Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 33, issue 4, 2023, pp. 122 - 131

EFFECTS OF AQUEOUS AND ETHANOLIC EXTRACTS OF *RUTA CHALEPENSIS* (SAPINDALES: RUTACEAE) ON WISTAR RATS

Rayene CHEDDAD[1*], Nour El Imen BOUBLATA[1], Youcef Islem HAMIDA[1], Wafa HABBACHI[1], Reda DJAOUAHDOU[2], Alina Iuliana TABIRCA[3], Valentin RADU[3]

[1]Laboratoiry of Applied Neuroendocrinology. Department of Biology, Faculty of Sciences, University BadjiMokhtar Annaba 23000, Algeria
[2]Faculty of Economics, Commerce & Management, University BadjiMokhtar Annaba 23000, Algeria
[3]Faculty of Economics, Valahia University of Targoviste, 130004 Targoviste, Romania

Abstract: The use of plant-based biopesticides is considered a promising alternative to chemical pesticides for human health. In this study, we investigated the effects of aqueous and ethanolic extracts of *Ruta Chalepensis* leaves. Experiments were conducted on Wistar rats (*Rattus rattus*), widely used as a model in pharmacology and toxicology. The extracts were administered orally for seven consecutive days. To evaluate anxiety-related behavior, three standard tests were performed: the open field, the elevated plus maze, and the forced swim test. Results showed that the extracts produced no significant behavioral changes. However, biochemical analyses revealed modifications in urea and blood glucose levels, while creatinine, cholesterol, and triglycerides remained unchanged. Hematological tests indicated a reduction in red blood cell count. In contrast, other blood parameters such as white blood cells, hemoglobin, and platelets were not affected. These findings suggest that *Ruta Chalepensis* may influence certain biochemical and hematological markers without altering behavior in rats.

Keywords: biopesticide, *Ruta chanlepnsis* L, Wistar rat, aqueous extract, ethanolic extract.

INTRODUCTION

Recent advances in industry and biotechnology have led to the creation of products that improve human living conditions. However, these advances have been accompanied by the creation of many physical, chemical and biotechnical agents that affect the environment. Over the past 50 years, human exposure to environmental and occupational chemicals has increased dramatically, and pesticides account for the largest share of these chemicals (Bretveld et al., 2006; Taib et al., 2014). Pesticides are reported to be among the most dangerous environmental pollutants, due to their carcinogenic, neurotoxic and endocrine-disrupting effects on living organisms, as well as their long-term effects on infertility and the immune system.

As throughout the Maghreb, Algeria is one of the Mediterranean countries with a long history of dealing with spontaneous plants. For decades, most work in Algeria has converged on the use of plant extracts as a means of controlling various pests (Aouinty et al., 2006; Bounechada & Arab, 2011; Habbachi et al., 2013; Habbachi et al., 2014; Merabti et al., 2015; Benhissen, 2016; Bekhakheche et al., 2017; Habbachi et al., 2019; Habbachi et al., 2020; Boublata et al., 2020; Habbachi, 2020; Boublata et al., 2021a, b, c, d; Habbachi et al., 2021; Saadane et al., 2021; Rahat et al., 2021; Bouzar et al., 2021; Saadane, 2022; Bouzar et al., 2022; Bouzar, 2023), which involve the use of biopesticides.

Biopesticides or botanical pesticides represent an ancient form of natural defense. The use of botanical pesticides reduces the risk of toxicity compared with synthetic pesticides (Anjarwalla et al., 2016). Several authors have confirmed the effect of biopesticides on insect mortality (Benhissen et al., 2019). Humans have used plant extracts as insecticides since before Roman times, a practice that continues to exist with many plant species known for their insecticidal properties (Balandrin et al., 1985). Plant products can be more rapidly degraded in the environment than synthetic compounds, and some may have enhanced specificity that can favor insects beneficial to the plant (Desneux et al. 2007).

This work focuses on using Rue d'Alep under the scientific name *R.chalepensis L* to synthesize biopesticides that are both effective on insects and have no effect on humans. It involves preparing aqueous and ethanolic extracts of *R.chalepensis L* and determining their effects on the behavior and biochemical parameters of Wistar rats.

