Original Article

CNS Depressant and Antinociceptive Effects of Different Fractions of *Pandanus Foetidus* Roxb. Leaf Extract in Mice

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Abstract

***Background*: Various parts of *Pandanus foetidus* Roxb. are used as traditional medicines. However, scientific reports concerning the effect of this plant on central nervous system (CNS) depression and analgesia are unavailable. This study investigated the CNS depressant and antinociceptive effects of *Pandanus foetidus* leaf extracts in a rodent model.**

***Methods:* The sedative and anxiolytic activities of *Pandanus foetidus* extract (500 g) were tested using behavioural models of Swiss albino mice, and the analgesic activity was assessed by formalin-induced pain and tail immersion tests at 200 mg/kg body weight of the mice. The data were analysed by a one-way ANOVA, a repeated measure of ANOVA and a non-parametric test (Kruskal-Wallis test) using the SPSS software. Acute toxicity was tested using an established method.**

***Results:* Compared with the aqueous fraction, the methanol, petroleum ether and chloroform fractions of the extract exhibited a more significant ($P < 0.001$) reduction of locomotor activity in the mice in the open field, hole-cross, and elevated plus maze (EPM). The methanol fraction maximized the duration of sleeping time caused by the thiopental sodium induction. The extract produced a significant step-down in pain, as shown by the paw licking time in the early and late phases of the formalin test. In the tail immersion test, the chloroform fraction maximally reduced the heat-induced analgesia. The extract was found to be non toxic.**

***Conclusion:* The methanol, petroleum ether, and chloroform fractions of *P. foetidus* have strong CNS depressant and antinociceptive effects and thus merit further pharmaceutical studies.**

Keywords: *Antinociceptive, CNS depressant, analgesic, anti-inflammatory*

Introduction

Anxiety and depression are major psychiatric conditions that are commonly found worldwide (1). Many people suffer from these conditions at some point in life. Although there are number of drugs for the treatment of anxiety and depression, their efficacy is very limited, and they are expensive. There is continuing research to develop highly efficacious, better-tolerated and cost-effective drugs. Plant-derived medicines across a wide spectrum have been advanced as novel sources of psychiatric therapies (1), which have been reflected in the large number of traditional medicines that have been researched and screened for their psychotherapeutic potential in rodent models.

Pain is predominantly a safety mechanism for the body; it occurs in the case of injury to any tissues and causes an individual to react to remove the pain stimulus (2). Analgesics relieve symptomatic pain, whereas they have no effect on the cause of pain (3). Additionally, they have many side effects, which make the drugs unfavourable for use as therapeutic agents. In this context, demand is increasing for novel compounds that possess better pain relieving potential and minimum side effects.

Pandanus foetidus Roxb. (Pandanaceae), locally known as kewa kata or keora, is a common hedge-plant without a proper stem that grows throughout Bangladesh, predominantly in the coastal mangrove forest region, Sundarban (4) and Chittagong. Leaves of this plant are used in therapies for leprosy, syphilis, scabies, small pox, and brain, and heart diseases (5,6). The leaves and

spadix are used to treat diabetes (6). In addition to its medical usage, the essential oil of *P. foetidus* is used in perfume. The root is considered to be diuretic, depurative, and stimulating (6). Recently, the crude ethanol extract of *P. foetidus* was studied for its phytochemical screening, antioxidative effects, total phenolic content, and analgesic properties. A remarkable 2,2,-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging effect was observed in this study (7). The in vivo antidiarrhoeal and cytotoxic potential of different fractions of *P. foetidus* leaves have been reported very recently (8). Previously, the CNS depressant effect of the methanol extract of *P. foetidus* leaves was investigated (9). Despite the promising results of the in vitro and in vivo studies, the plant has not been studied extensively. The antinociceptive effect of *P. foetidus* and the central nervous system (CNS) depressant effect of other solvent extracts of this plant have not been investigated; the fraction of this plant that possesses the most potential as a CNS depressant may be noticed through a comparison. This study investigated the antinociceptive and CNS depressant effects of four *P. foetidus* leaf fractions.

