Jordan Journal of Pharmaceutical Sciences, Volume 15, No. 4, 2022

Acute and Sub-Acute Oral Toxicity Assessment of Mixed Extract of *Trigonella Foenum-Graecum* Seeds and *Withania Somnifera* Root in Rats

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ABSTRACT

This study aimed at assessing the safety of a mixed extract of *Trigonella Foenum-graecum* seeds and *Withania Somnifera* root (TFWS), which effectively relieves male menopausal symptoms. To this end, male and female Sprague-Dawley rats were divided into the following groups and repeatedly administered TFWS orally for 90 days: control, low-dose (**500** mg/kg/day), intermediate-dose (1,000 mg/kg/day), and high-dose (2,000 mg/kg/day) groups. The animals were monitored for general symptoms; their body weights and electrolyte levels were measured; urinalysis, blood chemistry, biochemistry tests, and histopathological tests were performed to assess the toxicity of TFWS. The no-observed-adverse-effect level of TFWS was 2,000 mg/kg/day for all male and female rats. While in the TFWS-administered and control groups, most parameters were within the normal range; some rats in the high-dose group showed changes not induced by the test substance but which may be specific to an individual animal or may occur naturally. Thus, based on our findings, we consider that TFWS may be a safe, non-toxic substance for alleviating male menopausal symptoms.

Keywords: *Trigonella Foenum-graecum*, *Withania Somnifera*, rats, safety, toxicity.

1. Introduction

The incidence of hormone-related disorders such as menopausal symptoms, sexual dysfunction, and prostatic hypertrophy is expected to increase with the transition from aging to an aging society. Female menopausal symptoms are easy to identify and manage to owe to markedly reduced levels of estrogens, which are female sex hormones, which occur with aging. Male menopausal symptoms, on the other hand, are harder to identify and are often managed late since the levels of testosterone, which is a male sex hormone, decrease gradually. In general, testosterone levels in men start to decrease by 3.1-3.5 ng/dL per year from the age of 30 years.[1,2,3] Male menopause refers to the period after the age of 30 years during which various symptoms develop because of an aging-induced reduction in blood testosterone levels. These symptoms include low energy, erectile dysfunction, reduced muscle mass, abdominal obesity, and anxiety.[4,5]

Trigonella foenum-graecum, commonly known as fenugreek, is an annual plant in the family *Fabaceae*. It is cultivated worldwide as a semiarid crop[6], including Yemen[7], and its seeds have traditionally been used for medicinal purposes.[8,9] Fenugreek seeds have been used to treat kidney and bladder symptoms for a long period and have been recently found to increase energy, maintain blood glucose and insulin balance, and aid weight management.[10,11] Fenugreek seed extract has been reported to positively affect sexual health and quality of life through its anabolic activities and androgen activation.[12] The beneficial effects of the extract are attributed to the extract's ability to increase testosterone levels. Fenugreek

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Received: 26/7/2021 Accepted:20/2/2022.
DOI: https://doi.org/10.35516/jjps.v15i4.673

seed extracts may effectively treat menopausal symptoms in older men and have been reported to improve male menopausal symptoms by increasing the total and free testosterone levels inhibiting 5α-reductase and aromatase.[13]

Withania somnifera, commonly known as Ashwagandha, is an annual evergreen shrub in the *Solanaceae* family that grows in Yemen, it is widely utilized in Yemen, a traditional system of medicine in Yemen[7], and is deemed an "adaptogen," a herb that protects the body from stress and helps the body address the effects of stress. Ashwagandha has been shown to decrease cortisol levels in persons under chronic stress, restore healthy adrenal function, and normalize the sympathetic nervous system[14,15]. Ashwagandha root extract is used to treat sexual weakness, erectile dysfunction, and performance anxiety in men and has been advocated to ameliorate diminished sexual desire in women and all forms of sexual dysfunction[16-19], particularly where a depleted nervous system is playing a role.

While numerous studies have proven the beneficial health effects of newly developed or existing substances, they have been mostly limited to verifying only the positive effects of the active ingredients. Few studies have assessed the toxic effects of active ingredients when ingested. Hence, in this study, we assessed the safety of TFWS, which is a mixed extract of *T. Foenum-graecum* seeds and *W. Somnifera* roots that has been found to relieve male menopausal symptoms, by conducting a toxicity test through oral administration to rats.

2. Materials and methods

2.1. Procurement of test substance and extraction process

The seeds of *Trigonella Foenum-graecum* (fenugreek) were purchased from Yassin spices in Sana'a city, Yemen,and were collected from the Hajjahgovernmentt, Yemenfromg Jan to Feb 2020. Also, the roots of *Withania Somnifera* (Ashwagandha) were purchased by Yassin spices in Sana'a city, Yemen)and were collected from the Hajjah goverment, Yemen from March to July 2020. fenugreek seed and Ashwagandha roots were identified and authenticated by Dr. Hassan M. Sugail, Assistant professor of Plant Taxonomy, Department of Biology, Faculty of Science, Hajjah University, Yemen.