MATERIAL AND METHODS

Animals: Wistar rats

We used adult rats (*Rattus rattus*) of the Wistar strain from the Institute Pasteur in Algiers (Algeria), weighing between 200 and 250 grams. The rats were reared in plastic cages lined with sawdust and equipped with steel lids and water bottles in the animal house of the Biology Faculty of the University of Annaba. The rats were fed corn sticks, barley, and vitamin supplements. Sawdust was used as bedding and changed two or three times a week for all groups of animals.

Plant: *Ruta chalepensis L*

In this work, we tested the effect of the aqueous and ethanolic extract of *Ruta chalepensis (Rutaceae)* on the Wistar rat. *Ruta chalepensis L* is a group of the most widespread small shrubs (Günaydin & Savcib, 2005), powerful odor characterizes its leaves. They have a blue-green color, and their flowers are from 1 to 2 cm in diameter; in a cup of dark yellow color, it has 8 to 10

***Correspondence**: Rayene Cheddad, Laboratoiry of Applied Neuroendocrinology. Department of Biology, Faculty of Sciences, University BadjiMokhtar Annaba 23000, Algeria, phone number: +213 (0) 664 80 10 83, email: rayenecheddad@gmail.com*

stamens and an ovary, Its fruits are represented black fruit follicles (Houas, 2021). This plant is used in traditional medicine against rheumatism, fever, mental disorders, menstrual problems, epileptic seizures, hemorrhage and also to flavor food (Ouerghemmi et al., 2017) in agriculture against pests and insects (Houas, 2021). This plant is toxic in high concentrations (Daoudi et al., 2015).

Plant material collection site

Our plant was in its adult stage in May, and was harvested in June. It comes from Bani Souiden-commune de Mechroha- Wilaya de Souk-ahras (Algeria; 36° 21' 26'' north, 7° 50' 08'' east).

Preparation of *Ruta chalepensis L* extracts

For the ethanolic extraction, we mixed 100g of the plant powder with 1000ml of ethanol diluted to 70%, and left the mixture to stir for 24 hours in the shade.

This step was followed by filtration, and the filtrate obtained was passed through the rota-vape at 40°C and the oven at 45°C to obtain a dry extract (a powder) and evaporate the ethanol.

We mixed 100g of the powder with 1000ml of distilled water for the aqueous extract. Then, we placed the mixture on a magnetic stirrer for 24 hours, after which the mixture was filtered and then passed through the rota-vape and oven to reduce the volume of water and obtain a dry extract in powder form (Bohui et al., 2018).

Treatments

A total of forty-two Wistar rats were allocated into three experimental groups (n=14 per group; 7males and 7 females). The control group received 1 ml of destilled water by gavage. The second group was asministered 1 ml of *Ruta chalepensis* aqueous extract (100 g/l), while the third group received 1 ml of the ethanolic extract (2.5 g/l). All treatments were delivered once daily for seven consecutive days.

Rat behavior in different anxiety-provoking situations

The open field (Crawley, 1999; Palanza, 2001; Karl et al., 2003; Prut & Belzung, 2003; Elizalde et al., 2008), the elevated cross maze (Rodgers & Dalvi, 1997; Van-Gaalen & Steckler, 2000; Karl et al., 2003; Walf & Frye, 2007; Elizalde et al., 2008), and forced swimming (Porsolt et al., 1977; Detke et al., 1995; Karl et al., 2003; Elizalde et al., 2008; Alijanpour et al., 2019).

Behavioral studies for anxiety

Behavioral tests were conducted in the following sequence, with a 48-hour gap between each test to avoid interfering with the next one: open field (OF) test, high cross maze, and forced swim. After 20 minutes of acclimatization to the test room during the lighting phase, all tests were performed in a quiet room (between 10:00 and 12:00). Rats were returned to their rearing room after behavioral assessment.

