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Methanol extract of *Abrus precatorius* seeds on reproduction indices, hepatorenal profiles in female albino rats

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Abstract

Background *Abrus precatorious* is a member of the leguminous family with characteristic red and black seeds. The roots, leaves and seeds of this plant are used for different medicinal purpose. It principally carries flavonoids, triterpene glycosides, abrin and alkaloids. The plant had been mentioned for neuromuscular effects, neuroprotective, abortifacient, antiepileptic, anti-viral, antimalarial, antifertility, nephroprotective, immunomodulator, immunostimulatory properties, antiinflammatory activity, antidiabetic, and many others. The aim of this study was to investigate the anti-fertility activity of the methanol extract of *Abrus precatorius* seeds (APS) in female albino rats administered 45 and 50 mg/kg BW through oral gavage for 30 days. An *Abrus precatorius* seed (APS) was extracted (1:1 w/v) using methanol to obtain the crude extract. Liquid-liquid fractionation was performed on the crude methanol extract using solvents of different polarity.

Methods Eighteen female rats were divided into three groups each containing six animals was used for this experiment. Group one (control), received 1 ml of saline solution, group two received 45 mg/kg and group three received 50 mg/kg body weight by oral gavage daily for a period of 30 days. At the end of the study, Body weight, Organ Weight, Estrous cycle, Hormonal Concentrations, Oxidative Stress Markers, Enzymatic Activities, Biochemical indices (Liver and Kidney panel) and Hematological parameters were also evaluated.

Results Consequent upon the administration of *A.precatorius* seed methanol extract 45 and 50 mg/kg BW into the female rats resulted in changes pertaining to Body weight, Estrous cycle, Hormonal Concentrations, Antioxidant Enzymes, Serum Biochemical indices and Hematological indices significantly ($p < 0.05$) compared to control rats. Initial body weights are significantly ($p < 0.05$) indicated, whereas final weights were decreased significantly ($p < 0.05$). Organ weight changes i.e. Ovary, Liver and Kidney weights were found to be significantly ($p < 0.05$) decreased in APS administered rats compared to control. Estrous cycle study i.e. time duration of Proestrus, Estrus, Metestrus and Diestrus (in days) was significantly prolonged. Hormonal Concentrations represented by FSH, LH, Prolactin, Estradiol were significantly ($p < 0.05$) decreased, whereas Progesterone levels were significantly ($p < 0.05$) increased in APS administered female rats compared to control group of rats. All the antioxidant enzyme assayed CAT, SOD, GPx, GR and GST were found to be significantly ($p < 0.05$) decreased, whereas MDA concentration was found to be increased significantly ($p < 0.05$). Enzymatic activities and biomolecules includes Cholesterol and Ascorbic acid contents were

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significantly increased ($p < 0.05$), but G6PDH and Δ5 – 3β-HSD activities were found to be significantly ($p < 0.05$) decreased in APS administered group of female rats compared to control group. Biochemical indices of liver tissue presented by Bilirubin, SGPT, SGOT, Albumin and Globulin were found to be significantly ($p < 0.05$) increased whereas Alkaline Phosphate and Total Protein contents were found to be significantly ($p < 0.05$) decreased. The biochemical indices of kidney, i.e. Urea and Creatinine were significantly ($p < 0.05$) increased and Uric acid and Calcium contents were found to be significantly ($p < 0.05$) decreased in APS administered female rats compared to control group of rats. Hematological parameters including RBC, Hb, ESR and Clotting Time were found to be significantly ($p < 0.05$) increased but WBC levels were significantly ($p < 0.05$) increased in APS administered female rats compared to control group of rats.

Conclusion *A. Precatorius* seeds have anti-fertility effect on female rats.

