



RESEARCH ARTICLE

PHYTOCHEMICAL, ANTI-INFLAMMATORY, ANALGESIC, ANTIPYRETIC AND ACUTE TOXICITY OF *PSIADIA PUNCTULATA* GROWING IN YEMEN

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Abstract

Background: *Psiadia punctulata* growing in Yemen is used traditionally for different medicinal purpose, such as in casts of broken bones and for relief of pain, fever and abdominal pain.

Objective: To determine the chemical composition and to assess the anti-inflammatory, analgesic, and antipyretic activity of *P. punctulata* leaf extracts.

Method: Phytochemical screening of *P. punctulata* ethyl acetate and ethanol extracts were performed using chemical tests and thin layer chromatography. An acute oral toxicity study was conducted in rats by administering oral ethanol leaf extract of up to 5000 mg/kg. The anti-inflammatory activity of orally administered ethyl acetate and ethanol extracts (200, 400 mg/kg) and diclofenac (20 mg/kg) were evaluated using a formalin-induced inflammation rat model. The analgesic activity of orally administered ethyl acetate and ethanol leaf extracts (100, 200, 300 mg/kg), compared to diclofenac (20 mg/kg) were evaluated by a formalin-based test as well. The antipyretic activity of oral ethyl acetate and ethanol extracts (400 mg/kg) versus paracetamol (150 mg/kg) was assessed in Baker's Yeast-induced pyrexia rats.

Results: The phytochemical analyses indicated the presence of alkaloids, carbohydrates, steroids, phenolic compounds/tannins, phytosterols, saponins, gum and mucilage. The ethanol extract of the plant was apparently safe in rats at doses as high as 5000 mg/kg body weight. Time- and dose-dependent anti-inflammatory activity of the ethyl acetate and ethanol extracts (200 and 400 mg/kg) were clearly observed in rats. The results showed that both extracts exerted significant analgesic and antipyretic effects.

Conclusion: *Psiadia punctulata* possess anti-inflammatory, analgesic and antipyretic activities with a wide safety margin.

Keywords: Analgesic, antipyretic, anti-inflammatory, *Psiadia punctulata*, Yemen.

INTRODUCTION

Psiadia punctulata belongs to the family Asteraceae, is found in several African countries as well as in Yemen¹. The plant *Psiadia* Jacq. contain several species², three of which are found in Yemen including *Psiadia incanao*, *Psiadia punctulata* and *Psiadia schweinfurthii*³. Phytochemical studies of leaf exudate of *P. punctulata* showed presence of flavonoid, kaurenes and trachylobanediterpenes^{1,4}. Several studies have reported several biological activities for *P. punctulata*. For instance, it has been shown to exert cytotoxic activity against multiple types of cancer cell lines including breast cancer, cervix cancer, hepatocellular carcinoma, bladder carcinoma and

carcinoma of the pharynx. Antioxidant, antifungal, anti-Leishmanial and anti-malarial activities were also observed^{5,6}. The plant is traditionally used for different medicinal purpose in the Arab Peninsula. It is used by locals in casts of fractured bones. Also, the leaf and stem extracts are used to relieve pain and to speed recovery in foot injuries of villagers who often walk around barefooted. In east Africa (particularly in Kenya), leaf decoction offers several benefits including common cold and fever management and for protecting cattle against ectoparasites¹. The plant is also used for its analgesic activity, particularly for abdominal pain⁷. In Yemen, the species were found in Taiz, Sumara, Dhamar, Adhale, Hajja, Ibb, Shabwa and Hadramout. It is mostly added there to casts to speed up recovery of

bones. The aim of this study is to carry out the phytochemical screening of ethyl acetate and ethanol fractions of the leaf extract of *P. punctulata* in addition to assessing the anti-inflammatory, analgesic and antipyretic activity of both extracts. The oral acute toxicity of the ethanol leaf extract was also assessed *in vivo*.