Open field (OF) test

The open field (OF) test consisted of a square floor with a white background (70 cm x 70 cm), divided in two parts by black lines into 49 squares of equal size (10 cm x 10 cm): 24 squares on the periphery and 25 in the center. The floor was surrounded by transparent Plexiglas walls 30 cm high. The rat was placed in the center, and its behavior was recorded for 5 minutes by a video camera. The apparatus was cleaned with 70% ethanol after each rat (Prut & Belzung, 2003). This test was used to assess locomotor activity by counting the number of squares crossed, calculating the time spent and distance traveled in the periphery, the time spent and distance traveled in the center, and the time of immobility (Hall, 1934).

Elevated cross maze (EPM) test

The EPM test is a well-known device for testing anxiety in laboratory rodents. It consists of two open arms (50cm x10 cm) crossed at right angles with two closed arms (50cm x 10cm, surrounded by 40 cm-high walls). The device is raised 50 cm above the floor. The rat was placed in the center, facing one of the open arms, and its behavior was recorded for 5 minutes using a video camera. The device was cleaned with 70% ethanol after each rat (Prut & Belzung, 2003). The number of entries into the open and closed arms and the time spent in the open and closed arms were measured (Pellow et al., 1985).

Forced swimming test

The forced-swimming test examines depressive behavior in rats (Porsolt et al., 1977). The apparatus consisted of an aquarium (54 x 38 x 40 cm) filled with water at 25°C up to a height of 30 cm. At this height, the rat cannot use its lower limbs to stay at the surface, so it is subjected to forced swimming. The water temperature was maintained at 25°C using an electric immersion heater before the start of the experiment. The FST was conducted in two sessions, the pre-test and the test, separated by a 24-hour period. During the pre-test, the rat was placed for 15 min in a water-filled aquarium in which it could not escape, to create a mental depression (depressed session). On the day of the test, the rat was placed back in the aquarium for a minute session during which immobility, swimming, and climbing time were recorded as indicators of depressive behavior.

Effect on certain biochemical parameters:

Blood samples are taken from control adults and adults treated with the aqueous and ethanolic extract of the *R.chalepensis* plant for hematological and biochemical analyses (urea, creatinine, blood glucose, cholesterol, and triglycerides).

Data Analysis

The study data were analyzed using descriptive and comparative methods (variances analysis) on XLStat 2009 software. The multivariate analysis (MANOVA) with SPSS Statistics 22.0 enabled us to test treatment and sex effects on the rat behaviors tested and biochemical parameters.

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RESULTS

Effect of *Ruta chalepensis* aqueous and ethanolic extracts on anxiety behavior

Open field test

Statistical analysis indicates that treatment with the aqueous and ethanolic extracts of *Ruta chalepensis* does not produce any overall significant effect on the locomotor or anxiety-related behavior of male or female rats in the open field test (Table 1).

In males, the time spent in the central area shows a slight increase following administration of both extracts compared with the control group, suggesting a non-significant trend toward reduced anxiety-like behavior (Table 1). A similar pattern is observed for the distance traveled in the center, which appears higher in treated animals, particularly those receiving the ethanolic extract (Table 1).

In females, the time spent in the center displays an opposite pattern: treated animals tend to explore the central zone less than controls, which could indicate an increase in anxiety-like behavior, although the differences remain statistically non-significant. Distances covered in this zone generally follow the same tendency (Table 1).

Time spent in the periphery remains relatively stable across all groups, with only minor variations depending on the extract type and sex, further supporting the absence of a marked treatment effect on peripheral exploration. Distances traveled in the periphery also do not show clear treatment-related changes.

Finally, immobility duration varies slightly between treated and control animals, but again without revealing a consistent effect of the extracts in either sex.

Overall, the results suggest that the aqueous and ethanolic extracts of *Ruta chalepensis* do not exert a significant influence on the behavioral parameters assessed in the open field test.

Table 1.