Keywords *Abrus precatorius*, Infertility, Estrous cycle, Sex hormones, Antioxidant enzymes, Hematological, Biochemical parameters

Introduction

Herbal medicines are in great demand for preliminary health care due to their wide medicinal values without any side effects. The use of Herbal medicine has increased around the world due to its presumptive efficiency, availability and general acceptance. For basic medical care, almost 75% of the world's population uses herbal medicine. Moreover, the creation of infertility medications derived from plants is seen favorably due to its low toxicity and cost-effectiveness [1]. Plant extracts have a significant therapeutic effect in the treatment of numerous human diseases because of their secondary metabolites [2]. There is a vast array of physiologically active chemicals found in natural products, most of which are derived from plants. Despite significant advancements in contemporary medicine, the creation of novel medications from natural sources is still seen as crucial. 70,000 plants are thought to have therapeutic uses. Ayurveda treats a wide range of illnesses using about 2000 different plant species [3]. The Encyclopedia of Traditional Chinese Medicine lists 5757 plants that serve as the foundation for the Chinese medical system. A wide variety of therapeutic herbs are also used in the medical systems of Korea and Japan [4]. More than 2,000 herbal products of medicinal relevance are blanketed within the Indian Material Medica, four hundred of which might be derived from minerals or animals and the last four hundred from plant life [5]. India is dubbed the "botanical garden of the sector" for acceptable cause-it's miles arguably the area's largest producer of medicinal plant life. Ayurveda and different conventional medical systems prescribe healing medicinal capsules crafted from about 1250 Indian medicinal herbs [6]. The use of natural ingredients to deal with infertility is well known in conventional medicinal drug round the area. *Abras precatorius*, commonly known as precatori bean, rosary pea, or ratti, is an indigenous traditional medicinal plant, the stem of a plant whose powdered seeds are used as an oral contraceptive with the useful resource of Ayurvedic practitioners [7]. Regarding *A. precatorius* infertility remedy sports, the usage of seed extract in male rats has been stated, however there is no documented proof to be had in case of female rats.

A. precatorius seeds are also reported to have anticancer, antifertility, antitumor, antispermatogenic, antibacterial, antidiabetic, antioxidant, nephroprotective, antiarthritic, antimicrobial, and antimalarial activities [8]. *A. precatorius* is widely used in various traditional systems due to its potential effects on reproductive health as it contains several bioactive compounds including flavonoids such as abrin, precatorine, abraline, abrectine, abrusogenin, kaempferol, quercetin, and isorhamnetin [9]. These compounds exhibit different pharmacological properties and may contribute to effects on fertility [9].

Previous studies reported the antifertility activity of *A. precatorius* seed extracts in female rats and also in male rats where these researchers used the crude seeds, steroidal fractions or alcoholic extract of *A. precatorius* seeds for testing the antifertility effect [1]. Previous studies have been carried out on the extracts made from seeds and leaves of *A. precatorious*. The seeds have been shown to have anti fertility effect in males and females, also demonstrated its cytotoxic and anti-tumor effect. Antimicrobial activities of the aqueous extract of the seed of *A. precatorius* have also been studied. Investigated the anti-inflammatory effect of its seeds, the seeds are considered abortifacient and useful in treating diabetes and chronic nephritis [10]. The *A. precatorius* seeds and other parts of the plant are used traditionally as ornament, contraceptive and abortifacient in parts of central Africa and the Far East. The plant also has hypoglycemic effects. *A. precatorius* has potent anti-oxidant activity because of its phenolic and flavonoid constituents [11]. *A. precatorius* has been explored exhaustively for its ethnomedicinal, phytochemical, pharmacological, and ethnopharmacological applications. From the foregoing, it is evident that *A. precatorius* has been used ethnomedicinally as a valuable therapeutic agent for a variety of diseases.

Myriads of phytocompounds found in this plant are responsible for its pharmacological activities. However numerous therapeutic claims have been reported as the plant is gaining widespread popularity in terms of traditional medicinal uses. Therefore more investigations are proposed to validate these claims and even identify new bioactive components with potential therapeutic benefits [12]. Systematic studies are needed to understand the contraceptive mechanism of *A. precatorius* seed powder in rural women. As a first step, it is important to test the anti-reproductive effects of *A. precatorius* seed extract in female rats (Figure 1). Therefore, the aim of the present study was to evaluate the anti-fertility activity of Methanol extracts of *A. precatorius* seeds and to investigate the reproductive parameters such as hormonal profile (LH, FSH, estrogen), major reproductive organs and to investigate the impact on and other important organs.

Therefore, the present study was aimed to evaluate the effect of the methanol (70%) extract of *A. precatorius* seeds on reproductive, hepatic and renal functions in female albino rats.

Materials and methods

Chemical reagents

All other chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich., India.

