Evaluation of Nephroprotection and Reproductive Safety of *Astragalus spinosus* Extract Against Bisphenol A Toxicity in Pregnant Rats

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Abstract

Bisphenol A is an environmental pollutant that can lead to functional and histological disorders in the kidneys because of its toxic effects and oxidative stress, particularly during pregnancy. The current study aimed to evaluate the toxic effects of BPA on kidneys function and histological alterations in pregnant female rats, and highlighting the possible protective role of *Astragalus spinosus* in mitigating these effects. For 20 days, twenty-four female albino rats weighing 150-180 grams were divided into six groups (n = 4) at random control (G1), extract-only(G2), BPA treated 250 mg/kg (G3) and 500 mg/kg (G4) and combined treatment groups extract with BPA 250 mg/kg (G5) and extract with BPA 500 mg/kg (G6), the extract was taken orally with BPA that had been dissolved in olive oil, following the experiment, kidney tissues were preserved in formalin and prepared for histopathological analysis using standard methods, blood samples were also taken for biochemical investigation of kidney function, including urea creatinine. The results indicated that BPA caused clear disorders in the function and tissue of kidneys, with the more severe as the dosage increased, these disorders manifested as raised urea and creatinine levels, and histological alterations such as necrosis, glomerular atrophy, congestion, and epithelial cell destruction, it also had negative effects on fetuses, with some treated groups showing fetal distribution abnormalities, delayed fetal growth, and stillbirth, on other hand, the aqueous extract of the aerial parts of *Astragalus spinosus* revealed a partial protective effect on the kidneys, although some miscarriages were reported in the groups treated with it. According to the study's findings, BPA has detrimental effects on pregnancy and fetuses as well as functional and histological abnormalities in the kidney that worsen with increasing dosage, although some incidences of miscarriage were documented in the groups treated with the aqueous extract of the aerial portions of *Astragalus spinosus*, there was a partial protective effect against nephrotoxicity.

Keywords: Bisphenol A, *Astragalus spinosus*, Nephrotoxicity, Kidney function, Fetuses, Miscarriage, Stillbirth.

Introduction

Many common consumer goods contain Bisphenol A (BPA), an industrial chemical compound widely used in the production of epoxy resins and polycarbonate plastics. Due to its widespread use,

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environmental exposure to it has increased, making it one of the major pollutants of global health concern, because BPA can alter hormonal balance and affect a number of biological processes in the body, it is classified as an endocrine disruptor[11]. Recent research indicates that exposure to BPA is linked to a number of adverse consequences, including damage to the liver, nervous system, and kidneys. Given the kidneys' crucial role in filtering blood and removing toxins, they are among the organs most susceptible to these effects, according to a recent study, BPA exposure causes nephrotoxicity, characterized by oxidative stress, DNA damage, and activation of renal cell death pathways and fibroblasts, leading to acute kidney failure[12]. The primary cause of BPA's nephrotoxicity is elevated reactive oxygen species (ROS) generation, which leads to oxidative imbalance in cells and damages lipids, proteins, and DNA. Furthermore, BPA exacerbates kidney tissue damage by inducing inflammatory pathways and apoptosis. Exposure to this toxicant has been shown in experimental trials to cause distinct histological alterations, including as tube damage and glomerular dysfunction[10]. Pregnancy is one of the most sensitive periods for exposure to environmental pollutants, endocrine disruptors such as BPA can affect normal hormonal balance, potentially impacting reproductive health and pregnancy success, numerous studies have linked BPA exposure to various reproductive disorders, including an increased risk of fetal loss, miscarriage, and fetal growth retardation, therefore, studying its effects during pregnancy is crucial [3]. On the other hand, because medicinal plants contain active compounds such as flavonoids and saponins, which possess anti-inflammatory and antioxidant properties, interest in their use as a preventative measure against environmental pollutants has increased, several studies have shown that *Astragalus spinosus*, an important medicinal herb, contains potent biochemical capable of reducing oxidative stress and protecting tissues from damage, this plant has a promising protective effect [5]. The field of nephrotoxicity during pregnancy, particularly in pregnant rats, suffers from a severe lack of research, despite the abundance of studies addressing the harmful effects of BPA, furthermore studies investigating the protective effects of medicinal plants against BPA toxicity remain scarce, especially regarding the use of an extract of the aerial parts of *Astragalus spinosus*, and its relationship to histological and biochemical changes in the kidneys within a single experimental model, this study aims to evaluate the histopathological and biochemical changes induced by BPA exposure in pregnant rats and to elucidate the efficacy of an extract of the aerial parts of *Astragalus spinosus*, in preventing nephrotoxicity, with a focus on its potential role as an antioxidant in mitigating this toxicity.

