Turk J Pharm Sci 2019;16(1):88-95
DOI: 10.4274/tjps.87487

ORIGINAL ARTICLE

Subchronic Toxicity Assessment of Orally Administered Methanol (70%) Seed Extract of *Abrus precatorius* L. in Wistar Albino Rats

Wistar Albino Sıçanlarda Oral Olarak Uygulanmış *Abrus precatorius* L. Tohum Metanol (%70) Ekstraktının Subkronik Toksisite Değerlendirmesi

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ABSTRACT

Objectives: *Abrus precatorius* L. is a famous medicinal plant of the family Fabaceae and is widely used in traditional medicine for the treatment of various ailments. However, there are limited toxicological data available regarding its safety following repeated exposure; therefore, the present study was designed to evaluate the 28-day subchronic toxicity of methanol (70%) crude extract of *A. precatorius* seeds in adult Wistar albino rats.

Materials and Methods: A subchronic toxicity experiment was conducted by oral administration of graded doses (200 mg/kg and 400 mg/kg) of test extract daily for 28 days. Signs of toxicity, food and water consumption, body weight, and gross pathology as well as relative organ weight were evaluated. The toxic effects were also assessed using hematological and biochemical data followed by histopathological examination of various internal organs. All data collected were expressed as mean ± standard deviation. ANOVA followed by the Bonferroni test was used for data interpretation and $p<0.05$ was considered significant.

Results: No deaths or evident toxic signs were found during the experimental period. There were no significant differences in body weight, gross pathology, organ weight, or food and water consumption between the control and the treated groups. There were no treatment-related differences in hematological or biochemical indices. Moreover, no gross abnormalities or histological alterations were observed.

Conclusion: The methanol extract of *A. precatorius* seeds was nontoxic in subchronic intake at the dosages tested. Thus, this study is expected to be beneficial for clinical and traditional applications for safe consumption and to utilize *A. precatorius* as a remedy at a recommended dosage.

Key words: *Abrus precatorius*, subchronic toxicity, hematology, histopathology, biochemical indices

ÖZ

Amaç: *Abrus precatorius* L., Fabaceae familyasından ünlü bir şifalı bitkidir ve geleneksel tıpta çeşitli rahatsızlıkların tedavisinde yaygın olarak kullanılır. Bununla birlikte, tekrarlanan maruz kalmanın ardından güvenliği ile ilgili sınırlı toksikolojik veriler mevcuttur; bu nedenle, bu çalışma yetişkin Wistar albino sıçanlarında *A. precatorius* tohumlarının metanol (%70) ham ekstraktının 28 günlük subkronik toksisitesini değerlendirmek üzere tasarlanmıştır.

Gereç ve Yöntemler: Yirmi sekiz gün boyunca, günlük olarak test edilen dozlar (200 mg/kg ve 400 mg/kg) oral yolla uygulanarak bir subkronik toksisite deneyi gerçekleştirildi. Toksisite belirtileri, yiyecek ve su tüketimi, vücut ağırlığı ve makroskopik patolojinin yanı sıra nispi organ ağırlığı değerlendirildi. Toksik etkiler, hematolojik ve biyokimyasal veriler kullanılarak değerlendirildi ve bunu takiben çeşitli iç organların histopatolojik incelemesi yapıldı. Toplanan tüm veriler ortalama ± standart sapma olarak ifade edildi. Verilerin yorumlanması için ANOVA ve ardından Bonferroni testi kullanıldı ve $p<0.05$ anlamlı kabul edildi.

Bulgular: Deney süresince hiçbir ölüm ya da belirgin toksik belirti saptanmadı. Kontrol ve tedavi edilen gruplar arasında vücut ağırlığı, makroskopik patoloji, organ ağırlığı veya gıda ve su tüketimi açısından anlamlı fark yoktu. Hematolojik veya biyokimyasal indekslerde tedaviye bağlı fark yoktu. Ayrıca, hiçbir makroskopik anormallik veya histolojik değişiklik gözlenmedi.

Sonuç: *A. precatorius* tohumlarının metanol ekstraktı, test edilen dozajlarda, subkronik alımda toksik değildir. Bu nedenle bu çalışmanın, klinik ve geleneksel uygulamalarda güvenli tüketim için faydalı olması ve *A. precatorius*'un tavsiye edilen bir dozda ilaç olarak kullanılması beklenmektedir.