Seed collection and authentication

Abrus precatorius seeds (APS) were collected from a local market in Tirupati, Andhra Pradesh, India. The seeds were authenticated by faculty member from department of Botany, SVU College of Sciences, Sri Venkateswara University, Tirupati, and a voucher specimen (reference no. 520/Bot/SVU/2023) 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

The air dried seeds were crushed into fine powder. Two hundred and seventy grams (250 g) of powdered seed was extracted using 2 L of methanol (70%) in a soxhlet apparatus at 65^0C for 6 h. The extract obtained was concentrated by simple distillation and evaporation. The yield of the extract was determined and the dried extract was stored at 4^0C until used. Percent yield of all extracts were calculated using following formula:

$$\text{Yield } (\%) = \frac{\text{Weights of solvent free extract (g)}}{\text{Dried extract weight}} \times 100$$

For every sample triplicates flasks were used for extraction and results were expressed as mean ± standard deviation (SD) ($n=3$).

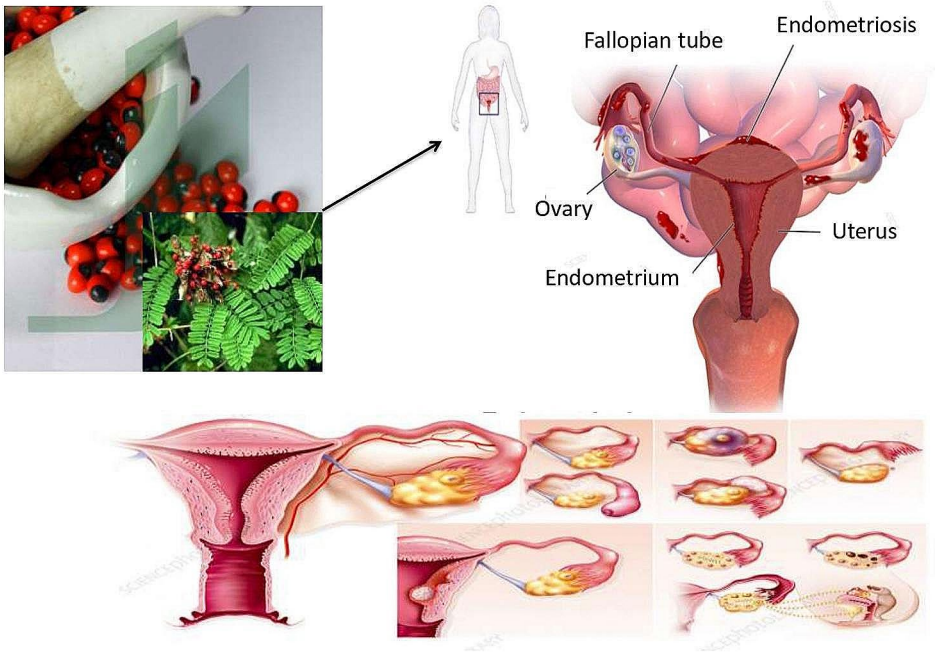

Fig. 1

The history of development of pharmaceutical dosage forms can be traced back to Charak Samhita, the first systematic documentation of Ayurveda. Ayurveda has recommended a comprehensive material medical including medicinal plants, minerals, metals, and products of marine and animal origin. However, the use of herbs has been given priority. Dried seeds of *A. precatorius* are grinded to powder and administered orally one teaspoonful once a day to cure the worm infestation for two days. In veterinary section of medicine, dried seed powder of Abrus is used for the treatment of fractures. The brightly-colored seed of Abrus attracts the children, as a result sometimes the children at the rural villages who don't have any knowledge about the plant and the origin of the seed they eat the seed and got attacked by the toxic effect of the seed of *A. precatorious* if the dose cross the safety limit. Boiled seeds are eaten in certain parts of India. They have a weight of 1/10th of a gram which is almost uniform, hence used as weighing unit [7].

Maintenance of experimental animals

Female albino rats weighing 120–180 g were used for this study. 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 h light/12 hours dark cycle) with standard diet (obtained from M/S Sai Durga Feeds, Bangalore) 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.