MATERIALS AND METHODS

P. punctulata was collected from the district of Bani Saifin Bani Moharam, Ibb city, Yemen in November 2017. The plant was identified by Dr. Abdul-Wali Al-Khulaidi (working at the Public Authority for Research and Agricultural Extension, Yemen). The specimen voucher of the plant was deposited in the department of pharmacology, Faculty of pharmacy, Sana'a University. The voucher number is pp¹⁷. Methanol 99.8% (Scharlae, Spain), ethyl acetate (HiMedia, India), formic acid (Fluka, Switzerland), paracetamol and sodium diclofenac (Shaphaco Pharmaceutical Ind.-Yemen), 0.9% NaCl (SMSO, Saudi Arabia), Tween 80 (UniChem, Beograd), and thiopental (Rotexmedica, Germany). Solvents/chemicals used were of standard analytical grade.

Extract preparation

The leaves of the plant were thoroughly cleaned then cut into small pieces before weighing them. Then the leaves were put in sufficient amount of ethyl acetate for 20 seconds to get an ethyl acetate extract then the leaves was soaked in ethanol at room temperature for 3 days to get an ethanol extract⁸. Filtration of extracts was performed filtered using Whatman No.1 filter paper. After that, the solvent was evaporated with a rotary evaporator in a water bath with temperature not exceeding 45°C. The extracts were stored in airtight containers at room temperature until time of use.

Phytochemical screening

Carbohydrates, alkaloids, fixed oils/fats, glycosides, poly phenols, tannins, sterols, peptides/proteins, saponins, gum and mucilage were screened in the ethyl acetate and ethanolic extracts using a standard phytochemical screening procedure as previously described⁹.

Animals

Mature male Albino rats, weighing 150-250 g were obtained from the animal house of the College of Science (at Sana'a University). The animals were put in individual cages with controlled light, temperature and humidity (six rats per cage). The animals were maintained on standard diet and tap water and put in a colony room. A 12/12 hr light/dark cycle and a temperature of 21±2°C were maintained before and during the experimentation period. The rats were acclimatized to the laboratory conditions for 48 h before experimentation. The experiments were approved by the Institutional Ethical Committee, Faculty of Medicine, Sana'a University (23/10/2017).

Acute oral toxicity

To investigate the acute toxicity profile of *P. punctulata*, the guidelines of the Organization for Economic Co-operation and Development (OECD) were followed¹⁰. In brief, 36 rats (6 animals in 6

groups) were given only water for 16 hours. The animals were then administered oral methanolic plant extracts in Tween 80 (1% w/v) at serial concentrations of 100, 1000, 2500, 4000 and 5000 mg/kg of body weight whereas the control group were fed the vehicle only. Several parameters were then monitored for 14 days¹¹ including physical signs (weight, physical appearance, eyes, mucous membranes, and fur/skin condition), neurological abnormality (behavior, tremors, diarrhea, salivation, seizures, and physical activity) and mortality. A large dose of sodium thiopentone (100 mg/kg intraperitoneal) was used to euthanize the animals at the end of experiment¹².

Evaluation of anti-inflammatory activity

Formalin-induced inflammation: This test was performed as described previously^{9,13}. Six groups (n = 6) of albino rats were used. All groups were injected with 2% freshly prepared formalin (10 µl) into the sub plantar region of right hind paw to induce inflammation. One hour prior to inflammation induction group 1 was administered 10 ml/kg water p.o. (vehicle), group 2 was administered 20 mg/kg diclofenac sodium p.o., groups 3 and 4 were fed ethyl acetate extracts 200 and 400 mg/kg p.o. (respectively) and groups 5 and 6 were given ethanol extracts 200 and 400 mg/kg p.o. (respectively). With a Vernier caliper, paw volume was measured prior to administration of inflammatory agent and then at predetermined time points (1, 2, 3 and 4 hours after formalin injection).

Anti-inflammatory activity of the extract was evaluated through this equation:

$$\text{Inhibition (\%)} = \frac{(\text{Vt} - \text{V0}) \text{ control} - (\text{Vt} - \text{V0}) \text{ treated group}}{(\text{Vt} - \text{V0}) \text{ control}} \times 100$$

The edema reduction was assessed using the following formula:

$$\text{Edema (\%)} = \frac{\text{Vt} - \text{V0}}{\text{V0}} \times 100$$

Where;

V0 represents rat paw volume prior to administration of formalin, Vt represents rat paw volume after formalin injection at a given time.