The fenugreek seed and Ashwagandha roots were dried separately under shade at room temperature following the published procedure[18], then they were finely powdered using a mill with ultra-centrifugal (Retsch ZM-200, Germany). The powdered seeds were kept in dark airtight container before extraction.

A 100 g of crushed fenugreek seed and 100 g of crushed Ashwagandha roots were extracted separately using ethanol 70% four times and then put on a shaker at 35 °C. After continues shaking for 3 days, the mixture was filtered through a No.1 paper filter (Whatman). The extract was transferred into a round flask and solvent was evaporated using rotary evaporator, (Rotavapor R-200, Büchi, Germany) at 40 °C. Finally, the yield of extraction was calculated using Eq. (1).

$$\text{Extraction yield}\left(\frac{V}{W}\%\right) = \frac{\text{Amount of extracted (ml)}}{\textit{Weight of dry sample used (g)}} \times 100 \quad (1)$$

The dry crude extract yield from fenugreek seed was 5.55 ± 0.05% (v/w), while the dry crude extract yield from Ashwagandha roots was 2.89± 0.03% (v/w).

Chemical composition of fenugreek seed and Ashwagandha roots referenced from PubChem were alkaloids amino acids, saponins, steroidal, flavonoids, fibers, lipids, coumarin, vitamins, minerals, mucilage, volatile oil, tannins and glycosides[20,21].

2.2. Laboratory animals and breeding environment

Five-week-old male and female rats, weighing 180-200g for males and 150-170g for females were purchased from Sana'a Zoo, Sana'a, Yemen. They were housed in stainless steel cages in a well-ventilated room in the animal house of the collage of Medical Sciences, Al-Razi

University. The animals were kept under controlled environmental conditions with free access to a standard laboratory diet and water ad libitum during the entire period of the study. During one week of acclimatization, the animals were monitored for any abnormal symptoms. Only animals deemed to be normal were used in the experiment. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH) 1978. Animal handling and all related procedures were carried out by the procedures approved by the research committee of the college of Medical Science, Al-Razi University, Sana'a, Yemen (021/CMS/2021).

2.3. Administration dose and experimental group composition

The laboratory animals were divided into control and TFWS-administered groups, with 10 animals per group. Each group was further divided into male and female groups. Ten rats were randomly allocated to a group such that their body weights were close to the mean body weight of the group. The animals were identified by tail marking.[15] Considering that the test substance was a natural extract, the administration dose was set to 2,000 mg/kg/day for the high-dose group, which is greater than the standard dose limit for repeated-dose toxicity tests (1,000 mg/kg/day); 1,000 mg/kg/day for the intermediate-dose group; and 500 mg/kg/day for the low-dose group. Thus, the animals were divided into four groups, including the control group.

2.4. Test substance preparation and administration

The test substance was prepared at concentrations of 2,000, 1,000, and 500 mg/10 mL/kg using water as the solvent for oral administration. Using a syringe with a zonde for enteral administration, the test substance was directly administered into the stomach. The volume administered was calculated as 10 mL/kg, based on the most recently measured body weight of the animals. The test substance was administered once daily for 90 days.

2.5. Observations

2.5.1. General symptom monitoring and weight measurement

All animals were monitored once daily for the type, onset, and severity of general symptoms and twice a day for fatal conditions or death. The animals were monitored for the entire course of TFWS administration (90 days). Body weights of all animals were measured on the first day of TFWS administration, once a week thereafter, and once on the day before the autopsy and on the day of the autopsy.

2.5.2. Urinalysis

Urinalysis was conducted for all five animals in each group 13 weeks after TFWS administration. Fresh urine (1 mL) collected from rats in metabolic cages for 3-4 h was used for the general analysis and urine sediment analysis. The total urine volume was measured based on the amount of urine that was continuously collected for 24 h.

2.5.3. Autopsy and blood collection

The rats were fasted for over 17 h before the day of the autopsy and anesthetized via isoflurane inhalation. Blood samples were collected from abdominal arteries. An autopsy was conducted on all organs, and the findings were recorded.

2.5.4. Hematology and serum biochemistry tests

A portion of the blood collected during the autopsy was added to a vacutainer tube (Vacutainer, BD, USA) containing EDTA-2K, an anticoagulant. General blood test parameters (White blood cell count [WBC], red blood cell count [RBC], hemoglobin concentration [HGB], hematocrit [HCT], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red cell distribution width [RDW], hemoglobin distribution width [HDW], platelet [PLT], mean platelet volume [MPV], and reticulocyte [RET]) and leukocyte (neutrophil [NEU], lymphocyte [LYM], monocyte [MONO], eosinophil [EOS], basophil [BASO], large unstained cells [LUC]) counts were measured using an automated hematology analyzer, K-96 (ADVIA 2120, Siemens, USA).