Experimental setup

Eighteen (18) female albino rats were used in this experiment. They were divided into three groups of six ($n=6$) animals each. The extract was orally (gavage) administered for a period of 30 days as follows;

Group 1: Control, saline solution (10 mL/kg of body weight).
Group 2: Methanol extract of *APS* (45 mg/kg/day BW).
Group 3: Methanol extract of *APS* (50 mg/kg/day BW) [10].

Measurement of body weight

Body weight of rat from each group was taken after treatment at every 10 days interval.

Tissue homogenization

At the end of the experiment, animals that were in the estrus phase of the estrous cycle were dissected after they were euthanized by diethyl ether. Ovary, Liver, Kidney were removed and placed on a cooled glass slide and their fats removed. A sample of 100 mg of tissue was homogenized in 1mL of Britton-Robinson buffer solution at pH7.4, a phosphate buffer (0.2 mol L^{-1}), containing sodium chloride (2.09 g), potassium chloride (0.09 g), calcium chloride (0.07 g), and magnesium sulfate (0.04 g). The homogenized tissues were centrifuged (ECCO, Germany) at 1000 rpm for 10 min at room temperature and their supernatants were isolated and used for determining Biochemical and enzyme activity.

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 30. 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.

Assessment of estrous cycle

The unstained smear method after Marcondes (2002) [13] was employed to determine the different stages of the estrous cycle.

Hormonal profiling

The stored serum was used to measure the level of follicle stimulating Hormone (FSH), luteinizing hormone (LH), Prolactin, Estradiol and Progesterone concentrations by Chemi Luminescent Immuno Assay (CLIA) and Enzyme Linked Immunosorbent Assay (ELISA) (Bangalore, India). The experiment was performed as per the manufacturer's instructions.

Oxidative stress markers

The Antioxidant Enzyme parameters such as Malondialdehyde (MDA) [14], Catalase (CAT) [15], Superoxide dismutase (SOD) [16], Glutathione peroxidase (GPx) [17], Glutathione reductase (GR) [18] and Glutathione S-transferase (GST) [18] were analyzed in homogenates of ovary.

Estimation of enzymes activities & serum biochemical parameters

Estimation of total cholesterol [19], total ascorbic acid content [20], Glucose-6- Phosphate Dehydrogenase activity [21] and Δ^5-3β- Hydroxy Steroid Dehydrogenase activity [22] were analyzed in homogenates of ovary.

The serum was assayed for assayed for Bilirubin, Glutamic oxaloacetic transaminase (SGOT) activity, Glutamic pyruvate transaminase (SGPT) activity, Alkaline

Table 1 Body weight of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Initial (g)	Final (g)
Control	175 ± 12.35^{a}	184 ± 14.79^{a}
APS-45	189 ± 14.64^{b} +8.00	174 ± 12.58^{b} -5.43
APS-50	191 ± 15.37^{b} +9.14	171 ± 11.35^{b} −7.06

All values are expressed as mean ± SEM, $n=6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p<0.05$

The significance were determined by One-way ANOVA was used to analyze the data

Table 2 Organ Weight of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Ovary (mg/gm bwt)	Liver (mg/gm bwt)	Kidney (mg/gm bwt)
Control	22.82 ± 1.63^{a}	3.29 ± 0.21^{a}	0.537 ± 0.03^{a}
APS-45	18.48 ± 1.35^{b} -19.01	3.08 ± 0.19^{b} -6.38	0.513 ± 0.02^{b} -4.46
APS-50	17.17 ± 1.14^{b} -24.75	3.02 ± 0.17^{b} -8.20	0.511 ± 0.02^{b} -4.84

All values are expressed as mean ± SEM, $n=6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p<0.05$

The significance were determined by One-way ANOVA was used to analyze the data

phosphatase (ALP) activity, Total protein, Albumin, Globulin, Urea, Uric acid, Creatinine, and Calcium using clinical autoanalyzer (Erba Magnum EM200) (Bangalore, India).

Hematological investigations

The blood or hematological parameters analysed were of follows [23]. The blood or hematological parameters were analyzed using an automated hematology analyzer (Procan Electronics, Model PE6800). The parameters included White blood cell (WBC), Red blood cell (RBC), Hemoglobin (Hb) concentration, erythrocyte sedimentation rate (ESR) and Clotting Time.

Statistical analysis

The data were analyzed with one-way ANOVA (analysis of variance), followed by the Tukey and Scheffe tests. Statistical analysis was done with SPSS v. 17.5 software; $p<0.05$ was considered as statistically significant difference.