Evaluation of the analgesic activity

Formalin test: Thirty two male albino rats were allocated into 8 groups (n=4). Group 1 (negative control) were given normal saline 10 ml/kg and groups 2, 3, 4 were treated with ethyl acetate extract at doses of 100, 200, 300 mg/kg, respectively. Groups 5, 6, 7 were administered ethanol extract at doses of 100, 200, 300 mg/kg, respectively. Group 8 (positive control) were treated with 20 mg/kg diclofenac sodium. Thirty minutes later, the rats were injected with 0.05 ml formalin 2.5% into the right hind paw, then placed immediately in separate plastic cages before injected paw licking time and frequency were recorded for 30 min¹³.

Antipyretic Test

Yeast-induced pyrexia model in rats: The test was performed as described earlier^{14,15}. Four rat groups (n = 5) were used. Group 1 (negative control) were administered 10 ml/kg normal saline whereas group 2 (positive control) were treated with 150 mg/kg paracetamol and groups 3 and 4 were treated with 400 mg/kg of the ethyl acetate and ethanol extract of

P. punctulata, respectively (all orally). Before inducing fever, baseline rat rectum temperatures for all rats were recorded with a digital thermometer. For pyrexia induction, subcutaneous injections of 20% w/v Baker's yeast suspension (10 ml/kg) were administered. Rectal temperatures were then taken after 19 hours. After that, normal saline, paracetamol, ethyl acetate, and ethanol extract were administered orally only to the rats with a 0.6°C (or 1°F) increase in rectal temperature. Rectal temperatures were again recorded in the first, second, third and fourth hours following treatments.

Statistical analyses

Statistically Package for Social Sciences (SPSS) version 11.5 was utilized for data analysis. Data are presented as means±Standard deviations (SD), Categorical variables were represented by frequencies and percentages. Paired T-test was implemented to test the significance of the differences between every two groups. Significance level was set at 0.05 and 0.01.

RESULTS

Phytochemical screening

The findings of the phytochemical analysis for the *P. punctulata* ethyl acetate, and ethanol extracts are illustrated in Table¹. Both extracts were positive for alkaloids, carbohydrates, steroids, poly phenols, tannins, phytosterols, saponins, gum and mucilage.

Acute toxicity test

The findings of the study indicate the safety of *P. punctulata* methanolic extracts on rats. Even at high doses of up to 5000 mg/kg, no apparent adverse reactions or toxicity were noted in rats. No physical, neurological, psychological abnormalities were recorded during the 2 weeks period post oral extract ingestion. The animal appeared physically active with no alterations in appearance, skin/fur, salivation, defecation, or sleeping patterns.

Table 1: Phytochemical screening of the ethyl acetate and ethanol extracts of *P. punctulata*.

Phytochemical screening		Ethyl acetate extract	Ethanol extract
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
Carbohydrates	Benedict's test	+	+
Fixed oils/ fats	Saponification	-	-
Steroids	Salkowsk's test	+	+
Anthraquinones	Borntrager's test	+	+
Phenolic compounds/tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Phytosterols	Mg and HCl reduction test	+	+
	Liebermann-Burchard's test	+	+
Proteins	Biuret test	-	-
Saponins	Foam test	+	+
Gum and Mucilage		+	+

+ = presence, - = absence, Mg = Magnesium, HCl = Hydrochloric acid.

Table 2: Anti-inflammatory activities of the ethyl acetate and ethanolic extract of *P. punctulata* leaves and diclofenac on formalin-induced edema in the right hind-limb of rats.

Time (hr)	Treatment	Ethyl acetate extract (mm) (% of inhibition)		Ethanolic extract (mm) (% of inhibition)		Sodium Diclofenac 20mg/kg
		200	400	200	400	
0	Control	2.98±0.09	3.23±0.20	2.93±0.08	2.98±0.07	2.96±0.05
1	3.91±0.20*	3.56±0.33*	3.78±0.22*	3.33±0.22*	3.33±0.18*	3.43±0.12*
2	4.13±0.21*	3.25±0.28 (9%)	3.46±0.20 (8%)	3.16±0.10* (5%)	3.15±0.15 (5%)	3.16±0.16" (8%)
3	4.13±0.21*	3.21±0.31 (10%)	3.25±0.19" (14%)	3.08±0.07" (8%)	3.05±0.05" (8%)	3.11±0.09" (9%)
4	4.76±0.45*	3.03±0.08" (15%)	3.25±0.19" (14%)	2.98±0.04" (11%)	3.00±0.06" (10%)	3.01±0.04" (12%)

* = $p \leq 0.05$ compared to value at zero time, " = $p \leq 0.05$ compared to value at 1 hour time

Also, no behavioral changes, neurological defects, comas or deaths were observed. These data show the relative safety of *P. punctulata* in living systems and indicate that the lethal dose 50 (LD₅₀) of *P. punctulata* methanolic extract in rats is above 5000 mg/kg.