Material and Methods

Plant collection

Astragalus spinosus was collected from the Al-Zalaya area in Salah Al-Din Governorate, Iraq. It is considered a wild plant, as shown in figure(1), by taking the aerial parts (stem, leaves, flowers and fruits) from the beginning of February until the end of March 2025.

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Figure 1: show *Astragalus spinosus* collected from the selected area

Preparation of the aqueous extract of *Astragalus spinosus*

An aqueous extract was prepared from the aerial parts (leaves, stem, flowers and fruits) of *Astragalus spinosus*. The plant specimens were collected, thoroughly cleaned by washing with distilled water, dried away from sunlight, and then finely ground using an electric grinder to obtain a fine powder. Ten grams of the powdered aerial parts were weighed and added to 100 ml of distilled water. The mixture was heated to a gentle boil and then boiled for 30 minutes with continuous stirring using a magnetic stirrer. The extract was then allowed to cool to room temperature and filtered through cheesecloth to remove any remaining solids. The filtrate was transferred to a centrifuge to remove any remaining impurities. Finally, the clear extract was collected, placed in glass bottles, and refrigerated.

Preparation of Bisphenol A

Bisphenol A (BPA) was obtained from MACKLIN in the form of white crystals stored in an opaque glass container. It was prepared by grinding the crystals in a ceramic mortar to obtain a fine powder, which was then dissolved in olive oil prepared by Savola Gide San.ve Tic.A. The oil was used as a solvent and carrier.

Collection of serum samples

Blood was drawn directly from the heart by pricking it with a medical syringe, blood samples were placed directly into gel test tubes for no more than two hours, the tubes were then transferred to a centrifuge at 3000 rpm for 15 minutes to obtain serum, after that, biochemical tests for kidney function creatinine and urea were performed using a spectrophotometer, according to the instructions of the kit manufacture (Randox/England)[21].

Animal preparation

The experiment was conducted in accordance with the ethical guidelines adopted by the Iraqi Society for the Protection of Animal Rights, which were approved by the Animal Research Ethics Committee under the approval reference number [SCITUA-0004] of the Iraqi Biological Society. The laboratory animals were obtained from the College of Veterinary Medicine at Tikrit University and were kept in cages for ten days to ensure there health and acclimatization to their environment. Twenty four adult female white rats, weighing between 150-180grams, were used in this experiment. The animals were divided into six groups of four animals in each cage, with two adult male white rats placed with each group, pregnancy was confirmed by the presence of a vaginal plug which was considered evidence of successful mating, and the gestation period was calculated up to day 19.The animals were dissected after anesthesia, humane and ethical considerations were taken into account when handling laboratory animals to minimize pain and stress as much as possible, at the end of the experiment, euthanasia was performed using an overdose of anesthesia, and biological and chemical waste, including materials containing BPA, was disposed of according to

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established biosafety and chemical safety guidelines.

First group (G1): The control group.

Second group (G2): Orally administered an extract of the aerial parts of the *Astragalus Spinosus* plant at a dose of 1.5 ml per day for each animal for 20 days

Third group (G3): Orally administered Bisphenol A 250 mg/kg dissolved in olive oil at a dose of 1 ml per day for each animal for 20 days.

Fourth group (G4): Orally administered Bisphenol A 500 mg/kg dissolved in olive oil at a dose of 1 ml per day for each animal for 20 days.