Anahtar kelimeler: *Abrus precatorius,* subkronik toksisite, hematoloji, histopatoloji, biyokimyasal indeksler

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Received: 03.11.2017, **Accepted:** 25.01.2018

INTRODUCTION

Medicinal plants offer numerous opportunities for the development of new drugs, as extract, pure compound, or derivative. The natural origin, however, does not guarantee their safety for medicinal purposes. Most herbal products used in folk medicine have strong scientific evidence regarding their biological activities. However, the main obstruction to the use of herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of drugs. Different toxicological study data like acute and subchronic on medicinal plants or their preparations should be obtained in order to increase the assurance of their safety in humans, particularly for use in the development of pharmaceuticals.[1,2]

Abrus precatorius L. (family: Fabaceae), locally known as "Gunja" or "Rati", is indigenous to India and is also found in other tropical and subtropical areas of the world. It is a beautiful, perennial, deciduous, twining woody vine with herbaceous branches and pinnate leaves. The flowers are short stalked, white to pink, and are borne in clusters. Fruits are oblong pods that contain characteristic red seeds with a black mark at the hilum/base. After maturation, these open before falling and curl back to reveal seeds. Seeds are of uniform weight (0.1 g) and therefore were used in the past as standard weighing units by jewellers for weighing silver and gold.[3,4] They are poisonous when taken internally and therefore are used after processing.[5,6] A number of biochemical constituents have been reported from *A. precatorius* seeds. These are rich in amino acids (like serine, alanine, valine, choline, and methyl ester); proteins (abrin and *A. precatorius* agglutinin), carbohydrates (galactose, arabinose, and xylose), flavonoids (abectorin, dimethoxycentaureidin-7-o-rutinoside, and precatorin I, II, and III), anthocyanins (delphinindin, pelargonindin, and cyaniding), and alkaloids (dimethyl tryptophan, methocation, picatorine, abrine, hypaphorine, choline, and trigonelline). The seed proteins are rich in most of the essential amino acids, and they are deficient only in cystine and threonine. Moreover, various triterpenoids, steroids, and fatty acids have also been isolated from *A. precatorius* seeds.[7-11] The principle poisonous component of the seed is abrin, an albumotoxin.[12] The plant has been used for therapeutic purposes since ancient times. The most widespread use of *A. precatorius* seeds is in the treatment of eye infections and as a potential contraceptive.[4] Dry seeds are powdered and taken one teaspoonful once a day for two days to cure worm infection. The seeds are considered purgative, emetic, aphrodisiac, diuretic, expectorant, refrigerant, vermifuge, febrifuge, laxative, and abortifacient. They are commonly used to cure leprosy, dysentery, jaundice, fainting, arthritis, paralysis, nervous disorders, ulcer, stiffness of the shoulder joints, tuberculosis, headache, diarrhea, sciatica, tetanus, rabies, convulsions, fever, cold, gastritis, snakebite, conjunctivitis, inflammations, and leucoderma in traditional and folk medicines. Hot water extract of seeds is taken orally to cure malaria. The seeds are nutritious and are eaten boiled in certain parts of India. Various African tribes use powdered seeds as oral contraceptives.[8,13-18] Seeds of this plant are also used to lower high blood pressure and relieve painful swellings. In veterinary medicine, seeds are used in the treatment of fractures.[8,10] *A. precatorius* seeds are also reported to have anticancer,[19] antifertility,[4] antitumor, antispermatogenic, antibacterial,[20,21] antidiabetic,[22] antioxidant,[23] nephroprotective,[24] antiarthritic,[25] antimicrobial,[19] and antimalarial activities.[26] Despite its popularity in folk medicine, its toxicity profile has not yet been explored. The present study was thus based on the subchronic toxicity of the methanol (70%) extract of *A. precatorius* seeds in Wistar albino rats. Subchronic toxicity tests were designed to examine the effects resulting from repeated exposure over a portion of the average life span of an experimental animal.

MATERIALS AND METHODS

Seed collection and authentication

Seeds of *A. precatorius* were collected from a local market in Bhopal, Madhya Pradesh, India. The seeds were authenticated by Dr. Zia-Ul-Hassan, Head of the Department, Govt. Saifia Science College, Bhopal, and a voucher specimen (reference no. 520/Bot/Saifia/2015) was deposited there for future reference. The seeds were cleaned and washed with distilled water in order to remove the impurities and were shade dried. The seeds were then coarsely powdered in a mixer grinder.