For serum biochemistry test, blood samples were

added to vacutainer tubes containing clotting activators and allowed to coagulate at room temperature for 10–15 min. Next, serum was obtained by centrifuging the blood samples for 10 min at 4,000 rpm (MF80, Hanil, Korea) and then analyzed using an automated hematology analyzer K-97 (KONELAB 20XT, Thermo Fisher Scientific, USA). Electrolyte levels were measured using the K-99 electrolyte analyzer (744 Na+/K+/Cl- Analyzer, Siemens, USA).

2.5.5. Organ weight measurement

For all animals, organs were harvested during the autopsy, and their weights were measured using a precision scale. The organs investigated in this measurement included adrenal, pituitary, testis, ovary, epididymis, thymus, prostate, spleen, kidney, heart, lung, brain, and liver.

2.5.6. Histopathological examination

The harvested organs were fixed in 10% neutral-buffered formalin. The fixed organs and tissues were subjected to a conventional processing procedure that involved trimming, dehydration, and paraffin embedding to prepare tissue sections. Sections were then cut and stained with hematoxylin and eosin. In a histopathological examination, all fixed organs and tissues from the control and high-dose groups were examined under a microscope. The organs and tissues studied in this examination included prostate, kidney, liver, spleen, lung, heart, and kidney. Organs observed as abnormal in these groups were also examined in the low- and intermediate-dose groups[22].

2.6. Statistical analysis

Mean values between the control and TFWS-administered groups were compared using parametric or non-parametric multiple comparison methods. Statistical analyses were performed using the SPSS 19.0 (IBM, USA).

2.6.1. Continuous data

One-way analysis of variance (ANOVA) was used to evaluate the significance of the differences in the mean body weight, feed consumption, blood chemical and biochemical parameter values, and organ weights. If a significant difference was found, the homogeneity of variance was evaluated using Levene's test. If the homogeneity of variance was satisfied, Duncan's multiple range test was used. Dunnett's t-test was used for heteroscedastic data.

2.6.2. Non-continuous data

Urinalysis results were expressed as severity after scale transformation as shown in Table 1, and statistical analysis was conducted. If significant differences were found in the Kruskal-Wallis H test, the Mann-Whitney U test was performed to confirm the statistical significance of the differences with respect to the control group.

3. Results

3.1 Toxicity criteria

The criteria were used to determine toxicity. The no-observed effect level (NOEL) is the maximum concentration at which a test substance does not cause toxic or pharmacological changes. The no-observed-adverse-effect level (NOAEL) is the maximum concentration at which a test substance does not induce adverse effects or lead to an evident disease. The lowest-observed-adverse-effect level (LOAEL) is the minimum toxic concentration at which a test substance induces adverse effects.

3.2. Body weight and abnormal symptoms

No deaths or unusual symptoms were observed in any group, including the control group, throughout the 90-day repeated oral dose toxicity study period. No significant differences in weight change were observed in any experimental group, compared to the control group (Figure 1,2).