Fifth group (G5): Orally administered Bisphenol A at a concentration of 250 mg/kg at a dose of 1 ml and an extract of the aerial parts of the *Astragalus Spinosus* plant at a dose of 1.5 ml per day for each animal for 20 days.

Sixth group (G6): Orally administered Bisphenol A 500 mg/kg at a dose of 1 ml and *Astragalus Spinosus* aerial parts extract at a dose of 1.5 ml per day for each animal for 20 days.

Histological Study

Rats were sacrificed by anesthetizing them with chloroform. They were then dissected, and the kidneys were removed and washed with distilled water, immediately after removal, the kidneys were fixed in a 10 % diluted formalin solution for 48 hours after the fixation period, it was removed from the formalin solution and washed with tap water for minutes to remove any remaining fixative, dehydration was then performed using a series of graduated alcohol concentrations (50%, 70%, 80% 90%) for three hours, place it twice in 100% absolute ethyl alcohol for one hour each time, afterwards, the clearing process was performed using xylene for 30 minutes, after the clearing process was completed, the infiltration process was carried out, in which the samples were transferred to glass beakers containing paraffin wax in a 1:1 ratio of xylene and paraffin with a melting point of 58-62 for two hours, the wax embedding process was then carried out in iron molds, and they were cut using a manual microtome to a thickness of 5 micrometers, and stained with hematoxylin-eosin.

Statistical analysis

The results were statistically analyzed using the Variance Analysis of Variance (ANOVA). The values in the table represent (Mean ± SD). Significant differences were determined according to Duncan's multiple range test, and comparisons were made at a probability level of ($P \leq 0.05$) for significant values[19].

Results and Discussion

Biochemical indicator results

Urea and Creatinine Concentration

The results of the current study, as shown in Table (1), indicate clear differences in serum urea levels between the different experimental groups. The $F_{=}$ value reached 3.03 at a significance level of $P_{=}$ 0.05, where the control group (G1) recorded an average of (47.00 ± 7.91), which represents the normal level of urea. The group with the aqueous extract of the aerial parts of the plant (G2) recorded an average of (48.63 ± 9.64), which indicates that no significant changes occurred compared to the control group. The group treated with BPA at a concentration of 250 mg/kg (G3) showed a clear increase in urea levels with an average of (68.90 ± 8.61), while the group treated with BPA at a concentration of 500 mg/kg (G4) recorded the highest increase in urea levels with an average of (87.60 ± 6.62), indicating a dose-dependent toxic effect. As for the groups treated with the plant extract with BPA, the aerial parts group

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with BPA at a concentration of 250 mg/kg (G5) recorded (50.93 ± 7.05), while the aerial parts group with BPA at a concentration of 500 mg/kg (G6) recorded (61.00 ± 8.57).

The results of the current study, shown in Table 1, indicate significant differences in creatinine concentration levels between the experimental groups. Serum creatinine concentration was clearly and significantly affected by the different treatments, as evidenced by the F=2.67 and P = 0.05 values. The mean creatinine concentration in the control group (G1) was (0.400 ± 0.0816), while the group treated with the aqueous extract of the aerial parts (leaves, stem, and fruits) of the *Astragalus spinosus* plant (G2) showed a mean of (0.500 ± 0.0816), indicating that the plant extract itself did not affect kidney function. Regarding the treatment with BPA (BPA), the group treated with 250 mg/kg BPA (G3) showed a mean of (1.6750 ± 0.0957), indicating a significant increase in creatinine levels compared to the control group and the other groups. This reflects a clear detrimental effect at this concentration. The 500 mg/kg BPA concentration (G4) recorded the highest average (2.400 ± 0.1000) with a dose-dependent increase. The groups treated with the plant extract with BPA showed a relative decrease in creatinine levels compared to the BPA group alone. The aerobic parts group with a concentration of 250 mg/kg (G5) recorded an average (0.955 ± 0.0273) and with a concentration of 500 mg/kg (G6) recorded an average (1.003 ± 0.173).