Materials and Methods

Reagents and chemicals

The reagents and chemicals used in this research were of analytical grade. Methanol, petroleum ether and chloroform were purchased as impure solvents to be purified through distillation. Diazepam, sodium thiopental, formalin and nalbuphine were collected from Sigma Chemical Co., 3050 Spruce St. St. Louis, MO, USA. Diclofenac sodium was supplied by GlaxoSmithKline, Chittagong.

The collection and identification of plant material

The *Pandanus foetidus* leaf samples were harvested from the Chittagong coastal area of Bangladesh during August and September 2012. The plant samples were identified as a taxonomical sample specimen (K 4031) and were preserved in the National Herbarium of Bangladesh for future reference.

Plant extract

The *Pandanus foetidus* leaves were cleaned with tap water, chopped, and oven-dried at 35–40 °C for one week. The plant leaves were ground into a powder (500 g) with a mechanical grinder (a Moulinex three-in-one grinder, China). The resulting powder was defatted by 100% hexane and successively extracted by methanol, petroleum ether, chloroform, and water. The filtrate obtained from the extractions was concentrated using a rotatory evaporator (RE200, Bibby Sterling, Ltd., UK) under reduced pressure. The concentrate was designated as a crude extract (yield 5.0%, w/w). The extracts were referred to as methanol extract (PFMEx), petroleum ether extract (PFPEx), chloroform extract (PFCEx) and aqueous extract (PFAEx).

Experimental animals

Six-week-old male or female albino mice (average weight, 25–30 g) were obtained from the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The mice were acclimatised to the laboratory conditions (room temperature 24 ± 2 °C, relative humidity 55–60%, and 12 h light and dark cycles). During the entire study period, the mice were caged individually and provided with a rodent pellet diet and water *ad libitum*. The animals were maintained and handled according to the protocol approved by the Institutional Ethics Committee (IAEC, Reference no IIUC/AE 04).

Animal grouping

The experimental mice were divided into six groups, and each group contained five mice. The rats in the normal control group received the vehicle (Tween 80, 1%). The test groups were administered a 200 mg/kg b.w. oral dose of methanol, petroleum ether, chloroform or aqueous extracts of *P. foetidus*, whereas the reference control group was treated with diazepam (1 mg/kg, intraperitoneally).

Neuropharmacological activity

Open field test

This test was conducted in accordance with a modified method of Kulkarni and Reddy (10) to measure the CNS depressant effect of *P. foetidus* extract in albino mice. This test apparatus consists of one-half-square meter floorboards that were divided into a number of squares, each alternately coloured white and black. The entire board was surrounded by a 40 cm high wall. The number of squares travelled by the mice was recorded for 3 min during each time interval at the 0, 30, 60, 90 and 120th min of the entire study period.

Hole-cross test

The hole-cross test was conducted according to an established protocol detailed by Takagi et al. (11) using a cage (30 × 20 × 14 cm^3) with a partition fixed in the middle. A 3 cm diameter hole

with a height of 7.5 cm was made in the centre of the cage. The number of visits of a mouse from one chamber to another through the hole was recorded for 3 min at the 0, 30, 60, 90 and 120 min of the oral *P. foetidus* extract administration.

Thiopental sodium-induced sleeping time

This test was conducted by the protocol described by Ferrini et al. (12). The test groups were administered sodium thiopental (by a 40 mg/kg intraperitoneal injection) 20 min after the administration of methanol, petroleum ether, chloroform, and aqueous extracts of *P. foetidus*, and the sleeping time was counted as the interval between the loss and regaining of the righting reflex. The reference control group was injected with diazepam (at 1 mg/kg intraperitoneally), and the normal control group was administered the vehicle (Tween 80, 1%).