Effects on rat behavior in the open field test

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	**AQ**	**ET**	**C**	**AQ**	**ET**	***p***	***p***	***p***
Time spent in the center (s)	11.06± 4.41	14.23± 3.33	18.00± 5.17	23.66±10.43	13.80± 4.88	7.97± 1.77	***0.83***	***0.52***	***0.86***
Distance traversed in the center	16.00±3.21	21.43± 3.47	26.86± 5.06	25.43±3.77	24.00±4.68	15.29± 3.60	***0.78***	***0.71***	***0.39***
Time spent in periphery (s)	288.94±4.41	285.77±3.33	285.86± 3.28	276.34±10.43	286.20±4.88	292.03± 1.77	***0.83***	***0.52***	***0.86***
Distance traversed in periphery	245.43±21.44	252.86±16.63	289.71± 21.56	293.71±22.70	298.43±11.44	268.29±8.31	***0.84***	***0.15***	***0.15***
Immobility time (s)	72.69±8.08	46.029±9.487	57.69± 13.89	49.29±6.43	56.23±9.63	40.11± 5.26	***0.89***	***0.07***	***0.33***

[**C** - *Control*; ♂ - *Male ;* ♀ - *female;* **AQ** - aqueous; ET - ethanolic][* significant; ** highly significant; *** very highly significant].

Elevated cross maze test

The results indicate that neither the aqueous nor the ethanolic extract of *Ruta chalepensis* exerts a significant influence on anxiety-related behavior in the elevated plus maze (Table 2). In males, treated animals displayed slightly greater exploration of the open arms compared with controls, but this increase was modest and not statistically meaningful (Table 2). Females showed inconsistent patterns, with treated groups exhibiting variable open-arm exploration that did not reflect a clear anxiolytic or anxiogenic effect (Table 2).

Behavior in the closed arms followed a similar pattern: small variations were observed between groups, but none suggested a treatment-related change in anxiety levels. Overall, the behavioral responses of both males and females remained largely comparable to those of the control animals (Table 2).

These results demonstrate that the plant extracts do not significantly alter the behavioral parameters commonly associated with anxiety in the elevated plus maze (Table 2).

Table 2.

Effects on rat behavior in elevated cross maze test

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	**AQ**	**ET**	**C**	**AQ**	**ET**	***p***	***p***	***p***
Time spent in open arms (s)	22.29 ± 7.10	28.37 ± 10.22	41.40 ± 4.87	57.26 ± 15.26	32.49 ± 13.42	42.43 ± 16.90	***0.73***	***0.12***	***0.65***
Number of entries in open arms	2.14 ± 0.46	2.43 ± 0.48	2.71 ± 0.36	3.00 ± 0.49	2.86 ± 0.94	3.29 ± 2.43	***0.53***	***0.37***	***0.97***
Time spent in	258.26 ± 12.36	210.94 ± 15.36	179.40 ± 13.03	183.60 ± 16.93	250.71 ± 13.79	206.57 ± 25.21	***0.09***	***0.74***	***0.05***

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	AQ	ET	C	AQ	ET	*p*	*p*	*p*
closed arms (s)									
Number of entries in closed arms	3.14 ± 0.67	3.71 ± 0.61	3.86 ± 0.40	5.57 ± 0.90	4.14 ± 1.16	4.29 ± 1.17	***0.58***	***0.39***	***0.54***

[**C** - *Control*; ♂ - *Male ;* ♀ - *female;* **AQ** - aqueous; **ET** - ethanolic][* significant; ** highly significant; *** very highly significant].

Forced swimming test

The final assessment of anxiety-related behavior using the forced swimming test demonstrates that treatment with either the aqueous or the ethanolic extract of *Ruta chalepensis* does not produce any significant behavioral changes in the animals (Table 3).

In males, swimming time shows slight variations between groups, with treated rats displaying marginal increases or decreases compared with controls; however, these differences are modest and not indicative of any meaningful treatment effect (Table 3). A similar pattern is observed in females, whose swimming durations remain largely comparable across all groups.

Immobility times also fluctuate between treated and control animals, but without forming a consistent trend.

Although some treated groups exhibit somewhat reduced immobility, these variations are small and do not suggest any antidepressant-like or anxiogenic activity linked to the extracts (Table 3).

Climbing behavior presents minor differences among the groups, with slight increases or decreases depending on sex and extract type. Nevertheless, these changes are inconsistent and remain within a range that does not reflect any significant pharmacological effect (Table 3).

Overall, the results of the forced swimming test confirm that the aqueous and ethanolic extracts of *Ruta chalepensis* do not exert measurable effects on the behavioral parameters typically associated with anxiety or depressive-like responses in rodents (Table 3).

