and MCHC are as comparable to the normal limits. The level of WBC has given increased value for male and PCV has put up similar values in both the sexes before and after the treatment.

Table II: Assessment of LD^{50} dose of *P. corylifolia*.

Dose (mg/kg)	Log Dose	Dead/ Total	% Dead	% Corrected	Probit
10	1.0000	0/6	0	4.17	3.26
50	1.6989	0/6	0	4.17	3.26
100	2.0000	0/6	0	4.17	3.26
250	2.3979	0/6	0	4.17	3.26
500	2.6989	0/6	0	4.17	3.26
1000	3.0000	0/6	0	4.17	3.26
1500	3.1761	0/6	0	4.17	3.26
2000	3.3010	0/6	0	4.17	3.26
3000	3.4771	2/6	33.3	33.3	4.58
4000	3.6021	4/6	66.6	66.6	5.42

Correction for 0% dead = 100(0.25/6); 100 % dead =100X (n-0.25/6)

Figure 1: Rats given with 2000 mg /kg of the extract showing no significant pathological changes at necropsy.



Sub-chronic toxicity

The results of *P. corylifolia* sub-chronic toxicity show that there were significant increases in body weight of female rats given extract at doses of 400mg/kg when compared to the control group. There was no statistically significant difference in the relative organ weights of any of the seed extracts tested. Every animal was monitored on a daily basis for signs of toxicity, body weight, neurological examination, and mortality (**Table III** and **Figure 2**).

On examination, the seed extract did not produce any significant differences in haematological markers (**Figure 3**). At the doses studied, the photomicrograph demonstrated normal architecture in the kidney and liver. On examination, the heart, lungs, spleen, uterus, and ovaries showed no morphological abnormalities in architecture. At the end of the 90-day observation period, all of the animals were necropsied for pathological abnormalities. A thorough post-mortem assessment of all of the animals indicated no major pathological alterations linked to the extract. **Table IV** shows the relative organ weights in detail as well as a summary of necropsy lesions. **Table V** shows the tabulation of lesions at necropsy.

Table III: Sub-chronic toxicity examination through neurological parameters.

S.No	Identification	Locomotor activity	Tail elevation	Ataxic gait	Head position
1	Head	Casual	Normal	None	Without tilt
2	Neck	Casual	Normal	None	Without tilt
3	Body	Casual	Normal	None	Without tilt
4	Tail	Casual	Normal	None	Without tilt
5	Colourless	Casual	Normal	None	Without tilt

Table IV: Relative organ weights.

S.No	Identification	Brain	Lungs	Liver	Kidney	Spleen	Ovary	Heart
1	Head	0.72	0.96	4.8	0.72	0.72	1.2	0.48
2	Neck	1.08	0.81	5.15	1.8	0.54	0.8	0.54
3	Body	91	0.91	4.56	0.68	0.45	1.36	0.45
4	Tail	0.5	0.74	4.7	1	0.5	0.75	0.5
5	Colourless	1.12	0.84	5.36	0.84	0.56	0.84	0.56
Mean ± S.D		0.866 ±0.26	0.854 ±0.08	4.914 ±0.33	0.864 ±0.17	0.554 ±0.10	0.99 ±0.26	0.506 ±0.04

Figure 2: Body weight of sub-chronic oral Toxicity of P. corylifolia by using albino wistar rat.

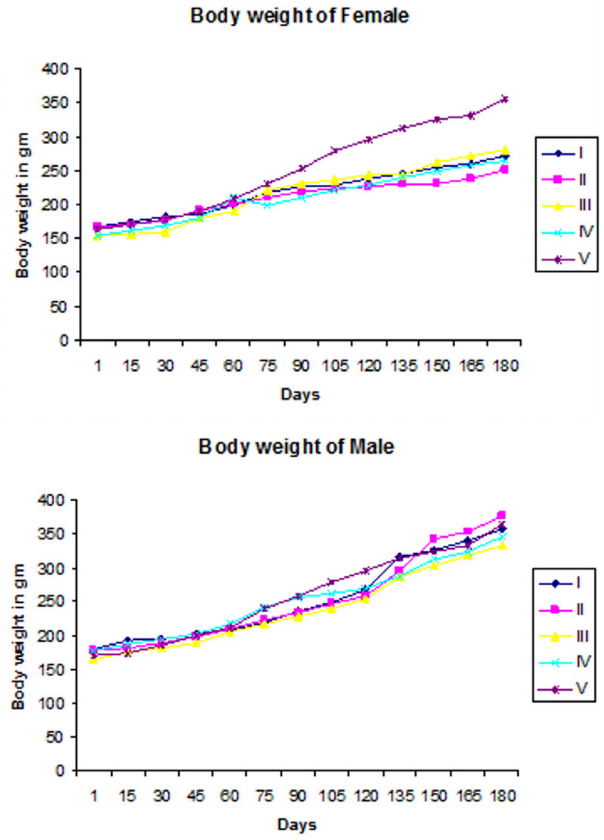


Table V: Tabulation of lesions at necropsy.