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Table 1:Show Average Creatinine and Urea levels for the study groups

Group symbol	Group name	Creatinine	Urea
G1	Control	0.400 ± 0.0816 e	**47.00 ± 7.91 de**
G2	*Astragalus spinosus* extract 1.5 ml	0.500 ± 0.0816 e	**48.63 ± 9.64 de**
G3	250 mg/kg 1 ml BPA	1.6750 ± 0.0957 b	**68.90 ± 8.61 b**
G4	500 mg/kg 1 ml BPA	2.400 ± 0.1000 a	**87.60 ± 6.62 a**
G5	BPA 250 mg/kg 1 ml + *Astragalus spinosus* ectract 1.5 ml	0.955 ± 0.0273 d	**50.93 ± 7.05 d**
G6	BPA 500 mg/kg 1 ml + *Astragalus spinosus* extract 1.5 ml	1.003 ± 0.173 d	**61.00 ± 8.57 c**

The different letters indicate a semantic difference (P < 0.05).

Tests for renal function, particularly urea and creatinine levels, were utilized to determine whether kidney disease or damage was present, in comparison to control group, the *Astragalus spinosus* aerial parts extract group, the 250 mg/kg BPA treated group, and the groups treated with the extract and BPA, our study's data demonstrate that a 500 mg/kg dose of BPA significantly increased serum renal function markers, kidney disease is the cause of the increased amounts of creatinine and urea.

The current study's findings are in line with other earlier research that showed exposure to BPA causes obvious abnormalities in renal function as well as elevated levels of creatinine and urea due to oxidative stress and kidney tissue damage, rats exposed to BPA had significantly higher levels of creatinine and urea, according to a study by [17]. The study's findings are also in line with recent research that demonstrated that exposure to BPA significantly raised nephrotoxicity markers due to elevated oxidative stress and

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free radical generation[1][8]]. According to[7] rats exposed to BPA had a marked rise in creatinine and a fall in renal clearance, these results were corroborated by[6] who showed that long term exposure to BPA causes systemic kidney diseases with distinct biochemical alterations in renal function markers. *Asragalus spinosus* extract is comparatively safe and does not produce nephrotoxicity, as evidenced by the plant extract group's lack of significant alterations as compared to the control group, this is consistent with research by[8] which shown that antioxidant-rich plant substances can preserve and stop detrimental biochemical alteration. The results indicate no significant differences between the two groups treated with BPA and the aqueous extract of the plant, the BPA group at a concentration of 250 mg/kg and the BPA group at a concentration of 500 mg/kg, this suggests that the aqueous extract of the aerial parts of *Astragalus spinosus* exhibited similar protective efficacy against both low and high doses of BPA, this may be attributed to the presence of active compounds in the aerial parts of the plant, which possess antioxidant properties that contributed to reducing kidney damage and maintaining creatinine levels within a similar range between the two groups [2].

Histological evaluation of the effect of BPA and the protective role of the *Astragalus spinosus*

G1: The results of the current study showed that the healthy control group exhibited a normal shape of the proximal convoluted tubule and the distal convoluted tubule, as well as a normal shape of the renal glomerulus and nephrons surrounded by bowman's capsule as in figure(2).

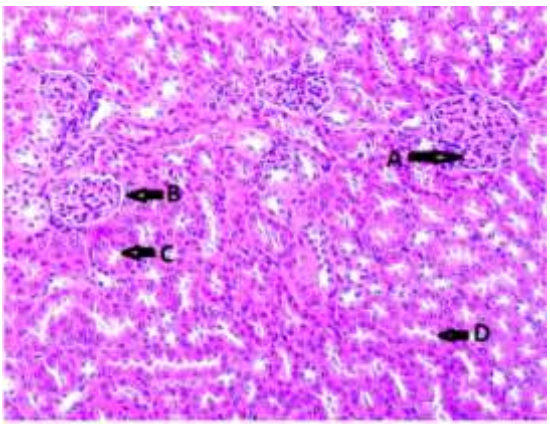

Figure2: show kidney tissue, control group, glomerulus (A), nephrons surrounded by bowman's capsule (B), proximal convoluted tubule (C), distal convoluted tubule (D) (H&E 10X).