Table 3.

Effects on rat behavior in the forced-swimming test

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	AQ	ET	C	AQ	ET	*p*	*p*	*p*
Swimming time (s)	211.37 ± 10.68	245.03 ± 20.45	217.71 ± 15.47	218.14 ± 14.56	228.51 ± 18.79	212.31 ± 17.04	***0.77***	***0.83***	***0.82***
Immobility time (s)	88.63 ± 10.68	55.03 ± 20.44	82.29 ± 15.47	81.86 ± 14.56	71.49 ± 18.79	87.69 ± 17.04	***0.77***	***0.83***	***0.82***
Climbing time (s)	23.31 ± 5.08	17.83 ± 4.29	32.74 ± 9.73	38.09 ± 10.95	22.96 ± 6.88	27.43 ± 10.05	***0.28***	***0.26***	***0.69***

[**C** - *Control*; ♂ - *Male ;* ♀ - *female;* **AQ** - aqueous; **ET** - ethanolic][* significant; ** highly significant; *** very highly significant].

Effect of *Ruta chalepensis* aqueous and ethanolic extracts on biochemical parameters

Analysis of the biochemical parameters indicates that treatment with the aqueous and ethanolic extracts of *Ruta chalepensis* produces only minimal variations in blood glucose, urea, creatinine, triglyceride, and cholesterol levels in both male and female rats (Table 4).

Blood glucose concentrations show slight fluctuations between treated and control groups. While males treated with the extracts tend to exhibit marginally lower glucose levels compared with controls, females display small increases depending on the extract type. Despite these variations, statistical analysis reveals that the only highly significant differences observed concern the comparison between sexes rather than the effect of the treatments (Table 4).

Similarly, urea levels show minor changes following treatment. Males receiving the ethanolic extract present a small increase in urea concentration, whereas treated females exhibit modest reductions relative to controls (Table 4). As with glucose, these differences primarily reflect sex-related variation, with statistical tests confirming highly significant differences between males and females, but not between treatment groups (Table 4)

Creatinine levels vary slightly between treated and control animals in both sexes but remain within a comparable range. Statistical analysis confirms the absence of significant differences between males and females receiving the plant extracts, indicating that treatment does not affect this renal parameter (Table 4).

For triglycerides, both treated males and females display small increases or decreases depending on the extract administered. However, these shifts are minimal, and no significant sex-related or treatment-related differences are detected (Table 4).

Cholesterol concentrations also fluctuate modestly across groups, with treated animals showing slight increases or decreases compared with controls. Despite these changes, statistical analysis indicates no significant differences between males and females treated with the aqueous or ethanolic extracts (Table 4).

Overall, the biochemical profile of treated animals remains largely similar to that of controls. The variations observed in blood glucose, urea, creatinine,

triglycerides, and cholesterol are minor, inconsistent, and not attributable to the administration of *Ruta chalepensis* extracts, suggesting that the plant does not induce notable metabolic or renal disturbances under the experimental conditions (Table 4).

Table 4.

Effects on biochemical parameters

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	**AQ**	**ET**	**C**	**AQ**	**ET**	***p***	***p***	***p***
Glycemia (g/l)	1.20 ± 0.02	1.11 ± 0.03	1.04 ± 0.02	0.92 ± 0.07	0.99 ± 0.10	1.00 ± 0.06	***0.69***	***0.00****	***0.09***
Urea (g/l)	0.32 ± 0.02	0.31 ± 0.03	0.35 ± 0.02	0.48 ± 0.04	0.44 ± 0.05	0.40 ± 0.02	***0.65***	***0.00*****	***0.19***
Creatinine (mg/l)	5.66 ± 0.28	5.07 ± 0.41	5.33 ± 0.61	4.54 ± 0.29	4.84 ± 0.21	4.93 ± 0.37	***0.90***	***0.08***	***0.49***
Triglycerides (g/l)	1.39 ± 0.19	1.29 ± 0.10	1.23 ± 0.07	1.45 ± 0.19	1.53 ± 0.22	1.42 ± 0.14	***0.80***	***0.22***	***0.24***
Cholesterol (g/l)	1.28 ± 0.03	1.47 ± 0.11	1.35 ± 0.08	1.21 ± 0.06	1.18 ± 0.02	1.39 ± 0.16	***0.42***	***0.18***	***0.84***

[**C** - *Control*; ♂ - *Male;* ♀ - *female;* **AQ** - aqueous; **ET** - ethanolic][* significant;** highly significant; *** very highly significant].