Results

Effects of APS in body weight changes

The final body weights of control and treated female rats showed a significant decrease ($p<0.05$) compared to the initial body weight. The body weights recorded in APS

Table 3 Estrous cycle of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Total duration in each phase (in days)			
	Proestrus	Estrus	Metestrus	Diestrus
Control	16 ± 0.91^{a}	18 ± 1.01^{a}	26 ± 1.32^{a}	57 ± 3.43^{a}
APS-45	22 ± 1.25^{b} +37.50	24 ± 1.37^{b} +33.33	34 ± 2.38^{b} +30.76	82 ± 5.47^{b} +43.85
APS-50	24 ± 1.36^{b} +50.00	26 ± 1.31^{b} +44.44	38 ± 2.56^{b} +46.15	86 ± 5.84^{b} +50.87

All values are expressed as mean ± SEM, $n=6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p<0.05$

The significance were determined by One-way ANOVA was used to analyze the data

both administrations decreased significantly compared to the control group ($p<0.05$) (Table 1).

Effects of APS in organ weight changes

APS at both doses presented showed a significant reduction ($p<0.05$) in the weight of the ovaries, liver and kidneys compared to the control group (Table 2).

Effects of APS in estrous cycle study

Estrous cycle analysis revealed APS in both administrations, the duration of Proestrus, Estrus, Metestrus and Diestrus in APS in both administrations significantly increased ($p<0.05$) compared to the control group (Table 3).

Effects of APS in hormonal effects

The mean values of reproductive hormone levels were presented in Table 4. Serum levels of FSH, LH, Prolactin, and Estradiol significantly decreased ($p<0.05$) in APS Administered group compared to control group. Serum Progesterone concentration significantly increased ($p<0.05$) in the APS-treated group compared to the control group.

Effects of APS in antioxidant enzyme effects

Antioxidant parameters, measured in the ovarian tissue of the rat groups, are shown in Table 5. APS administration significantly increased ($p<0.05$) ovarian MDA compared to the control group. The concentration of ovarian CAT, SOD, GPx, GR, GST significantly decrease ($p<0.05$) in APS in both administered rats compared to the control group.

Effects of APS in enzymatic activities effects

The Cholesterol and Ascorbic acid contents in the ovarian tissues showed to be significantly increased ($p<0.05$) in APS administered group compared to the control group. Ovarian G6PDH and Δ^{5}-3β-HSD showed a

Table 4 Hormonal concentrations of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (ng/ml)	Estradiol (ng/ml)	Progesterone (ng/ml)
Control	7.36 ± 0.48[a]	6.18 ± 0.39[a]	148.52 ± 9.64[a]	487.29 ± 36.25[a]	46.72 ± 2.92[a]
APS-45	4.87 ± 0.29[b] -33.83	4.12 ± 0.22[b] -33.33	106.47 ± 6.23[b] -28.31	349.84 ± 24.86[b] -28.20	68.93 ± 4.61[b] +47.53
APS-50	4.38 ± 0.25[b] -40.48	3.84 ± 0.19[b] -37.86	103.63 ± 6.01[b] -30.22	337.69 ± 22.42[b] -30.70	73.27 ± 4.94[b] +56.82

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

Table 5 Oxidative stress markers of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	MDA (μmol/g protein)	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	GR (U/mg protein)	GST (U/mg protein)
Control	7.07 ± 0.42[a]	4.97 ± 0.24[a]	9.75 ± 0.51[a]	3.84 ± 0.23[a]	4.62 ± 0.31[a]	6.49 ± 0.36[a]
APS-45	10.28 ± 0.53[b] +45.40	2.85 ± 0.16[b] -42.65	6.28 ± 0.32[b] -35.58	2.27 ± 0.13[b] -31.51	3.03 ± 0.19[b] -34.41	2.82 ± 0.16[b] -56.54
APS-50	11.12 ± 0.63[b] +57.28	2.46 ± 0.10[b] -50.50	5.97 ± 0.26[b] -38.76	2.03 ± 0.11[b] -47.13	2.81 ± 0.15[b] -39.17	2.56 ± 0.14[b] -60.55