Preparation of methanol (70%) seed extract

Dried coarse powder of *A. precatorius* seeds (250 g) was defatted with petroleum ether and the marc remaining was extracted successively with methanol (70%) using cold maceration. The filtrate obtained was evaporated in a rotary evaporator under reduced pressure and was vacuum dried. The dried extract was packed in an air-tight container, labeled, and stored in a refrigerator (2-4°C) until needed for the experiment.

Ethical approval

The use of animals for the present study was reviewed and approved with approval reference no. PBRI/IAEC/PN-412 by the Institutional Animal Ethical Committee of Pinnacle Biomedical Research Institute (PBRI) Bhopal, Madhya Pradesh (Reg. No. 1283/PO/c/09/CPCSEA) and they were maintained as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India.

Selection of animals

For the purpose of subchronic toxicity studies, adult Wistar albino rats weighing about 94-178 g of both sexes were used. The animals were obtained from the animal house of PBRI.

Maintenance of experimental animals

The animals were kept in properly numbered large polypropylene cages with a stainless steel top grill. They were maintained under standard laboratory conditions (24±2°C; 50±5% humidity; 12 hours light/12 hours dark cycle) with standard diet (Hindustan Lever, Mumbai, India) and water *ad libitum* and were acclimatized to the laboratory conditions for a week before starting the experiment. Paddy husk was used as bedding material and it was changed twice a week.

Preparation of test sample

A suspension was made in 0.1% carboxymethyl cellulose solution and administered orally to the animals. The administration was done by straight type oral feeding needle. Individual doses were calculated on the basis of animal weight.

Subchronic toxicity study

Experimental setup

In order to investigate the adverse effects of repeated daily exposure to methanol (70%) crude extract of *A. precatorius* seeds, a subchronic toxicity study was carried out as per Organisation for Economic Co-operation and Development guidelines 407.[27] To determine the dose-related toxic effects, doses of 200 mg/kg/day and 400 mg/kg/day body weight of crude seed extract of *A. precatorius* were administered for the experimental period of 28 days. Eighteen rats of each sex were randomized into three groups of six animals each. The animals were marked to permit individual identification. Group 1 was the control group and received orally normal saline. Group 2 and Group 3 were orally administered 200 mg/kg/day and 400 mg/kg/day body weight of crude methanol seed extract of *A. precatorius*, respectively.

Clinical signs and mortality

During the 28 days of the experimental period, all the animals were observed once daily for toxicity signs and mortality immediately after dosing and up to 4 h after dosing.

Weekly body weight

Individual body weights of rats were recorded initially and every week and the individual doses were adjusted according to body weight to maintain the target dose level for all experimental animals.

Weekly food and water consumption

Food and water consumption parameters were also taken into consideration during the study. Consumption of food and water was measured initially and weekly for each rat.

Blood sample collection

At the end of the experimental period, blood samples were collected under diethyl ether anesthesia from all animals through a retro-orbital plexus puncture using capillary tubes on day 29. The blood from each animal was collected in ethylenediaminetetraacetic acid (EDTA) and non-EDTA tubes to determine hematological and biochemical parameters, respectively. For biochemical analysis, the blood samples were allowed to coagulate for 30 min and the clear serum was separated by centrifuging at 3000-4000 rpm using a cooling microcentrifuge for 15 min. The serum was introduced into new tubes and stored at -20°C until analyzed.

Hematological investigations

The blood collected in EDTA-containing tubes was taken immediately for hematological investigation. The blood or hematological parameters were analyzed using an automated hematology analyzer (Procan Electronics, Model PE6800). The parameters included hemoglobin (HGB), red blood cell (RBC) count, hematocrit (HCT), RBC distribution width, mean cell volume, mean cell HGB concentration (MCHC), white blood cell count, lymphocytes, granulocytes, platelet (PLT), PLT distribution width, mean PLT volume, and PLT large cell ratio.

Biochemical estimations

The effect of seed extract of *A. precatorius* on the activity of the serum biochemical enzymes was estimated calorimetrically. All the biochemical investigations were performed on an automated biochemical analyzer (Rapid, Model Star 21). The biochemical estimations evaluated were as follows:

a) Kidney function tests: blood urea nitrogen, creatinine, uric acid,

b) Liver function tests: total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatise, total proteins,

c) Lipid profile: total cholesterol, triglycerides (TG), high density lipoproteins, low density lipoproteins.