Effect of *Ruta chalepensis* aqueous and ethanolic extracts on immune parameters

Analysis of hematological parameters indicates that treatment with the aqueous and ethanolic extracts of *Ruta chalepensis* produces only minor and inconsistent variations in white blood cells, red blood cells, hemoglobin, and platelet counts in both male and female rats.

White blood cell counts show fluctuations between treated and control groups. Males receiving the aqueous extract display an increase in leukocyte numbers, whereas those treated with the ethanolic extract exhibit values closer to the control levels. In females, the pattern is reversed, with slightly lower counts in the aqueous-treated group and values comparable to controls in the ethanolic-treated group. Despite these variations, statistical analysis reveals no significant differences between males and females (Table 5).

Red blood cell counts also vary across groups, with a slight reduction observed in aqueous-treated males compared with controls, while ethanolic-treated males show a modest increase (Table 5). In females, treated groups exhibit small decreases relative to controls. Statistical analysis reveals significant differences between animals receiving the two types of extracts, although these variations remain within physiological ranges (Table 5).

Hemoglobin concentrations display modest changes in both sexes. Treated males tend to show slightly higher hemoglobin levels compared with controls, while females exhibit small fluctuations that do not follow a clear trend. Statistical analysis confirms the absence of significant differences between males and females treated with either extract (Table 5).

Platelet counts vary between treated and control groups, with aqueous-treated males showing a reduction and ethanolic-treated males displaying values similar to controls. In females, platelet numbers remain largely stable across all groups. Statistical results indicate no significant differences in platelet counts between sexes or across extract types (Table 5).

Overall, hematological profiles remain broadly comparable between treated and control animals. The slight variations observed in leukocytes, erythrocytes, hemoglobin, and platelets do not reflect any consistent or biologically significant effect of *Ruta chalepensis* extracts under the conditions of this study (Table 5).

Table 5.

Effects on immunological parameters.

	♂			♀			Treatment	Sexe	Treatment * Sexe
	C	**AQ**	**ET**	**C**	**AQ**	**ET**	***p***	***p***	***p***
WBC (*10⁹/L)	6.66 ± 1.52	14.30 ± 4.11	8.84 ± 0.57	9.10 ± 0.78	6.52 ± 0.34	8.36 ± 1.01	***0.59***	***0.11***	***0.13***
RBC (*10¹²/L)	7.57 ± 0.22	6.93 ± 0.17	7.84 ± 0.14	7.48 ± 0.16	7.06 ± 0.21	7.06 ± 0.21	***0.02****	***0.08***	***0.05***
HB (g/dl)	13.50 ± 3.01	16.04 ± 0.61	16.62 ± 0.41	16.18 ± 0.32	15.64 ± 0.34	16.04 ± 0.61	***0.61***	***0.69***	***0.43***
PLT (*10⁹/L)	989.50 ± 96.00	608.40 ± 63.37	950.20 ± 91.19	1000.40 ± 78.03	1028.60 ± 80.25	1028.60 ± 80.25	***0.61***	***0.69***	***0.43***

[**C** - *Control*; ♂ - *Male;* ♀ - *female;* **AQ** - aqueous; **ET** - ethanolic][* significant; ** highly significant; *** very highly significant].

DISCUSSION

The increasing interest in natural plant-derived products is largely driven by concerns regarding the potential side effects of synthetic compounds, which can be harmful to human health and the environment. Consequently, many pharmaceutical companies have sought to develop herbal extracts with well-characterized therapeutic and toxicological profiles (Baraka & Al-Zakrawi, 2012). In this context, the present study investigated the effects of aqueous and ethanolic extracts of *Ruta chalepensis* on behavioral, biochemical, and hematological parameters in Wistar rats.