Anti-inflammatory Activity

Injecting rats with 0.1 ml 2% formalin into the right hand foot pad resulted in a local inflammatory reaction and edema. The size of edema increased gradually with time following the injection compared to zero time in group one and reached a maximum after 4 hours of injection (Table 2). Prior administration of diclofenac (positive control) led to a marked decrease in the inflammation and in the size of edema compared to time zero. The anti-inflammatory activity of

diclofenac started within 2 hours, and the effect peaked after 4 hours post injection resulting in a 12% reduction in edema. The results showed that the ethyl acetate extract (200, 400 mg/kg b.w.) possessed anti-inflammatory activity. At 4 hours post injection, the two tested doses decreased the size of edema significantly (15% and 14% reduction respectively) compared to the edema size at 1 hour. Additionally, the ethanol extract (200 and 400 mg/kg) resulted in less anti-inflammatory effect and inhibited edema by 11% and 10%, respectively.

Analgesic Activity

Formalin-induced pain in the right hind-limb of rats was utilized to evaluate the analgesic activity of three doses of each extract at multiple doses (100, 200, 300

mg/kg). These extracts were compared with the analgesic effect exerted by the powerful non-steroidal anti-inflammatory drug diclofenac (20 mg/kg). Table 3 showed the extracts at different doses significantly decreased both licking time and frequency compared to those of the control group. Highest percent decrease in licking time (57%) was achieved at 200 mg/kg dose of ethanol extract. The percent reduction induced by diclofenac sodium (20 mg/kg) was about 61%. Interestingly, both extracts were also effective in alleviating pain. For instance, the 300 mg/kg ethyl acetate extract induced a 58% percent of reduction in licking frequency while the 300 mg/kg ethanol extract induced a 45% reduction. The analgesic activity recordings showed that the reduction of liking time and licking frequency by both extracts was generally not dose-dependent.

Antipyretic Activity

As shown in Table 4, subcutaneous injections of animals with 20% w/v Baker's yeast suspension (10 ml/kg) led to significant elevation in rectal temperatures after 19 hrs. In group one (treated with normal saline), rectal temperature continued to elevate for 2 hours before decreasing in the 3rd and 4th hours post normal saline treatment. In the positive group, treated with the antipyretic drug paracetamol (150 mg/kg), the rectal temperature significantly decreased

in all selected time points compared to time zero. Also, the rectal temperature of the group treated with the ethyl acetate (400 mg /kg) extract significantly decreased in all time points except in the 4th hour, compared to the negative control group. On the other hand, the rectal temperature of the group that was given the ethanol extract (400 mg/kg), significantly decreased in the first two hours but not in the 3rd or 4th hours post treatment.

DISCUSSION

In this study, the phytochemical screening of *P. punctulata* leaves as well as the biological activities of the plant leaf extracts, including anti-inflammatory, analgesic, antipyretic activities and acute toxicity were investigated. The preliminary phytochemical analysis of the ethanol and ethyl acetate leaf extracts indicated the presence of chemical constituents which may contribute to its claimed medicinal activities. The chemicals detected using chemical test included alkaloids, carbohydrates, steroids, bitters, poly phenols, tannins, flavonoids, sterols, saponins, gum and mucilage. To assess the anti-inflammatory activity exerted by *P. punctulata* leaves, formalin-induced inflammation rat model was utilized.

Table 3: The analgesic activity of the ethyl acetate and ethanolic extracts of *P. punctulata* leaves and diclofenac on formalin-induced pain in the right hind-limb of rats.