Gross pathology and relative organ weight

Following blood collection, the animals were anesthetized and dissected out. Immediately the liver, kidneys, heart, spleen, stomach, adrenal glands, duodenum, brain, colon, and lungs were excised, freed of fat, washed in cold saline, blotted with clean tissue paper, and observed for gross pathological changes. Then the organs were weighed in grams using a calibrated balance. The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Body weight of rat on sacrifice day}} \times 100$$

Histopathological examination

Defined samples of the liver, heart, kidneys, spleen, lungs, brain, stomach, and duodenum were collected for histological studies. The tissues were fixed immediately in 10% formalin for at least 24 h, dehydrated through a series of ethanol solutions, and embedded in paraffin. Then 4-5-µm-thick sections were cut in a rotary microtome and were stained with hematoxylin and eosin for photomicroscopic observation. All histopathological changes were examined by a pathologist. The microscopic features of the organs of both treated groups were then compared with those of the control group.

Statistical analysis

All the experimental data collected from various subchronic parameters were statistically analyzed using SIGMA STAT-3.5 software and expressed as mean ± standard deviation. ANOVA followed by the Bonferroni test was used for interpretation of data. $P<0.05$ was considered significant.

RESULTS

Clinical changes and mortality

Daily administration of methanol (70%) seed extract of *A. precatorius* at both 200 mg/kg and 400 mg/kg for 28 days did not induce any evident symptom of toxicity in rats of either sex.

No deaths or evident clinical signs were observed in any groups during the study period.

Body weight changes

Oral administration of *A. precatorius* seed extract to experimental animals for 28 successive days caused no statistically significant changes in body weight compared with the control group (Figure 1).

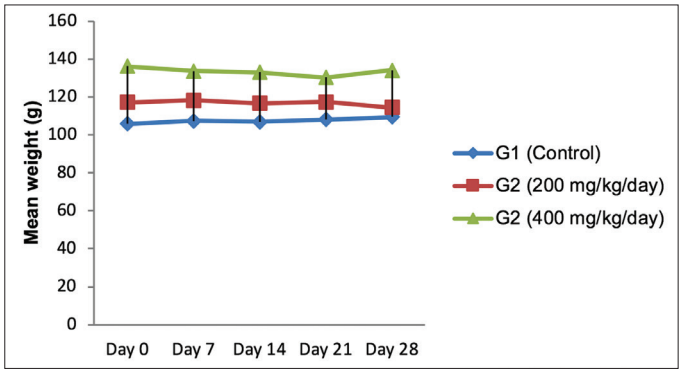

Figure 1. Changes in body weight of rats during the subchronic toxicity study

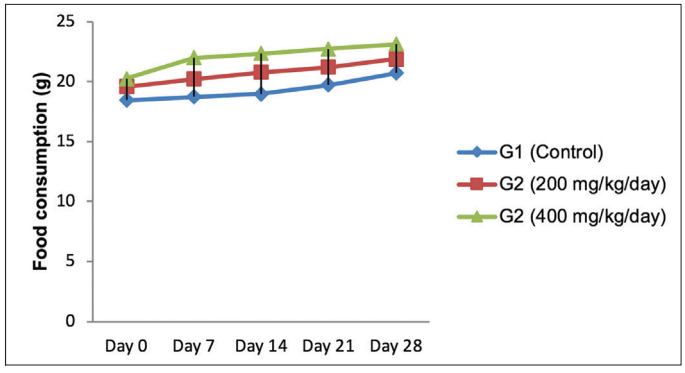

Figure 2. Changes in food consumption of rats during the subchronic toxicity study of methanol (70%) seed extract of *Abrus precatorius*

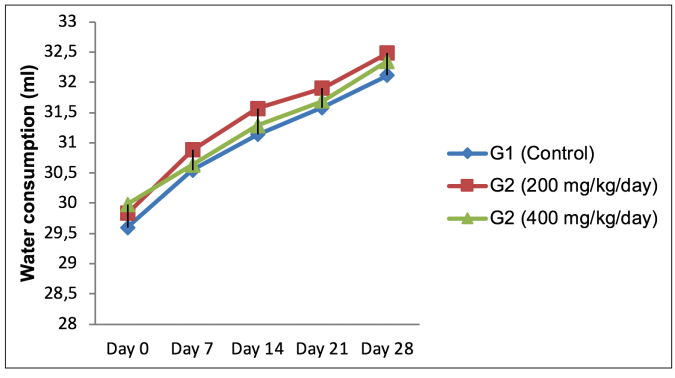

Figure 3. Changes in water consumption of rats during the subchronic toxicity study of methanol (70%) seed extract of *Abrus precatorius*

Food and water consumption changes

Oral administration of extract at 200 and 400 mg/kg body weight for 28 days caused no significant alterations in these indices compared to the control group (Figures 2 and 3).