Organ	H	N	B	T	C
Liver	NAD	NAD	NAD	NAD	NAD
Lungs	NAD	NAD	NAD	NAD	NAD
Heart6	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD
Stomach	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD
GIT tract	NAD	NAD	NAD	NAD	NAD
Lymph nodes	NAD	NAD	NAD	NAD	NAD
Testis/ovary	NAD	NAD	NAD	NAD	NAD
Uterus	NAD	NAD	NAD	NAD	NAD

Where, NAD: No abnormal changes detected; H: head; N: neck; B: back; T: tail; and C: colourless.

Histopathology

Histopathological examination of the Heart, Lungs, Liver, Spleen, Pancreas, Kidney, Genital organ, GIT and Leg part of treated and control groups were done. Histopathological observations were carried out for both control and treated group of animals. At the highest dose of 500 mg/kg the results are given for those groups of animals of 90 days' observation after administration of *P. corylifolia* extract can be seen in **figures 3-5**. At 500 mg/kg, the liver architecture showed very minor quiescent inflammatory alterations (Plate 1-E1). At 500 mg/kg, the photomicrograph demonstrated mild acute tubular necrosis with hyperplasia in kidney (Plate 2-E2). However, histology of the GIT tract, heart, spleen, lungs, uterus, and ovaries in the treated groups revealed no aberrant architecture (Plate 1 (A, B, C, D) and 2 (E, G, F, H).

Discussion

According to a recent estimate, nearly 75% of people around the world, mainly in poor nations, rely on traditional medicine and its practice for their health care needs[23]. In rats, the extract's oral median lethal dose (LD_{50}) was determined to be greater than 5000 mg/kg body weight. This indicates that the extract is basically non-toxic when administered acute (orally)[24]. Increased body weight and relative organ weight are usually considered non-toxic effects of extract on animals, resulting in increased food and water consumption[25]. When compared to the control group, the extract generated a rise in body weight, indicating that the extract was rather safe for the rats.

Toxicity experiments were carried out to find out the pharmacological level (lethal dose) but they did not produce any toxic symptoms in the animals till the dose 2000 mg/kg but at higher doses i.e. 3000 mg/kg and 4000 mg/kg exhibit that 80% of the animals died. So that from the result it may be concluded that the doses of 500 mg/kg and 1000 mg/kg (LD_{50}) might be the appropriate safe doses to be administered to the mice in order to assess the pharmacological effects[26]. Whereas the sub-acute toxicity studies exhibit mild abnormal changes were observed in treated animal's liver and kidney organ in comparison with control. Biochemical and haematological parameters were not significantly different between the control and experimental groups of rats[27]. In histopathological studies the pictures do not show any significant shift in the architecture of the heart, lungs, spleen, uterus and ovary of respective organ.

The kidney is a vital organ in the body that helps to maintain homeostasis by performing osmoregulatory functions (electrolyte and blood pressure management, acid-base balance maintenance)[28]. Urea is a by-product of protein metabolism that is eliminated entirely through the kidneys[29], whereas creatinine is a by-product of muscle metabolism that is similarly expelled solely through glomerular filtration[29]. As a result, creatinine, urea,

Figure 3: Pre and Post Haematological reports of sub-chronic oral toxicity of *P. corylifolia* by using albino wistar rats.