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

Table 6 Enzymatic activities of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Cholesterol (μg/mg)	Ascorbic acid (μg/mg)	G-6-PDH (IU/mg)	Δ^5 -3β-HSD (IU/mg)
Control	48.79 ± 2.86[a]	66.35 ± 4.32[a]	4.03 ± 0.26[a]	1.26 ± 0.06[a]
APS-45	74.35 ± 4.85[b] +52.38	88.76 ± 6.13[b] +33.77	2.47 ± 0.14[b] -38.70	0.65 ± 0.03[b] -48.41
APS-50	81.62 ± 5.92[b] +67.28	89.57 ± 6.74[b] +34.99	1.92 ± 0.11[b] -52.35	0.59 ± 0.02[b] -53.17

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

significant decrease ($p < 0.05$) between the control and experimental groups (Table 6).

Effects of APS in biochemical changes

Biochemical parameters of liver and kidney tissues were presented in Tables 7 and 8, respectively APS in both rats administered groups showed a significant increase ($p < 0.05$). Where as in serum biochemical parameters such as Bilirubin, SGPT, SGOT, Alkaline Phosphate, Albumin, Globulin, Urea and Creatinine, showed a significant increase between the control and experimental groups. A significantly decrease ($p < 0.05$) in serum Total Protein, Uric acid, Calcium were APS in both both treated rats compared to the control group.

Table 7 Biochemical indices (Liver panel) of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Bilirubin mg/dl	SGPT IU/L	SGOT IU/L	Alkaline Phosphate IU/L	Total Protein mg/dl	Albumin mg/dl	Globulin mg/d
Control	0.72 ± 0.03[a]	27.18 ± 1.34[a]	34.62 ± 1.75[a]	192.85 ± 9.83[a]	5.67 ± 0.28[a]	2.64 ± 0.11[a]	2.59 ± 0.10[a]
APS-45	0.94 ± 0.05[b] +30.55	39.36 ± 1.98[b] +44.81	47.37 ± 3.02[b] +36.82	137.69 ± 7.41[b] -28.60	3.42 ± 0.18[b] -39.68	3.72 ± 0.19[b] +40.90	3.68 ± 0.18[b] +42.08
APS-50	0.98 ± 0.06[b] +36.11	42.13 ± 2.97[b] +55.00	49.85 ± 3.14[b] +43.99	131.87 ± 6.83[b] -31.62	3.04 ± 0.16[b] -46.38	3.95 ± 0.21[b] +49.62	3.85 ± 0.20[b] +48.64

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

Table 8 Biochemical indices (Kidney panel) of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Calcium mg/dl
Control	22.38 ± 1.35^{a}	0.87 ± 0.05^{a}	3.57 ± 0.19^{a}	7.98 ± 0.45^{a}
APS-45	33.64 ± 1.96^{b} +50.31	0.51 ± 0.03^{b} -41.37	4.96 ± 0.25^{b} +38.93	4.36 ± 0.21^{b} -45.36
APS-50	35.86 ± 2.02^{b} +60.23	0.47 ± 0.02^{b} -45.97	5.13 ± 0.36^{b} +43.69	4.08 ± 0.20^{b} -48.87

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

Effects of APS in Hematological effects

The Hematological parameters of the treated groups and the control group are presented in Table 9. The APS in both treated rats showed a significant decrease ($p < 0.05$) in RBC, Hb, ESR and Clotting Time compared to control rats, while it significantly increase ($p < 0.05$) in WBC compared to control rats.

Discussion

There are very few and speculative studies on the impact of plant products on the female reproductive system and fertility. There has never been a bigger need for contraception from the standpoint of public health [24].

The present study was conducted to investigate the anti-fertility effect of APS in two different dose administrations i.e. 45 and 50 mg/kg BW in to female albino rats and showed that the body weight of female rats decreased because of estrogen and progesterone. In the present study, rats given APS, decreased ovarian, liver and kidney functions. A significant reduction in ovarian in the administration of APS at these dose levels may be due to the absence or decreased availability of ovarian steroid hormones and gonadotropins [25]. The liver weight of APS administrations rats was significantly decreased due to loss of water and glycogen [26]. In the present study, the organ weights of kidneys of the APS administrations rats were significantly decreased from those of the control rats. This may be because some of the bioactive substances in APS studies have weight loss properties [27]. However, APS causes significant reduction in the weights of liver and kidney, suggesting the risk of acute toxicity [28].