Hematological effects

The seed extract at both the investigated dose levels had a good hematological tolerance. Hematological parameters of extract-treated rats were not significantly different from those of the control group. Hematological parameters of the test groups and the control group are presented in Table 1.

Biochemical changes

A study of biochemical parameters showed that the seed extract of *A. precatorius* did not induce any harmful biochemical effects in the experimental animals. No significant alterations were observed in the serum concentrations of renal markers, hepatic markers, or lipid profile of extract-treated animals. The results of the biochemical analysis are summarized in Table 2.

Gross pathology and relative organ weight changes

Gross pathology of the liver, kidneys, heart, spleen, stomach, adrenal glands, duodenum, brain, colon, and lungs of treated rats did not show abnormalities in terms of color or texture as compared to the controls. In this study, the relative organ weight of each organ recorded in the treatment groups did not show a significant difference compared to the controls. Relative organ weights of all three groups are shown in Table 3.

Histopathological changes

The light microscopy examinations of the selected organs (heart, liver, lungs, kidneys, spleen, stomach, intestines, and brain) of the extract-treated groups and the control group are shown in Figure 4. The histopathological examination showed normal cytoarchitecture and absence of any gross pathological lesion in the organs of the control as well as the extract-treated rats.

DISCUSSION

Plants provide a wide variety of biochemical components useful to mankind. These substances can be extracted and used in the preparation of drugs or the plant itself can be used directly as a medication.[28] However, the main obstacle to the use of traditional herbal plants is the lack of proper clinical and scientific data in support of the safety of drugs. Plants contain some toxic substances and it is better to evaluate them according to standard procedures and their effects on different parameters in order to establish the safety of the plants.[29] Toxicity tests are carried out effectively on either rats or mice due to their availability and low cost and the wealth of toxicology data in the literature already available for these species.[30] Considering the numerous reported therapeutic potentials of *A. precatorius* seeds as an alternative medicine effective for various diseases, a safety profile was established through a subchronic toxicity study, as a guide for the management of its application and usage in herbal preparations. This study will serve to prevent exposing humans to potential toxicity-related health risks while using *A. precatorius* seeds.

Table 1. Effect on hematological parameters of rats administered with methanol (70%) seed extract of *Abrus precatorius*

Hematological parameters	Group 1 (Control)	Group 2 (200 mg/kg/day)	Group 3 (400 mg/kg/day)
Erythrocyte indices			
HGB g/dL	11.6833±0.99401	11.4167±0.78191	10.8067±0.50006
RBC (×10/µL)	6.495±0.34827	6.135±0.59779	5.73833±0.87959
HCT (%)	33.0333±11.56484	24.2667±6.294619	20.63333±7.247912
RDW (fL)	39.16667±2.361967	34.8333±4.399021	35.4±4.642916
MCV (fL)	59.6±2.442676	55.06667±4.454461	55.13333±1.966949
MCHC (g/dL)	30.73333±4.006106	37.23333±2.910708	36.69833±5.43031
Thrombocyte indices			
PLT (×10/µL)	164.666±30.4119	249.666±127.853	279.5±146.459
PDW (%)	9.066667±0.827983	9.23333±0.696818	8.7±1.001665
MPV (fL)	9.716667±1.439232	9.716667±0.982203	8.28333±1.166786
P-LCR (%)	20.03333±5.948856	20.68333±2.319064	18.46667±2.1723
Leucocyte indices			
WBC (×10/µL)	4.71666±3.92785	3.21666±1.66675	5.69833±2.40555
LYM (×10/µL)	3.1±2.20605	1.71666±0.43365	3.05±1.16868
LYM (%)	71.2±13.27717	61.23333±15.20961	55.05±8.147137