G2: The results of this group showed no negative effect of the aqueous extract of the aerial parts of *Astragalus spinosus* after a one-month treatment period, as the renal medulla, glomerulus, proximal convoluted tubule, and distal convoluted tubule appeared normal as in figure (3).

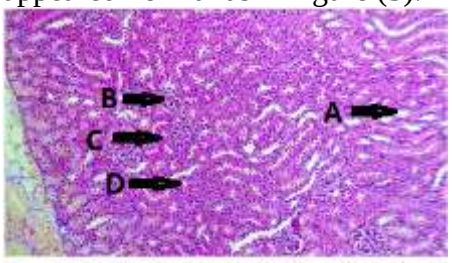

Figure3: show Kidney tissue, *Astragalus spinosus* group for 30 days, renal medulla (A), glomerulus (B), proximal convoluted tubule (C), and distal convoluted tubule (D) (H&E 4X).

G3: The results of this study showed the presence of an inflammatory focus around the convoluted tubule in the renal cortex. We also note the presence of fibroblasts around the convoluted tubule and close to the renal glomerulus, tubular scaling, vascular congestion, red blood cells, destruction of Bowman's capsule wall, and glomerular atrophy in the renal cortex. It also showed an enlargement of Bowman's space, in addition to the rupture of the capsule and the hypertrophy and necrosis of the tubular epithelial cells, as shown in Figure(4).

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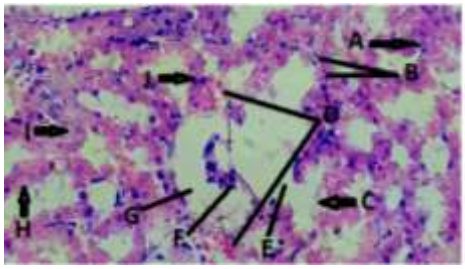

Figure4: show Kidney tissue, BPA 250 mg/kg group, inflammatory focus (A), fibroblasts (B), tubular desquamation (C), vascular congestion and the appearance of erythrocytes (D), bowman's capsule wall destruction (E), glomerular atrophy in the renal cortex (F), dilatation of bowman's space (G), nuclear necrosis (H), hypertrophy and necrosis of the tubular epithelial cells (I), and pyknosis (J) (H&E 40X).

G4 :The histological changes in this group showed damage resulting from a 500 mg/kg dose of BPA, including necrosis of the renal tubules, columnar matter, deterioration of cytoplasmic vacuoles, thickening of the tubular basement membrane, dilation of the renal tubules, degeneration of epithelial cells, and vascular congestion. Red blood cells resulting from hemorrhage were also observed, as shown in Figure (5).

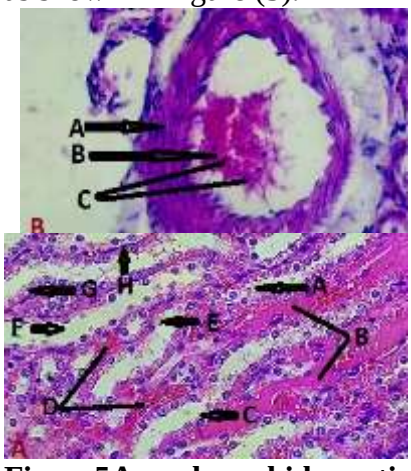

Figure5A : show kidney tissue, BPA 500 mg/kg group, nuclear necrosis (A), casts (B), cytoplasmic vacuolar degeneration (C), vascular congestion and appearance of erythrocytes (D), degeneration of epithelial cells (E), dilatation of renal tubules (F), necrosis (G), and thickening of the basement membrane (H). B: arterial wall thickening (A), thrombus containing neutrophils(B), neutrophils (C) (H&E 40X).