In the estrous cycle study, there was a significant increase in the duration of proestrus, estrus, metestrus and diestrus in APS administered rats. Disruption of the estrous cycle can be caused by APS administration of estrogen/progesterone. Exogenous estrogen (or estrogen-like) causes a decrease in gonadtrophins (FSH, LH) through a negative feedback mechanism [29]. This will cause anovulation and a decrease in the weight of the ovaries [11]. This interruption of the estrous cycle may be due to the effect of APS administration on the ovary which controls the ovarian functions and the estrous cycle through ovarian and extra-ovarian hormones. The diestrous phase is maintained by the activity of the corpus luteum which produces progesterone in the absence of pregnancy and may be responsible for maintaining the diestrous phase [30].

The results obtained pertaining to hormonal concentrations in the present study demonstrates that consequent up on APS administration into female rats, causes a significant ($p < 0.05$) decrease in serum concentration FSH, LH, Prolactin and Estradiol and a significant decrease in progesterone in APS administered rats compared to control group. FSH and LH levels may reduce the number of developing follicles and increased the number of atretic follicles in the ovary, since gonadotropic hormones were directly or indirectly responsible for the growth and development of follicles [31]. Prolactin levels were significantly increased in the APS administrations compared to the control group. These findings gain supports from earlier reports that the combination of increased prolactin and suppressed LH secretion is due to the prolongation of the estrus cycle. An imbalance in endogenous estrogen and progesterone levels may be responsible for Anti-implantation activity [32]. Progesterone levels were significantly increased in the APS administrations

Table 9 Hematological parameters of female albino rats administered *Abrus precatorius* seeds methanol extract

Dose (mg/Kg)	RBC (Count $\times 10^6$/mm^3)	WBC (Count $\times 10^3$/mm^3)	Hb (gm/dl)	ESR (mm/hr)	Clotting Time (sec)
Control	5.37 ± 0.22^{a}	4.02 ± 0.19^{a}	11.76 ± 0.69^{a}	2.26 ± 0.17^{a}	36.58 ± 2.26^{a}
APS-45	3.26 ± 0.17^{b} -39.29	5.57 ± 0.23^{b} +38.55	8.34 ± 0.48^{b} -29.08	1.52 ± 0.11^{b} -32.74	24.23 ± 1.65^{b} -33.76
APS-50	3.15 ± 0.13^{b} -41.34	5.84 ± 0.26^{b} +45.27	7.98 ± 0.33^{b} -32.14	1.43 ± 0.09^{b} -36.72	23.81 ± 1.47^{b} -34.90

All values are expressed as mean ± SEM, $n = 6$ rats in each group

+ and – percent increase and decrease respectively over control

Values with different superscript letter are significantly different from each other $p < 0.05$

The significance were determined by One-way ANOVA was used to analyze the data

compared to the control group. APS caused the survival of the corpus luteum and dilated its granulosa lutein cells' smooth endoplasmic reticulum (SER) that responds to high secretion of progesterone [33]. Administration of APS increases serum prolactin levels and significantly decreases estradiol, progesterone, FSH and LH. This shows a negative effect on follicle maturation and ovulation and therefore shows that the substance can be used as a contraceptive [10].

The data obtained in the study were based on the effects of APS Administration on ovarian antioxidant enzyme levels in female albino rats showed that MDA was significantly increased in APS Administration rats compared to controls. MDA is an end-product of lipid peroxidation. Lipid peroxidation is known to be the most damaging effect of free radicals on the cell. It has been reported that APS causes oxidative damage in the ovarian tissue, increasing the concentration of MDA [34]. In the ovaries of rats receiving APS, the levels of CAT, SOD, GPx, GR and GST decreased compared to the control group. The over production of oxygen free radicals during ovulation leads to the activities of CAT, SOD, GPx, GR, GST enzymes. These results indicate that the decrease in the level of antioxidant enzymes may be one of the factors that lead to infertility in female rats. Reports show that an increase in the ROS levels is associated with successful ovulation [35].