Treatment	Dose mg/kg	Licking time (sec) (% of inhibition)	Licking frequency/30min (% of inhibition)
Control		15.9±2.3	35.8±4.2
Ethyl acetate extract	100	10.3±0.6* (35%)	28.9±1.0* (19%)
	200	8.5± 0.6* (47%)	23.5±3.1* (34%)
	300	9.9±0.9* (38%)	15±1.0* (58%)
Ethanol extract	100	12.1±0.9* (24%)	20.2±4.6* (44%)
	200	6.9±0.1* (57%)	21±4.5* (41%)
	300	10.8±0.8* (32%)	19.8± 4.5* (45%)
Diclofenac	20	6.2± 0.4* (61%)	24.8±1.2* (31%)

* = $p \leq 0.05$ compared to control

This method is known to predict the potential of a test agent to combat acute inflammation by diminishing the action of the inflammatory autocooids involved and has extensively been used to evaluate the anti-inflammatory potential of plant extracts in several studies^{16,17}. When injecting formalin in rat paws, a biphasic local inflammatory response is induced. The first (early) phase is associated with neurogenic pain whereas the second (late) phase involves the activation of inflammatory processes driven by the release of local mediators¹⁸. Local inflammatory mediators produced during the late phase of inflammation include prostaglandins, serotonin, histamine, bradykinin and other cytokines¹⁹.

The present work established the anti-inflammatory activity of *P. punctulata* leaves *in vivo*. Indeed, both ethyl acetate and ethanol plant leaf extracts, at two different doses of 200-400 mg/kg b.w., displayed marked anti-inflammatory effect as shown by decreasing size of formalin-induced rat paw edema in rats. The paw edema reduction exhibited by the plant extracts appeared dose-independent as using a 400

mg/kg concentration did not have a favorable anti-inflammatory effect compared to that of using half that concentration (200 mg/kg). For instance, four hours post formalin injection, the edema reduction in the groups treated with the 200 mg/kg and 400 mg/kg ethyl acetate extracts were 15% and 14% respectively. Notably, the prominent reduction in edema and inflammation by the extracts started after 2 hours following formalin injection and continued throughout the entire observation period of 4 hours, which indicates their efficacy in alleviating the late phase of inflammation if taken orally. The shown two-hour time gap before the manifestation of the anti-inflammatory activity of extracts are likely ascribed to the time needed for the bioactive agents to distribute in body fluids (and then distribute into target sites) or the time needed for biotransformation inside the body to form active metabolites endowed with the anti-inflammatory activity. The anti-inflammatory effect of *P. punctulata* is possibly mediated by inhibiting prostaglandins and other inflammatory mediators. However, more investigations are needed to confirm that.

Inflammation is a natural biological response to insults and involves activation of various enzymes and local autacoids, cell migration, tissue breakdown and repair. Edema in an inflamed tissue occur due to increased capillary permeability of water and albumin in plasma. Migration of white blood cells, particularly neutrophils also occur from plasma into the injured area²⁰. The enzyme responsible for the biosynthesis of the most important inflammatory mediators "prostaglandins" from the natural precursor arachidonic acid is called Cyclooxygenase (COX). There are two types of COX available in human tissues. COX-1 produces basal amounts of physiological prostaglandins that are needed for several hemostatic functions in the body. COX-2, on the other hand, is induced in response to inflammatory conditions to produce inflammatory prostaglandins that are responsible for the regular signs of inflammation (redness, edema, itching etc)²¹.

To determine the potential of *P. punctulata* to combat pain, paw licking time and frequency were recorded after injecting formalin in the right hind paw of rats. This test is used to demonstrate the involvement of both central and peripheral pathways of analgesia and

offers the advantages of mimicking clinical human pain, sensitivity to agents with modest analgesic activity, and sensitivity to commonly used analgesics like NSAIDs²². The findings of our investigation showed the ability of *P. punctulata* leaf ethyl acetate and ethanol extracts to significantly raise pain threshold as compared to control as shown by the reduction in limb licking time and frequency. Similar to what was observed with the anti-inflammatory effect (discussed above), the analgesic effect of the extracts was dose-independent. Pain usually results from tissue damage and is defined as an unpleasant sensation induced by the release of endogenous mediators including prostaglandins that are synthesized by the action of COX on arachidonic acid.

Analgesics usually exert their pharmacological effect by acting on the central nervous system (CNS) or on peripheral tissues. Non-steroidal anti-inflammatory drugs (e.g. diclofenac) and simple analgesics (e.g. paracetamol) are thought to exhibit their analgesic activity by blocking biosynthesis of prostaglandins, either in the CNS or peripherally^{23,24}.

Table 4: Antipyretic activity of EtOAc and EtOH (400 mg/kg) extracts of *P. punctulata* leaves and Paracetamol (150 mg/kg) in Yeast-induced pyrexia model in rats.