Figure 1. Bodyweight changes in male rats

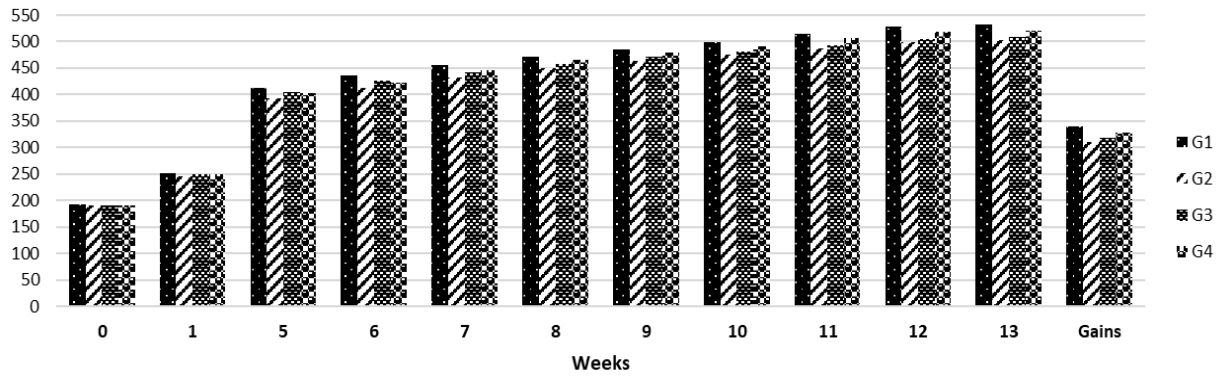
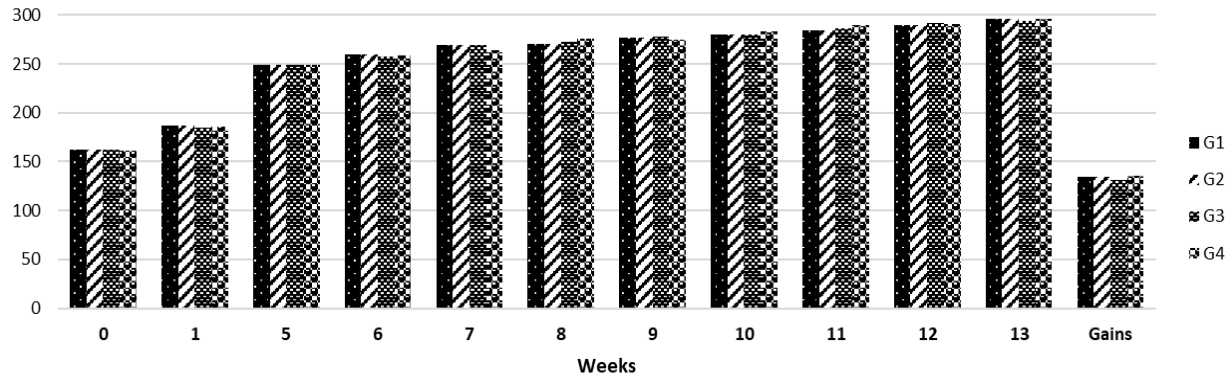


Figure 2. Bodyweight changes in female rats



3.3. Urinalysis and blood test

No significant findings and differences in urinalysis results were found between the experimental and control groups (Table 1). No unusual findings or significant differences in the general blood test results and reduction in leukocyte count were observed between the male experimental and control groups (Table 2). While the red blood cell counts were significantly reduced in the female high-dose group (2,000 mg/kg/day) compared to that in the control group, the reduction was small and within the normal range and thus deemed a change unrelated to the test substance ($P < 0.05$) (Table 2).

Table 1. Urinalysis of male and female rats

Tests	Results	Severity	Groups (mg/kg/day)							
			Male				Female			
			G1	G2	G3	G4	G1	G2	G3	G4
GLU	-	0	5	5	5	5	5	5	5	5
BIL	-	0	5	5	5	5	5	5	5	5
KET	-	0	4	3	2		5	5	4	5
	+/-	1	1	1	1	3			1	
	1+	2		1	2	2				
SG	≤1.010	1					2			
	1.015	2	5	2	1	2	2	3	3	
	1.020	3		3	3	1	1	2	1	5
	1.025	4				2			1	
	1.030	5			1					
pH	8.0	3	1		1	1	3	1	1	
	8.500	4	4	5	4	4	2	4	3	4
	≥9.0	5							1	1
PRO	-	0	1	1			4	4	3	3
	+/-	1	2		2		1		2	1
	1+	2	2	3	1	3		1		1
	2+	3		1	1	1				
	3+	4			1	1				
UROa)	0.2	0	5	5	5	5	5	5	5	5
NIT	-	0	5	3	5	5	5	5	5	4
	+	1		2						1
OB	-	0		1	3	2	5	5	5	5
	+/-	1	5	3	2	3				
	1+	2		1						
LEU	-	0	3	2	1	3	5	4	5	3
	+/-	1	2	1	1			1		2
	1+	2		1	1					
	2+	3		1	2	2				
No. of animals			5	5	5	5	5	5	5	5

a) The unit of urobilinogen is Ehrlich unit (EU)/dL. GLU, glucose; BIL, bilirubin; KET, ketone body; SG, specific gravity; PRO, protein; URO, urobilinogen; NIT, nitrite; OB, occult blood; LEU, leukocyte.