Behavioral assessments were conducted using validated models for anxiety in rodents, including the elevated plus maze and the open-field test. The open-field paradigm allows the evaluation of exploratory activity and general locomotor behavior, while also assessing nervous excitability under conditions of mild stress and isolation (Crawley, 1985; File & Fernandes, 1994). Our results indicate that *R. chalepensis* extracts did not significantly affect any parameters measured in the open-field test. These findings are consistent with previous studies, such as Gbaj et al. (2019), who reported that aqueous extracts of *Citrus reticulata* possess anxiolytic potential without altering locomotor activity, likely via modulation of GABAergic pathways.

Similarly, in the elevated plus maze, which measures anxiety-like behavior by exploiting rodents' natural aversion to open and elevated spaces (Pellow et al., 1985; Lister, 1987; Onaivi et al., 1990), we observed no significant differences between treated and control animals of either sex. These results align with Molina-Hernández et al. (2004) and Celso et al. (2013), who demonstrated that herbal preparations, including *Citrus aurantium* essential oil, do not alter anxiety-related parameters in maze tests, possibly due to interactions among the components of plant extracts that maintain normal biological activity (Galindo et al., 2010).

In the forced swim test, a well-established model of depression-like behavior in rodents (Porsolt et al., 1977; Kirby & Lucki, 1997; Petit-Demouliere et al., 2005), immobility is interpreted as a measure of behavioral despair. Our study showed no significant differences in swimming, climbing, or immobility times between treated and control animals. These results are consistent with prior findings in rats treated with *Casimiroa edulis* (Molina-Hernández et al., 2004) and suggest that *R. chalepensis* does not induce depressive-like behavior under the experimental conditions. Nevertheless, the study provides preliminary evidence of potential beneficial effects on depression, consistent with reports of *R. chalepensis* reducing stress-associated behavioral symptoms (Alam Khan & Riaz, 2014).

Biochemical analyses revealed that *R. chalepensis* caused significant differences in blood glucose and urea levels between males and females, whereas creatinine, cholesterol, and triglyceride levels remained unaffected. The increase in blood glucose may be attributed to the inhibitory effects of quinoline compounds in *R. chalepensis* on α-glucosidase and α-amylase, as previously reported (Shaban et al., 2018; Park & Lee, 2015). Sex-specific differences in urea levels are consistent with studies on other medicinal plants affecting renal excretion (Ntchapda et al., 2015; Ntchapda et al., 2016). The lack of significant changes in lipid and renal parameters indicates that the extracts do not exert overt metabolic or toxic effects under the tested doses (Merghem, 2015).

Hematological analyses demonstrated that *R. chalepensis* had no effect on hemoglobin, platelet counts, or white blood cell levels, suggesting that the extracts do not impair erythropoiesis, thrombopoiesis, or immune function. These findings are in agreement with previous studies on *Ruta montana*, which also showed no alterations in hematological parameters (Merghem, 2015; Lubran, 1989). Interestingly, we observed a significant difference in red blood cell counts between animals treated with aqueous and ethanolic extracts, a result supported by Othman (2004), who reported increases in certain erythrocyte parameters following *R. chalepensis* administration.

CONCLUSION

The possibility of using secondary plant substances to replace chemical pesticides has attracted great interest, with some studies showing various biological activities of plant-based preparations.

In this work, we have chosen *Ruta chalepensis*, a plant with a proven track record against various orders of insects. Our results show that this plant has no significant effects on the general behaviour of animals treated with aqueous and ethanolic extracts.

Concerning immune parameters, we reported no effect on white blood cells, platelets, and hemoglobin levels. However, there was a significant difference in red blood cell counts between animals treated with aqueous and ethanolic extract.

Regarding biochemical parameters, our results show that the aqueous and ethanolic extracts of *R.chalepensis* caused a significant difference in urea and blood glucose levels between treated males and females. At the same time, they had no effect on the other parameters (creatinine, triglycerides, cholesterol).

AUTHORS CONTRIBUTIONS

All authors equally contributed to this study. R.C., N.E.I.B., Y.I.H., W.H., R.D., A.I.T., V.R., designed and carried out the experimental study and writed the manuscript.

FUNDING

This research was not funded by any institution, industrial group, or any other party.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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