G5: In this group, the results showed protection of kidney tissue, but it was partial, as evidenced by desquamation of tubular epithelium, aggregation of inflammatory cells, and nuclear necrosis as in figure(6).

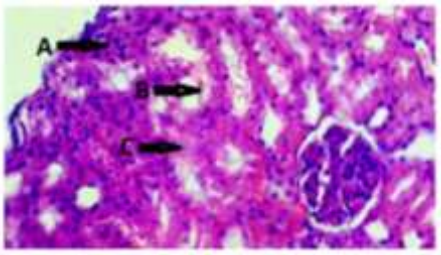

Figure6: show kidney tissue, BPA 250 mg/kg and *Astragalus spinosus* group, aggregation of inflammatory cells (A), desquamation of tubular epithelium (B), and nuclear necrosis (C) (H&E 40X).

G6: The results of this group showed atrophy and fragmentation of the glomerulus, dilatation of bowman's space, tubular desquamation, and appearance of congestion and hemorrhage as in figure (7).

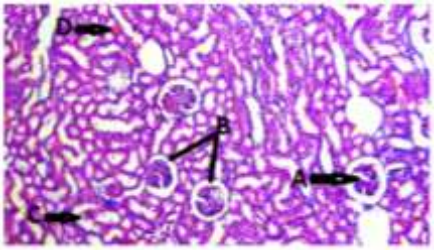

Figure7: show kidney tissue, BPA 500 mg/kg with Astragalus spinosus group, atrophy and fragmentation of the glomerulus (A), dilatation of bowman's space (B), tubular desquamation (C), congestion and hemorrhage (D) (H&E 10X).

The result of the current study showed that the aqueous extract of the aerial parts of the *Astragalus spinosus* plant did not induce any histological changes in the kidney. The renal cortex retained its normal structure, and glomeruli and proximal and distal convoluted tubules remained intact, indicating the safety of the extract and the absence of any toxic effects on kidney tissue, this is attributed to the

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plant's content of active compounds with antioxidant and anti-inflammatory properties, such as flavonoids and saponins, which contribute to maintaining the integrity of kidney cells and reducing oxidative stress[13]. On the other hand, the group that received 250 mg/kg of BPA showed distinct pathological alterations, including inflammatory foci, tubular epithelial desquamation, fibroblasts, erythrocytosis. Vascular congestion, bowman's capsule wall rupture, nuclear necrosis, glomerular atrophy with capsular dilatation, and epithelial cell hyperplasia and necrosis, these changes indicate acute kidney damage resulting from oxidative BPA toxicity, leading to increased reactive oxygen species production, impaired cell membrane permeability, mitochondrial damage, and eventually results in renal cell death, these finding are consistent with those of [17] which indicate glomerular atrophy, tubular necrosis, capsular dilatation, vascular congestion and inflammatory infiltration in rats treated with BPA. These finding were further supported by a study by[6] which demonstrated the presence of tubular epithelial cell desquamation, collagen fiber deposition, and renal tissue damage associated with activation of inflammatory pathways and oxidative stress.

The group that receiving 500 mg/kg of BPA showed more severe lesions, including nuclear necrosis, cast formation, basement membrane thickening, tubular dilation, epithelial cell damage, and vascular congestion, this indicates that the severity of renal damage is dose-dependent, with higher doses leading to more severe disruption of glomerular filtration and tubular reabsorption, casting is thought to result from the accumulation of proteins and dead cells within the tubular lumen due to sever epithelial cell lysis, these findings are consistent with the study by[14]which verified that BPA a mostly affects proximal tubular cells and causes tubular degeneration and cellular senescence, this is also consistent with the study by[16] which showed that exposure to BPA leads to oxidative stress, fibrosis and damage to the glomeruli and tubules in the kidney.