Elevated plus maze test

The elevated plus maze test uses a device with two open-arms (5 × 10 × 0.5 cm^3) and two closed-arms (5 × 10 × l5 cm^3). The arms are beamed from a platform (5 × 5 cm^2), and they form a plus sign that is 0.40 m above the surface. The experimental animals were tested once and kept alone for 5 min after 1 h of treatment to observe the entries of the mice into the closed and open arms. The time spent during each entry was recorded. To remove unwanted smells, the maze was cleaned after every use. If all of the paws of a mouse entered an arm, the movement was considered an entry (13). The test groups were administered similar oral doses as those described for the hole-cross test. The doses were administered 1 h before the experiments. Diazepam was used as a reference control in this test.

Antinociceptive activity

Formalin-induced nociception

Twenty microlitres of 2.5% formalin in saline was subcutaneously injected in the hind paw of the mice 30 min after the injection of diclofenac sodium (for a reference control, 10 mg/kg intraperitoneally) and the oral administration of methanol, petroleum ether, chloroform and aqueous extracts of *P. foetidus* to the reference control group and the test control groups, respectively. The normal control group was injected subcutaneously with 20 µL of 2.5% formalin. A pain response was counted as the time spent licking and biting the injected paw. The data were expressed as the total licking time in two phases; the early phase represents licking for 0–5 min, and the late phase represents licking for 15–30 min following the formalin injection (14).

Tail immersion method

In the tail immersion method, the normal control group was treated with Tween 80 (1% at 10 mg/kg). The reference control group was subcutaneously injected with the reference drug, nalbuphine, at 10 mg/kg b.w. The test control groups were treated with methanol, petroleum ether, chloroform, and aqueous extracts of *P. foetidus* at the same dose described above. The mice were screened, and those that failed to respond within 60 sec were not used for the assay. In this assay, the lower portion (3 cm) of the tail of the mice was dipped into a water bath at 55 °C. The reaction was counted as the tail withdrawal time (sec) from the water, which was recorded at before (0) and at 30, 60 and 90 min after the test sample dosing. Thermal injuries to the experimental mice were prevented by allowing a maximum immersion time of 15 sec (15).

Acute toxicity test

The albino mice for the acute toxicity study were acclimated to the standard laboratory conditions (humidity 55–60%, temperature 24 ± 1 °C). Five mice were orally treated with *P. foetidus* extract at a single dose of 0.5, 1.0, 1.5, and 2.0 g/kg b.w. The mice were fasted overnight prior to the treatment with the extract. Once the extract was administered, food was withheld for the next 3–4 h. Each individual animal was kept in observation for the first 30 min after dosing and then for 24 h (with particular care during the first 4 h) and were thereafter examined daily for 14 days to record any delayed toxicity. The cage-side observation (once daily) included observing changes in the fur and skin, mucous membrane and eyes, circulatory and respiratory rates, and CNS as well as the autonomic changes. The effective therapeutic dose was recorded as one-tenth of the median lethal dose (LD50 > 2.0 g/kg) (16).

Statistical analysis

The results were presented as the mean (SD). The data were analysed by a non-parametric test (the Kruskal-Wallis test), a one-way analysis of variance (ANOVA) and a repeated measure of ANOVA followed by the Duncan Multiple Range Test using statistical software (statistical package for social sciences, SPSS, Version 22.0, Data Editing for Windows, IBM Corporation, USA). The values of $P < 0.05$ were considered statistically significant.

Results

Open field test

The *P. foetidus* leaf (200 mg/kg body weight) of the methanol, petroleum ether, chloroform and aqueous fractions showed significant suppression in the mice, as observed in the number of squares across which a mouse travelled from 30 min to 120 min (P = 0.001). Among the fractions, the methanol and petroleum ether fractions showed more significant effects than did the aqueous and chloroform fractions (Figure 1).