Treatment Groups	Temperature (C°)					
	BBT	0	1	2	3	4
Normal Saline	37.98±0.1	38.6±0.1*	38.7±0.5	38.7±0.2	38.18±0.4 ^b	38.2±0.4 ^b
Paracetamol (150 mg/kg)	37.78±0.3	38.6±0.4*	37.1±0.4 ^{ab}	37.2±0.3 ^{ab}	37.3±0.2 ^{ab}	37.9±0.2 ^{ab}
Ethyl acetate extract	38.32±0.4	38.9±0.*	37.7±0.2 ^{ab}	37.6±0.2 ^{ab}	37.7±0.4 ^{ab}	38.0±0.4 ^b
Ethanol extract	38.08±0.4	38.7±0.3*	37.6±0.4 ^{ab}	37.9±0.4 ^{ab}	38.3±0.5 ^b	38.1±0.4 ^b

* = $p < 0.05$ compared to basal body temperature. a = $p < 0.05$ compared to control groups at zero time, b = $p < 0.05$ in compared to zero time of same group. BBT: Basal body temperature.

Thus, it is possible that the *P. punctulata* leaf extracts act by inhibiting PG synthesis or action, just like simple analgesics and NSAIDs. Taken into account that several CNS depressants in high doses have the potential to produce analgesia by merely suppressing the brain activity independent of their effect on prostaglandins and inflammatory mediators²⁵, the test extracts sedative profile on rats was observed. No apparent sedative action on rats was noted as shown by their normal physical activity during the test period. Therefore, sedation is likely not a contributor to the analgesic activity of *P. punctulata*.

In addition to the aforementioned effects, both ethanol and ethyl acetate extracts of *P. punctulata* leaves demonstrated marked anti-pyretic potential as shown by the inhibiting rise of temperature in the rat yeast model. The effect was evident in the first two hours but then faded in the 3rd and 4th hour of the investigation period, indicating a short antipyretic effect. The antipyretic effect of the extracts may be ascribed to inhibiting the synthesis of endogenous pyrogenic prostaglandins and thus decreasing their levels in serum and thus in the CNS, especially that these extracts also exhibited an analgesic and anti-inflammatory potential. The pharmacological properties shown by *P. punctulata* in the present work provide the support for the use of *P. punctulata* leaves for pain, fever and inflammation, as commonly practiced in traditional medicine. Although, it is not yet

clear where this medicinal activity comes from, it has been reported that phenolic compounds inhibit prostaglandin synthesis and thus elicit an analgesic effect²⁶. In addition, natural antioxidants (e.g. tannins, flavonoids) have the potential to bind free radicals released by leukocytes in response to tissue injury, and thus may suppress inflammation. Other potential contributors to the activity arises from the presence of saponins as previous reports on other plant species indicated that saponins exert both analgesic and anti-inflammatory activities. Natural products are often looked to as a promising source for bioactive agents with superior safety profile, compared to synthetic drugs. The present work showed that ethanol extracts of *P. punctulata* appeared safe to rats. Oral administration of ethanol extracts of *P. punctulata* leaves for up to 5000 mg/kg resulted in no detectable toxic signs in body weight, physical activity, behavior, or mortality rate²⁵. However, people should be cautious when the leaves are used in oral preparations, until absolute safety is confirmed in humans.

CONCLUSIONS

In conclusion, the results of this study provide evidence for the anti-inflammatory, analgesic and antipyretic activity of *P. punctulata* leaves claimed in Yemeni folk medicine. Although the mechanism is not clear, it is possible that these activities arise from blocking

prostaglandin biosynthesis of prostaglandins by *P. punctulata*. These observed pharmacological activities may be ascribed to the presence of one or more of the detected bioactive constituents: alkaloids, carbohydrates, steroids. Although this study provides scientific justification for the ethno-medicinal uses of *P. punctulata*, more research needs to be done to ascertain the safety of the plant in humans and to decipher its therapeutic molecular mechanisms.

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AUTHOR'S CONTRIBUTIONS

Al-Mahbashi H: writing original draft, pharmacological experimentation, interpretation of data. **Moharram BA:** methodology, statistical analysis, conceptualization. **Al-Maqtari T:** editing, methodology. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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