Table 2. Hematological values of male and female rats

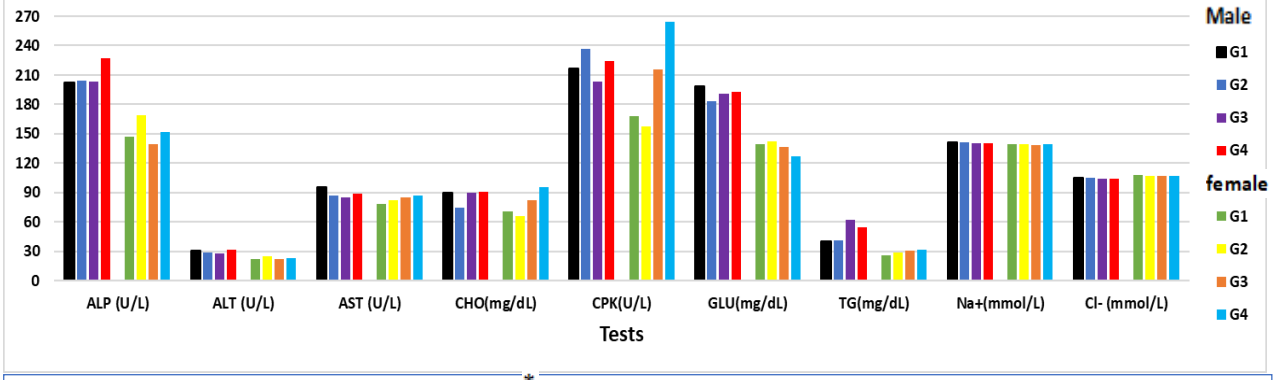
Tests	Units	Male groups (mg/kg/day)				Female groups (mg/kg/day)			
		G1	G2	G3	G4	G1	G2	G3	G4
WBC	103/µL	3.44 ± 1.07	2.85 ± 0.82	3.17 ± 0.88	3.52 ± 0.87	1.70 ± 0.69	1.92 ± 0.59	1.86 ± 0.70	2.12 ± 0.71
RBC	106/µL	8.64 ± 0.26	8.51 ± 0.51	8.61 ± 0.30	8.60 ± 0.49	8.09 ± 0.35	7.87 ± 0.22	7.89 ± 0.33	7.72 ± 0.36*
HGB	g/dL	15.3 ± 0.88	15.4 ± 0.56	15.4 ± 0.70	15.2 ± 0.64	16.3 ± 0.39	16.0 ± 0.44	16.2 ± 0.46	16.0 ± 0.36
HCT	%	43.8 ± 1.18	43.4 ± 1.09	43.7 ± 1.23	43.4 ± 1.23	42.7 ± 1.02	41.3 ± 0.94*	41.8 ± 1.37	41.7 ± 0.84*
MCV	fL	50.8 ± 2.10	51.2 ± 2.27	50.8 ± 1.97	50.5 ± 2.02	52.8 ± 1.53	52.6 ± 0.67	53.0 ± 1.01	54.1 ± 3.48
MCH	Pg	17.7 ± 1.31	18.1 ± 1.10	17.9 ± 1.13	17.7 ± 1.10	20.2 ± 0.82	20.3 ± 0.43	20.5 ± 0.76	20.8 ± 1.36
MCHC	g/dL	34.8 ± 1.64	35.4 ± 1.21	35.2 ± 1.03	35.0 ± 1.02	38.3 ± 0.71	38.7 ± 0.55	38.7 ± 1.05	38.4 ± 0.59
RDW	%	13.4 ± 0.81	12.9 ± 0.53	12.9 ± 0.63	12.8 ± 0.49	11.7 ± 0.93	11.4 ± 0.45	11.3 ± 0.52	11.8 ± 1.37
HDW	g/dL	2.83 ± 0.40	2.71 ± 0.32	2.61 ± 0.29	2.68 ± 0.41	2.35 ± 0.26	2.30 ± 0.10	2.27 ± 0.13	2.35 ± 0.08
PLT	103/µL	865 ± 279	929 ± 10	980 ± 69.4	940 ± 74.6	996 ± 173.1	981 ± 164.4	1009 ± 114.9	1094 ± 161.9
MPV	fL	7.5 ± 0.73	7.2 ± 0.40	7.1 ± 0.37	7.1 ± 0.31	7.4 ± 0.56	7.4 ± 0.58	7.1 ± 0.36	7.3 ± 0.41
RET	%	1.73 ± 0.32	1.59 ± 0.29	1.51 ± 0.25	1.49 ± 0.27	1.91 ± 0.41	2.04 ± 0.33	1.81 ± 0.36	1.92 ± 0.41
NEU	%	22.8 ± 8.37	24.7 ± 9.12	24.0 ± 8.67	22.0 ± 3.84	22.1 ± 18.48	18.7 ± 4.71	18.6 ± 7.20	19.4 ± 9.84
LYM	%	71.3 ± 8.56	70.2 ± 8.74	70.3 ± 9.29	72.3 ± 4.88	71.1 ± 23.99	75.7 ± 4.84	76.8 ± 6.83	75.1 ± 11.07
MONO	%	2.7 ± 0.71	2.6 ± 0.64	2.9 ± 0.81	2.9 ± 0.90	3.9 ± 5.28	2.4 ± 0.72	2.2 ± 0.86	2.5 ± 0.83
EOS	%	2.2 ± 0.79	1.7 ± 0.48	1.8 ± 0.73	1.8 ± 0.50	2.4 ± 0.79	2.5 ± 0.91	1.7 ± 0.90	2.1 ± 0.96
BASO	%	0.1 ± 0.07	0.1 ± 0.05	0.1 ± 0.09	0.1 ± 0.07	0.1 ± 0.10	0.1 ± 0.10	0.1 ± 0.08	0.1 ± 0.11
LUC	%	0.9 ± 0.58	0.7 ± 0.32	0.9 ± 1.01	0.9 ± 0.41	0.5 ± 0.39	0.6 ± 0.34	0.7 ± 0.37	0.7 ± 0.39
No. of animals		10	10	10	10	10	10	10	10

WBC, white blood cell, RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; HDW, hemoglobin concentration distribution width; PLT, platelet; MPV, mean platelet volume; RET, reticulocytes; NEU, neutrophils, LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; LUC, large unstained cells.