HGB: Hemoglobin, RBC: Red blood cell count, HCT: Hematocrit, RDW: Red blood cell distribution width, MCV: Mean cell volume, MCHC: Mean cell hemoglobin concentration, PLT: Platelet, PDW: Platelet distribution width, MPV: mean platelet volume, P-LCR: Platelet large cell ratio, WBC: White blood cell count, LYM: Lymphocytes, values are expressed as mean ± standard deviation of six animals

Table 2. Effect on serum biochemical parameters of rats administered methanol (70%) seed extract of *Abrus precatorius*

Parameter	Group 1 (Control)	Group 2 (200 mg/kg/day)	Group 3 (400 mg/kg/day)
Renal markers			
BUN (mg/dL)	15.07667±2.22464	14.38183±6.688322	15.72833±3.021062
Creatinine (mg/dL)	0.72533±0.15052	0.73833±0.18193	0.7675±0.114176
Uric acid (mg/dL)	2.461833±0.427353	3.426167±1.670978	3.0385±1.280693
Hepatic markers			
Total protein (g/dL)	5.509667±0.500666	7.0215±1.125476	6.672±2.130268
Total bilirubin (mg/dL)	0.23645±0.64627	0.26395±0.17536	0.29115±0.088835
ALP (IU/L)	157.6767±33.2871	168.4167±31.08203	165.5667±20.95387
AST (IU/L)	167.1333±31.34372	172.6322±23.588	164±13.46601
ALT (IU/L)	39.673±9.803	47.595±9.872	51.55333±10.47761
Lipid profile			
Total cholesterol (mg/dL)	44.09667±7.449652	49.15833±12.1925	42.51333±4.353549
Triglycerides (mg/dL)	52.765±54.1075	58.12833±8.903721	50.46±10.90015
HDL (mg/dL)	48.59333±5.753485	49.58±12.66136	43.44±10.9195
LDL (mg/dL)	22.3919±5.490329	21.6345±5.390373	20.42133±6.257871

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatise, HDL: High density lipoproteins, LDL: Low density lipoproteins, values are expressed as mean ± standard deviation of six animals

Subchronic toxicity was tested with repeated administration of 200 mg/kg body weight and 400 mg/kg bodyweight of methanol (70%) seed extract of *A. precatorius*, and its effects in terms of clinical signs, bodyweight, food and water consumption, gross pathology and organ weight, hematological and biochemical parameters, and histopathology were noted.

Mortality, behavioral signs, body weight, and food consumption are very sensitive indicators to assess the toxicity of a substance.[31] In the present study, all the test animals were physically active during the test period. There were no signs of toxicity or mortality in rats treated with the extract. The lack of significant changes in body weight, food intake, or

Table 3. Effect on relative organ weight of rats administered methanol (70%) seed extract of *Abrus precatorius*

Organ name	Control	200 mg/kg/day	400 mg/kg/day
Liver	3.360333±0.202464	3.342167±0.160833	3.601167±0.22121
R. Kidney	0.5415±0.031154	0.530833±0.042148	0.524167±0.035611
L. Kidney	0.511667±0.018696	0.500333±0.034238	0.524167±0.035611
Heart	0.425333±0.055733	0.473333±0.052908	0.482667±0.074033
Spleen	0.437167±0.054066	0.374333±0.072304	0.497667±0.047971
Stomach	1.135333±0.083194	1.010333±0.136626	0.963833±0.145387
Adrenal glands	0.0375±0.007588	0.033±0.00611	0.0295±0.006922
Duodenum	0.8595±0.211821	0.9065±0.111439	0.754333±0.125298
Brain	1.293±0.192562	1.3015±0.125279	1.127333±0.071818
Colon	0.4825±0.179648	0.635833±0.151786	0.657333±0.082265
Lungs	1.074167±0.070416	1.0225±0.21034	1.040333±0.181494