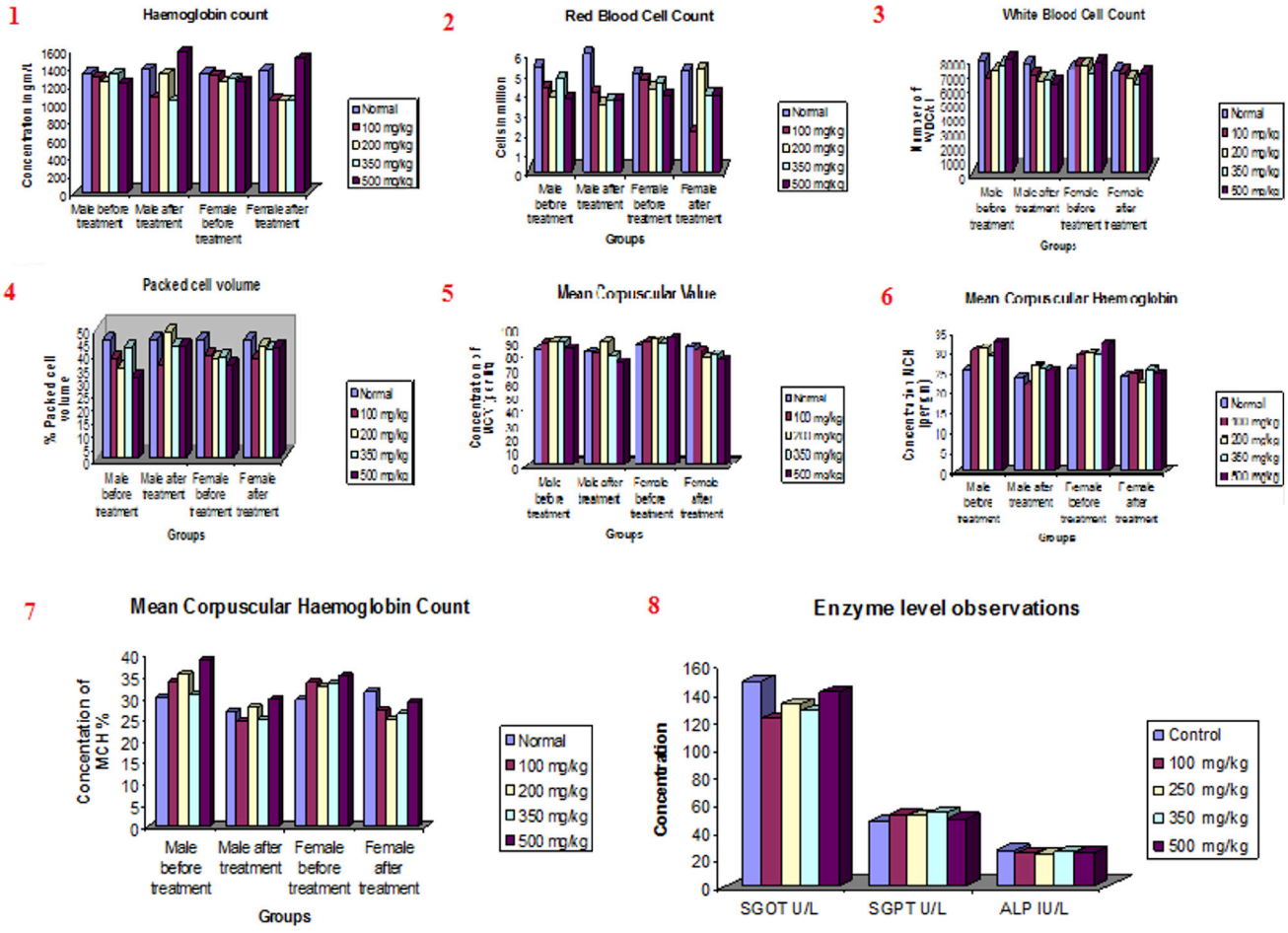


Figure 4: Photomicrographs of a section of tissues (H & Ex250) of Wistar rat's oral administration of hydroalcoholic extract *P. corylifolia*: Heart (A), Lungs (B), Spleen (C), and Liver (D) and Liver (D1).