3.4. Serum biochemistry and electrolyte tests

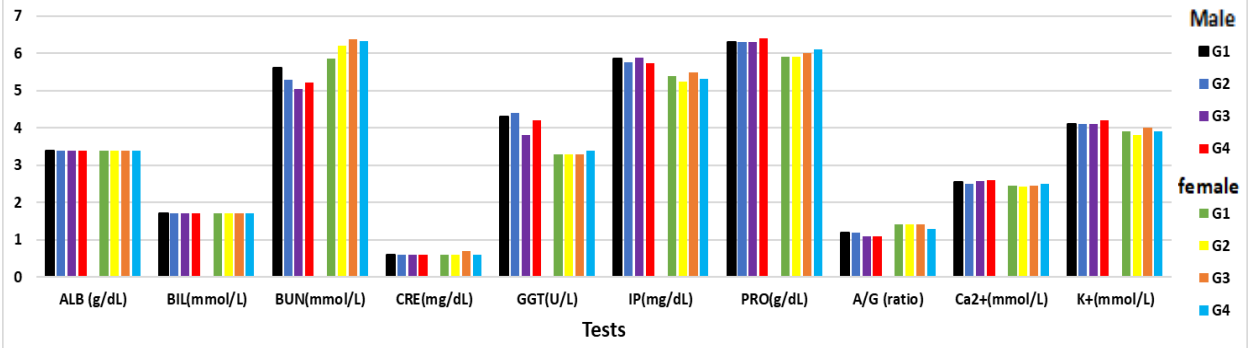
No unusual findings or significant differences in the results of the serum biochemistry test and electrolyte measurements were observed between the male experimental and control group. While the total cholesterol (CHO) level was significantly higher in the female high-dose group (2,000 mg/kg/day) than in the control group, the increase was small and within the normal range and thus deemed unrelated to the test substance ($P < 0.05$) (Figure 3&4).

Figure 3. Serum biochemical values of male and female rats (ALP, ALT, AST, CHO, CPK, GLU, TG, Na+ & Cl-)



ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase;

Figure 4. Serum biochemical values of male and female rats (ALB, BIL, BUN, CRE, GGT, IP, PRO, A/G, Ca+ & K+)



ALB, albumin; BIL, bilirubin; BUN, blood urea nitrogen; CRE, creatinine; GGT, gamma-

3.5. Organ weight measurement

No unusual findings or differences in organ weights were observed between the male experimental groups and the control group. While the absolute weight of the liver significantly increased in the female high-dose group (2,000 mg/kg/day) compared to that in the control group, the increase was within the normal range and deemed unrelated to the test substance ($P < 0.05$). While the relative weight of the heart significantly increased in the high-dose group (2,000 mg/kg/day) compared to that in the control group, the increase was within the normal range and deemed unrelated to the test substance ($P < 0.05$) (Figure 5, 6).