The groups treated with the plant extract along with BPA a showed relative improvement compared to the groups treated with BPA alone, in the group receiving a 250 mg/kg dose of the extract, damage was limited to inflammatory aggregates, epithelial desquamation, and limited nuclear necrosis, indicating that the plant extract provided partial protection to the renal tissue, this protective effect is attributed to the ability of the phenolic compounds and flavonoids in Astragalus to inhibit lipid peroxidation, reduce the inflammatory response, and enhance antioxidant system such as glutathione and superoxide dismutase, this finding supports the study by[9] which demonstrated that the total flavonoids in Astragalus helped protect the glomerular filtration barrier, reduce renal cell damage, and decrease inflammation and oxidative stress.

However, the group that received 500 mg/kg with extract showed persistence of some pathological changes such as glomerular atrophy, capsular dilation, congestion, and hemorrhage, indicating that the protective effect of the extract was not sufficient to counteract the high toxicity of the high dose of BPA, this result is consistent with that[17] reported, which showed that natural antioxidants can improve tissue damage caused by BPA, but to varying degrees depending on the severity of the damage and the dose used.

Evaluation of the toxic effects of BPA and the protective efficacy of *Astragalus spinosus* in pregnant rats

G1: The results of control group showed symmetrical distribution of embryos in the uterine horns and consistency in size as in figure(8).

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Figure8: show control group

G2: The results of this group showed that all the replicates were prone to miscarriage, as they showed that there was a difference in the distribution of the number of embryos in the uterine horns, and also showed that there was one horn containing only embryos as in figure(9)(10)(11).

Figure9,10,11: Show miscarriage, difference distribution number of embryos, and on horn containing only embryos of the.

G3: The results of this group showed equal distribution and consistent size in the uterine horns as in figure(12).

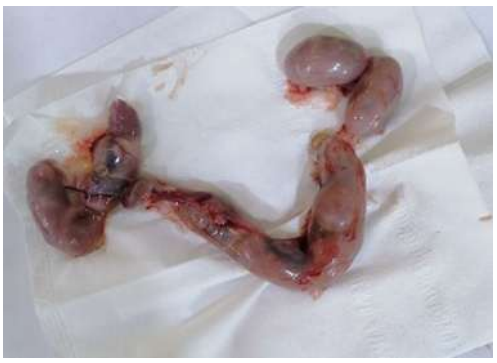

Figure12: show equal distribution and consistent size in the uterine horns.

G4: The results of this group showed that some embryos were dead and their embryonic development was incomplete, unlike the rest of the embryos in the same pregnant rat as in figure(13).

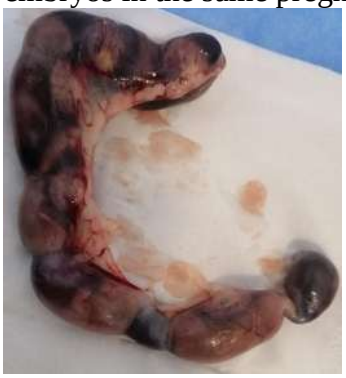

Figure13: show that some embryos were dead and their embryonic development was incomplete, unlike the rest of the embryos in the same pregnant rat.

G5: The results of this group showed that there was a difference in distribution, in addition to the presence of embryos that did not develop embryonically, and also a difference in their size as in figure (14).

Figure14: show that there was a difference in distribution, in addition to the presence of embryos that did not develop embryonically, and

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also a difference in their size.

G6: The results of this group showed that there was a difference in the distribution and size of the embryos, in addition to the fact that they were dead, and that there were spaces in the uterine horns that were devoid of embryos as in figure(16).

Figure16: show that there was a difference in the distribution and size of the embryos, in addition to the fact that they were dead, and that there were spaces in the uterine horns that were devoid of embryos.

According to the current study's findings, extract from *Astragalus spinosus* aerial portions significantly disrupted pregnancy by causing miscarriages and uneven embryo distribution between the uterine horn, with one horn having embryos while the other did not, this point to either poor embryo implantation or a disturbance in the hormonal milieu required for a successful pregnancy, these result are in line with research by[18]which showed that several Astragalus species have abortifacient properties, lowering progesterone levels and raising the rates of embryo resorption and abortion pregnant mice.