In our study, the effect of APS Administration on ovary enzymes activity levels in female albino rats showed that ovarian tissue cholesterol and ascorbic acid increased in rats receiving APS. The result of ovarian stimulation with some gonadotrophic hormones and the reduction of ascorbic acid from the ovary, as well as the reduction/elimination of the secretion of gonadotrophic hormones and ovarian atrophy are also present. Therefore, in the present study, the concentration of cholesterol and ascorbic acid in the ovaries of the treated rats indicates ovarian hypofunction (hypofunction of ovarian steroidogenic activity). In our study, for ovarian tissue in rats receiving APS, the levels of G-6-PDH and Δ^5-3β-HSD decreased. It has been shown that gonadotrophins, by activating the metabolism of G-6-PDH, increase the production rate of NADPH, necessary for the hydroxylation reaction and the formation of gonadal steroids from cholesterol in the ovarian tissue. Thus, G-6-PDH is an important factor in ovarian steroidogenesis. It has been shown that, in corpora lutea animals, there is a positive relationship between progesterone synthesis and increased activity of Δ^5-3β-HSD and G-6-PDH in the luteal tissue. The Δ^5-3β-HSD is an important enzyme in the production of steroid hormones. The presence of the enzyme indicates that the steroidogenic activity of the tissue and estrogen synthesis in increased amount is associated with heightened Δ^5-3β-HSD and G-6-PDH activities in the follicular granulose cells of the polycystic ovaries and in the ovaries of rats [36].

So far a small number of plants have been reported to have antifertility potential out of them only a few have been reached at clinical evaluation. Thus, hunting for herbal materials with the capacity to disturb the endocrine function of the hypothalamic pituitary-gonadal axis, then it regulates the strategies of infertility. Due to the administration of the principle compounds with the female rats. There exists a strong relationship between female reproductive system with infertility through the use of plant materials. Sufficient association exists between blood components and infertility has been established in limited cases [37].

The effects of the APS appear to be specific to the reproductive system and not accompanied with systemic toxicity in the female rats. This is evident from the lack of change in the whole body and organ weights and kidney and liver, as well as hematological parameters.

The results showed that APS administration affected many biochemical functions of blood serum, liver and kidney. In the present study, liver function showed that Bilirubin, SGPT, SGOT, Albumin and Globulin were increased, while Alkaline Phosphate and total protein levels were decreased in the APS compared to control group, which is evinced through the liver damage and serum enzyme levels in the presented study. Such damage can be linked to the permeability of hepatocyte membranes, the result of the generation of certain lesions following the association of glycidamide with the same function of membrane proteins [38]. Another important aspect of this study is related to kidney function parameters such as urea and creatinine. Decreased levels of uric acid and calcium, which indicate kidney function. Kidney function may be due to the decreased functional capacity of tubular secretion [39].

In this study, the hematological analysis such as reduction of RBC, Hb, ESR and Clotting Time and increase of WBC in rats receiving APS compared to controls. It has anti-fertility properties but it disturbed the hematological parameters due to which a loss of body weight observed in the treated rats [40].

Conclusions

The current study shows that the effect of APS administrations on the estrous cycle, hormonal concentrations, antioxidant enzymes (Tissue), enzymatic activities (Tissue), serum biochemical indices (liver and kidney panel) and hematological parameters significance disturbance in ovary tissue, liver and kidney. The main effect of APS administration likely to disrupt the estrous cycle pattern, reduce the serum levels of gonadotropins, which subsequently affect folliculogenesis and steroidogenesis in the ovarian tissue of female rats. The results of the present

study provide evidence of the infertility effects of action of APS administration and its effects on female rats. Hence this study is justified.

Abbreviations

ALP	Alkaline phosphatase
APS	*Abrus precatorius* seeds
CAT	Catalase
ESR	Concentration, Erythrocyte Sedimentation Rate
FSH	Follicle Stimulating Hormone
G-6-PDH	Glucose-6- Phosphate Dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione S-transferase
Hb	Hemoglobin
LH	Luteinizing Hormone
MDA	Malondialdehyde
RBC	Red blood cell
SGOT	Glutamic oxaloacetic transaminase
SGPT	Glutamic pyruvate transaminase
SOD	Superoxide dismutase
WBC	White blood cell
Δ5 – 3β-HSD	Δ5 – 3β- Hydroxy Steroid Dehydrogenase

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
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Competing interests
The authors declare no competing interests.

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