Figure 5. Absolute organ weights of male rats

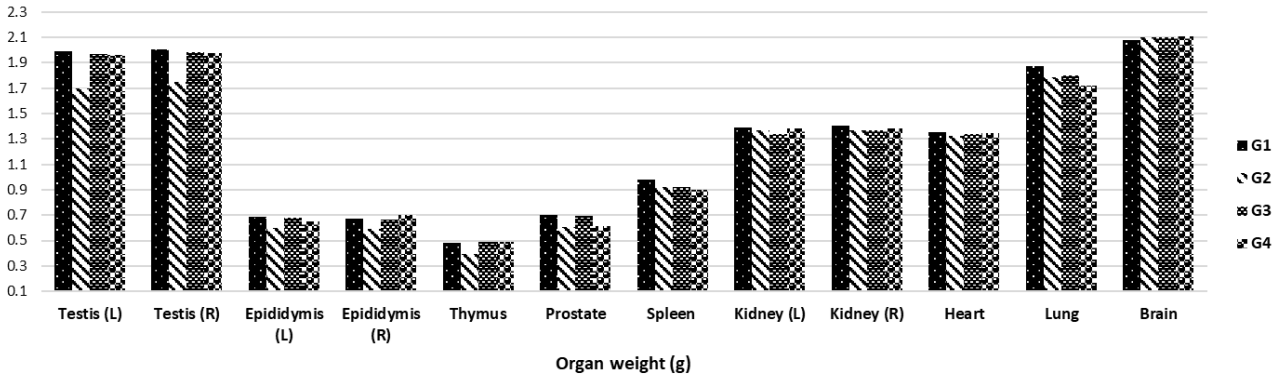
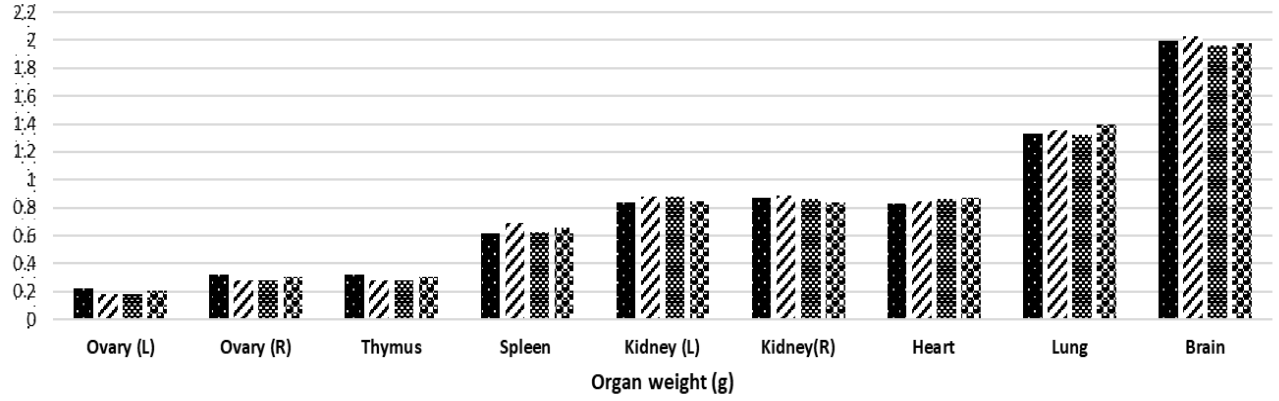


Figure 6. Absolute organ weights of female rats



3.6. Histopathological examination

Inflammatory cell infiltration, local inflammation, and congestion were observed in the prostate, kidney, liver, spleen, and heart of some animals in the male high-dose group (2,000 mg/kg/day). These symptoms were also observed in the control group and thus deemed unrelated to the test substance (Table 8). Inflammatory cell infiltration and local inflammation were observed in the kidney, liver, and heart of some animals in the female high-dose female group (2,000 mg/kg/day). The same symptoms were observed in the control group and thus deemed unrelated to the test substance (Table 4).

Table 3. Histopathological findings of male and female rats

Organs	Observed signs	Male groups (mg/kg/day)				Organs	Observed signs	Female groups (mg/kg/day)			
		G1	G2	G3	G4			G1	G2	G3	G4
Prostate	Inflammatory cell infiltration	4			4	Kidney	Inflammatory cell infiltration				1
Kidney	Inflammatory cell infiltration	3					Hyaline casts				1
	Regeneration	1					Mineralization	1			
Liver	Focal inflammation	2			2	Liver	Focal inflammation	3			3
Spleen	Congestion	2			2	Spleen	Congestion	1			
Lung	Mineralization	1				Heart	Inflammatory cell infiltration	1			
Heart	Inflammatory cell infiltration				1						
No. of animals		10			10			10			10

4. Discussion

Sexual expression is a normal and healthy part of human behavior. Positive sexual experiences are related to health and well-being throughout life; hence, there is a need to think about sexual health as not simply the absence of sexual disorders, but as a key factor affecting the quality of life[23]. FSD is characterized by problems in the psychophysiological variations combined with the "sexual response cycle." These variations are often due to underlying neurovascular, hormonal, or psychogenic aetiologies[24].

TFWS has previously been found to relieve various male menstrual symptoms by improving testosterone levels. [12,13,25,27] In this 90-day repeated oral dose toxicity study, the NOEL of TFWS was determined to be 2,000 mg/kg/day for both male and female rats. The maximum recommended starting dose of TFWS is 32.25 mg/kg/day or 1,935 mg/day for an adult weighing 60 kg. This is approximately five-fold higher than the proposed dose limit of 400 mg/day for an adult weighing 60 kg.

Male rats showed changes unrelated to the test substance, which determine the NOEL, in histopathological examinations.[28] Some rats in the high-dose groups showed inflammatory cell infiltration in the prostate and kidney; however, these symptoms were also observed in the control group. Furthermore, mild inflammation in the prostate and kidneys can occur naturally with aging. Since local liver inflammation, splenic congestion, and inflammatory cell infiltration in the heart observed in the experimental groups were also observed in the control group, these changes were not deemed to have been induced by the test substance.