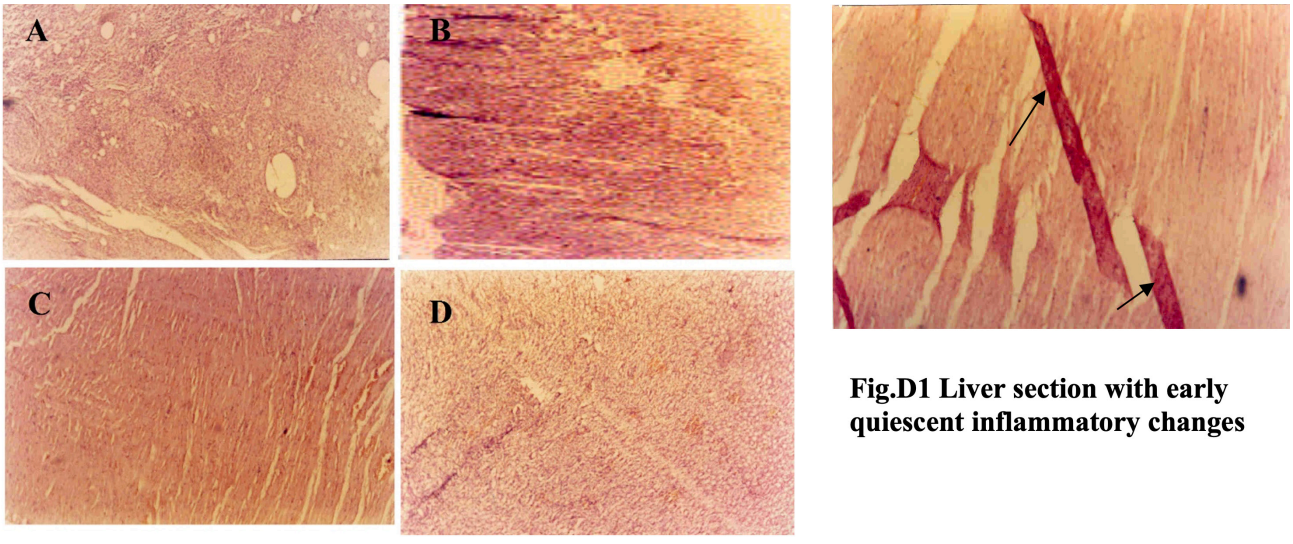
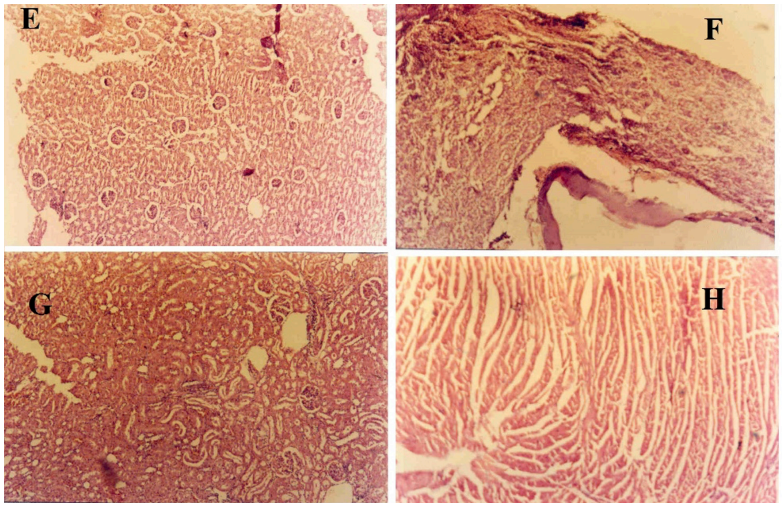


Fig.D1 Liver section with early quiescent inflammatory changes

Figure 5: Photomicrographs of a section of tissues (H & E×250) of Wistar rat's oral administration of hydroalcoholic extract *P. Corylifolia*. Kidney (E), Kidney (E1), Uterus and Ovaries (F), GIT tract (G), and Uterus with histology of ovaries and fallopian tubes (H).



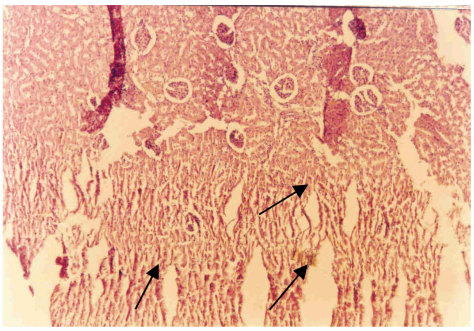


Fig.E1 Kidney with acute tubular necrosis

sodium, potassium, and chloride levels are utilized to assess kidney function[29]. There is no significant increase in the serum levels of renal biochemical parameters tested indicates that the medications tested have no effect on kidney function.

The liver is the most important organ in the living system for drug and xenobiotic metabolism. Biomarkers for assessing liver function include alanine transaminase, aspartate transaminase, and alkaline phosphatase[27,29]. The substantial increases in the level of AST might be an indicator of liver damage, which is suggestive of hepatotoxic impact. Although ALT and AST are commonly employed in the assessment of liver damage caused by extract or any hepatotoxic chemical (since they produce hepatocyte inflammation, cellular leakage, and cell membrane destruction)[30,31], an elevated level of ALT is more specific for liver-related injuries or disorders[32]. A high AST level, on the other hand, can indicate liver damage, myocardial infarction, and muscular injury[33]. ALT is only found in minute amounts in the liver, but it is secreted in the bile, and modest intra-hepatic biliary obstruction causes a significant increase in serum ALP[34]. When testing involves rats, the study of haematological parameters is significant in determining the harmful effect of a chemical since it has a better predictive value of toxicity in humans. When the extract-treated groups were compared to the control group, there was no significant difference in hematological parameters. After 90 days of oral treatment of the seed extract to rats, histopathological analysis revealed that the normal morphology of the heart, lungs, spleen, uterus and ovaries, and the GIT tract were not changed by the examined seed extracts, but liver and kidney had a mild unfavorable effect, suggesting that the extract could be harmful to the liver and kidney.

Conclusion

The hydroalcoholic seed extract of *P. Corylifolia* is a safe drug when taken in small doses; however, repeated use of extracts, may cause toxic effects on certain organs such as the liver and kidney.

Conflict of interests

The authors have no conflict of interest.

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