Values are expressed as mean ± standard deviation of six animals

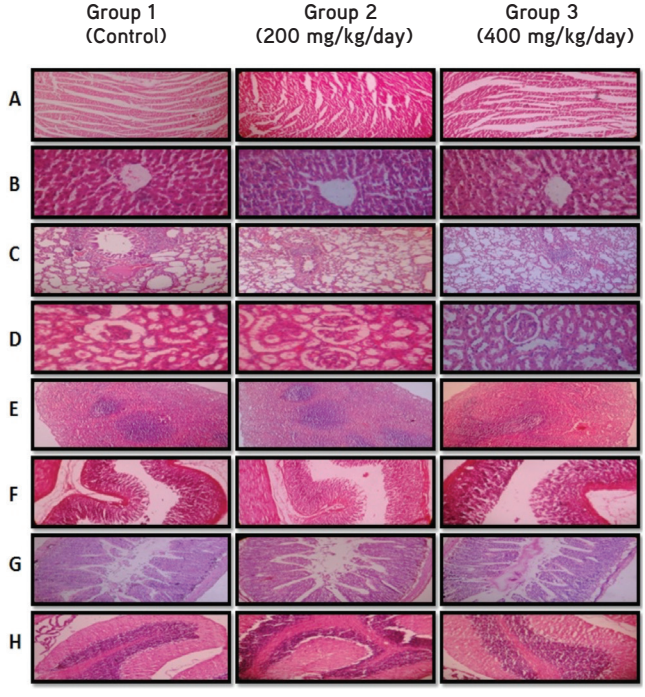

Figure 4. Effect of methanol (70%) seed extract of *A. precatorius* on various rat organ histomorphologies in the subchronic toxicity study (A: Heart, B: Liver, C: Lungs, D: Kidneys, E: Spleen, F: Stomach, G: Intestines, H: Brain)

water consumption of the treated animals also indicates the nontoxicity of the extract.

According to a previous report, the hematopoietic system is one of the most sensitive targets for toxic substances.[32] Hematological analysis easily detects the abnormalities in body metabolic processes and reveals very important information about the response of the body to injury, deprivation, and/or stress.[30] However, in the present study the mean value of each parameter was within the normal limits and this further supports the nontoxic nature of the extract.

The biochemical investigation showed that no significant modifications of assessed parameters occurred in the rats. The levels of enzymes in the liver and kidneys of all groups of rats stayed within normal ranges, which demonstrated that the test extract had no membrane-labializing effect on these organs. Enzyme activities in tissues are mostly employed as markers to ascertain early toxic effects of substances administered to experimental animals. Alterations in the lipid profile also show the efficacy and safety of plant extracts. Any deviation in the concentration of lipids can provide information on the status of lipid metabolism as well as the predisposition of animals to atherosclerosis.[33] Cholesterol and TG are the lipid parameters associated with coronary artery diseases and are usually used to ascertain hyperlipidemic conditions.[34] In the present study, the normal restoration of lipid parameters in the serum levels of extract-treated rats not only indicates the nontoxic nature of the extract, but also implies that the tested extract may not dispose the animals to cardiovascular risk.

Analysis of organ weight in toxicology studies is an important endpoint for identification of potentially harmful effects of chemicals. A main requirement in toxicological experiments is the ability to assess the effects of xenobiotics on specific organs.[35] In the present study, no changes were observed in the gross examination of the organs of treated animals when compared to the control group. The relative organ weight of the liver, kidneys, heart, spleen, stomach, adrenal glands, duodenum, brain, colon, and lungs recorded in the treatment groups did not show a significant difference compared to the control group. This may imply that the extract did not alter the secretory ability of the organs. It is also possible that the extract did not cause any cellular constriction or inflammation of the organs, which would have resulted in a decrease or increase in their weight, respectively.

Histopathological examination of the selected organs provides information to strengthen the findings of hematological and biochemical parameters. Microscopic examinations of sections

of the heart, liver, lungs, kidneys, spleen, stomach, intestines, and brain of all groups revealed no detectable abnormalities.

CONCLUSIONS

Following subchronic treatment, the methanol (70%) seed extract of *A. precatorius* was well tolerated and produced no toxicity signs, lethality, impairments in body and organ weights, or changes in food or water consumption. The extract also produced no signs of hepatotoxicity, nephrotoxicity, or hematotoxicity or detectable abnormalities in the lipid profile or histology of internal organs. Generally, all the values remained within the normal limits and did not suggest toxic effects in subchronic treatment. The present study, therefore, demonstrates the nontoxic nature of the test extract and thus 200 and 400 mg/kg doses may be used in phytomedical formulations with a low risk of adverse effects. However, further investigations such as to determine its effect in pregnant animals as well as trials in other animals towards the development of drugs from *A. precatorius* should be performed to test its claimed traditional therapeutic value.

Conflict of Interest: No conflict of interest was declared by the authors.

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