Female rats showed changes unrelated to the test substance, which determine the NOEL, in the blood biochemistry test, organ weight measurement, and histopathological examination.[29,30] The serum biochemistry test revealed that the total CHO level increased by 26% in the high-dose group (2,000 mg/kg/day) compared to that in the control group but was still within the normal range. The absolute weight of the liver increased by 10.3% in the high-dose group compared to that in the control group but was still within the normal range. The histopathological examination revealed inflammatory cell infiltration in the kidney in one rat in the high-dose group. Inflammatory cell infiltration can also occur naturally with aging. A few rats in the high-dose

group showed local liver inflammation; however, this was also observed in the control group. The overall test results for the experimental groups were within the normal range compared to those obtained for the control group; however, a few changes unrelated to the test substance were observed. These changes are not induced by the test substance, but are rather specific to certain animals or are natural phenomena. Based on these findings, we considered TFWS to be a safe and non-toxic substance.

5. Conclusion

A repeated oral dose toxicity test on rats was performed in this study to assess the safety of a mixed extract of TFWS, which has been shown to relieve menopausal symptoms in men. There were no deaths or unusual symptoms in any of the groups, including the control group. Weight change, eye examination results, urinalysis results, feed consumption, blood test results, and histopathological examination results were all normal. When compared to the control group, all test results in the experimental group were within the normal range. The NOEL was determined to be 2,000 mg/kg/day in both the female and male high-dose groups administered TFWS at 2,000 mg/kg/day, which is higher than the standard 1,000 mg/kg/day dose limit for repeated dose tests, indicating that TFWS is safe and non-toxic within a certain dose range. As a result, our findings suggest that TFWS could be a safe, non-toxic treatment for male menopausal symptoms.

Acknowledgments

The author is thankful to Al-Razi University and Amran University for their support. Also would like to thank the Yassin spices for their support in providing test substances (fenugreek seed and Ashwagandha roots).

Ethics approval and consent to participate

All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH) 1978. Animal handling and all related procedures were carried out by the procedures approved by the research committee of the College of Medical Science, Al-Razi University, Sana'a, Yemen (021/CMS/2021).

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تقييم السمية الفموية الحادة وشبه الحادة للمستخلص المختلط من بذور الحلبة *Trigonella Foenum-graecum* وجذور العبب *Withania Somnifera* في الفئران

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ملخص

هدفت الدراسة الحالية إلى تقييم سلامة المستخلص المختلط من بذور نبات الحلبة وجذور نبات العبب، والذي يخفف وبشكل فعال أعراض سن اليأس عند الذكور. ولتحقيق اهداف الدراسة، تم تقسيم ذكور وإناث الجرذان (40 جرذ) إلى مجموعة تحكم وثلاث مجموعات تجريبية اعطيت المستخلص المختلط من بذور الحلبة وجذور العبب بشكل متكرر عن طريق الفم لمدة 90 يومًا: مجموعة التحكم، مجموعة الجرعة المنخفضة (500 ملجم/كجم/يوم)، مجموعة الجرعة المتوسطة (1000 ملجم/كجم/يوم) ومجموعة الجرعة العالية (2000 ملجم/كجم/يوم). تم مراقبة الحيوانات بحثًا عن أي أعراض عامة؛ تم قياس أوزان الجسم ومستويات الايونات؛ وأجريت اختبارات تحليل البول، وكيمياء الدم، واختبارات الكيمياء الحيوية والنسيجية–المرضية لتقييم سمية المستخلص المختلط من بذور الحلبة وجذور العبب. كان مستوى التأثير الضار غير الملحوظ لـ 2000 ملجم/كجم /يوم من المستخلص المختلط من بذور الحلبة وجذور العبب لجميع الفئران من الذكور والإناث. بينما في المجموعات الاخرى التي ادريت بـ المستخلص المختلط من بذور الحلبة وجذور العبب ومجموعة التحكم، فقد كانت معظم المؤشرات ضمن النطاق الطبيعي؛ أظهرت بعض الفئران في المجموعة ذات الجرعات العالية تغييرات لم تحدثها مادة الاختبار ولكنها قد تكون خاصة بحيوانات فردية أو قد تحدث بشكل طبيعي. وبالتالي، وبناءً على النتائج التي توصلنا إليها، نعتبر أن المستخلص المختلط من بذور الحلبة وجذور العبب قد يكون مادة آمنة وغير سامة للتخفيف من أعراض سن اليأس عند الذكور.

الكلمات الدالة: بذور نبات الحلبة، جذور نبات العبب، الجرذان، السمية، الامان.

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تاريخ استلام البحث 2021/7/26 وتاريخ قبوله للنشر 2022/2/20.