The homogeneous distribution of embryos throughout the uterine horns in the BPA 250 mg/kg group may suggest that the dose had no discernible teratogenic consequences during pregnancy, however, [15] found that exposure to BPA is linked to reproductive abnormalities and negative effects on embryonic and placental development, suggesting that BPA is an endocrine disruptor that can impact reproductive processes even at low concentration. The group exposed to a 500 mg/kg dose of BPA exhibited a higher incidence of stillbirths and premature birth, indicating a clear dose-related effect of this substance, this is attributed to BPA's ability to induce oxidative stress, disrupt hormonal balance, and damage the placenta and uterine blood vessels, leading to fetal growth restriction and death, these findings are supported by a recent studies demonstrating that BPA exposure disrupts fetal and placental growth, causing growth restriction due to its negative impact no uterine and placental blood vessels[4][20].

The groups treated with BPA in combination with an extract of plant's aerial parts showed increased severity of the effects, including variations in embryo size, incomplete development, the presence of dead embryos, and vacuoles within the uterine horns, this suggests a possible synergistic effect between BPA and the plant compounds, leading to increased embryo toxicity and embryo onset absorption, these findings are consistent with the study by[22] which demonstrated that BPA induces oxidative stress and apoptosis in the placenta, resulting in fetal growth restriction and some embryonic death.

Conclusions

In addition to its detrimental effects on pregnancy

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and fetuses, the study found that BPA clearly and harmful effects on kidney function and tissue in pregnant female rats, with an increase in dosage, these symptoms become more sever, a partial protective effect against BPA induced nephrotoxicity was demonstrated by the aqueous extract of the aerial parts of *Astragalus spinosus*, however, the documented abortion cases in the extract treated groups suggest that more research is necessary to assess the extract's safety during pregnancy.

Recommendations

1- Carry out additional research to elucidate the harmful processes of BPA on fetuses and kidneys.

2- Determine appropriate dosages and assess the effectiveness and safety of the aqueous extract of *Astragalus spinosus* aerial parts during pregnancy.

3- Limit exposure to BPA because of its detrimental effects on fetal development and kidney function, particularly in pregnant women.

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Republic of Iraq
Ministry of Higher Education and Scientific Research
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Scientific Research Ethics Committee

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وزارة التعليم العالي والبحث العلمي
كلية العلوم - جامعة تكريت
لجنة اخلاقيات البحث العلمي

11 \ 5 \ 2026

Ethical Approval letter

Dear **Rihab Ataallah mohemeed** and **Ass.Prof.Dr.Wurood Mohammed Mutar**

We are pleased to inform you that your research project titled **"Renal toxicity and protective potential of Astragalus spinosus aerial parts extract against Bisphenol A exposure in pregnant female rats"** has been granted ethical approval by the Research Ethics Committee under the approval reference number **[SCITUA-0004]**. After a thorough review of your research proposal, the committee determined that it meets the ethical standards, principles, and guidelines adopted by our institution for conducting research, which are aligned with the updated World Medical Association Helsinki Ethics Principles for research and include human or animal participants or sensitive data (as applicable).

Please ensure that the study is conducted in accordance with the approved protocol and that any significant modifications to the study design or procedures are submitted to the Committee for further review and approval.

With our best regards

Prof.Dr.Haleh Abdulkhaliq Awadh
Member

Prof.Dr.Nagham Qasim Kadhim
Member

Ass.Prof.Dr.SalihY. Darweesh
Member

Ass.Prof.Dr.Firas Faris Rija
Member

Ass.Prof.Dr.Hayder Mudheher Abbas
Member

Ass.Prof.Dr.Reem Adeeb Mohammed
Member

Prof. Dr. Firas Shawqi Alghuri
Head of Scientific Research Ethics Committee
11 / 5 / 2026

Tikrit University
College of Science
11 MAY 2026
Scientific Research Ethics Committee

For more information, please visit the website of the Scientific Research Ethics Committee at College of Science/Tikrit University through the following link: http://csci.tu.edu.iq/index.php/ar/
All inquiries should be sent via the official email: cosethicswit@tu.edu.iq.

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