Hole-cross test

The results showed that all fractions of *P. foetidus* significantly (P = 0.046) decreased the number of holes crossed by the mice from its initial value over a period of time. All of the fractions perceptibly decreased the locomotor activity of the experimental mice from the 3rd to 5th observation hour (60 to 120 min) at 0.2 g/kg b.w. The CNS-depressant functions were significantly (P = 0.037) reduced with time. The locomotor activity was maximally suppressed by the methanol and chloroform fractions, and the results were significant compared with those obtained using diazepam, the reference drug (Figure 2).

Thiopental sodium induced sleeping time

All of the *P. foetidus* fractions decreased the time for the onset of action and increased the length of the mice sleeping time, which was comparable to the results of the control. The methanol fraction produced a more significant effect on the duration of sleep than did the standard drug, diazepam (Figure 3). A Kruskal-Wallis H test showed a statistically significant difference in the onset of sleep among the drug treatments, $\chi2(4) = 2\ 0.544$, $p < 0.001$. The Duncan Multiple Range Test (DMRT) indicates that the control and PFME groups differed significantly from the other treatments. There was no significant difference between PFAQ and PFPE. In addition, PFCF and diazepam had the same effect on the sleep duration.

Elevated plus-maze (EPM) test

The oral dosing of the fractions of *P. foetidus*, except that of the aqueous fraction, at 200 mg/kg b.w. exhibited a moderate increase in the entry into the open arms of the elevated plus-maze (EPM). The standard anxiolytic drug, diazepam (1 mg/kg), showed a very significant increase in the time spent in the open-arm segment of the maze (P = 0.013), which shows an anxiolytic action (Figure 4). In the extracts, the percentage of entries into the open-arm segments and the time spent in the open-arm segments were close to those resulting from the administration of diazepam.

Antinociceptive activity

Formalin-induced nociception

In the formalin test, the oral dosing of the *P. foetidus* methanol extract fraction displayed a significant decrease in the paw licking times in the early and late phases compared with that of the reference drug, diclofenac sodium. The Kruskal-Wallis H test showed a statistically significant difference (P < 0.001) with each other in the early and late phases (Figure 5). The chi-squared value for PFME was 27.759 in the early phase and 28.251 in the late phase of the PFME group, which showed a strong significance of P < 0.001. Other treatments were found to be significant compared with the reference control, diclofenac sodium.

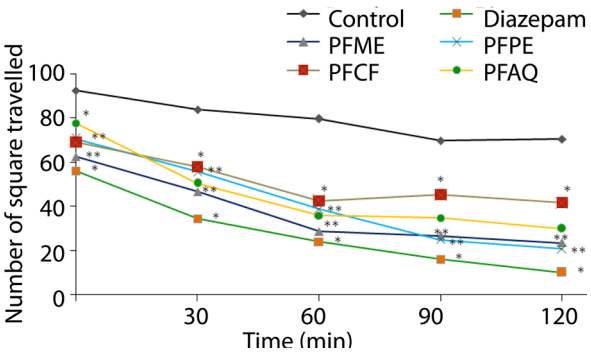

Figure 1: CNS depressant activity of the different fractions of *P. foetidus* leaf extract on Open Field Test in mice.

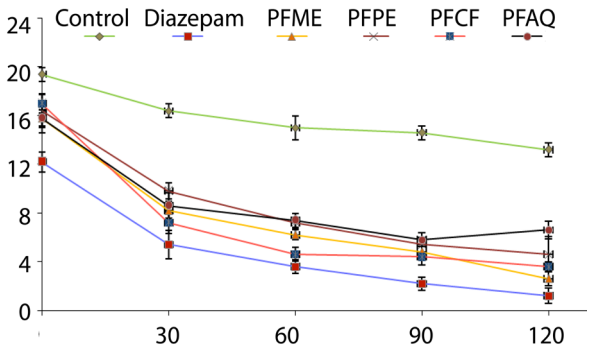

Figure 2: CNS depressant activity of the different fractions of *P. foetidus* leaf extract on Hole cross test in mice.

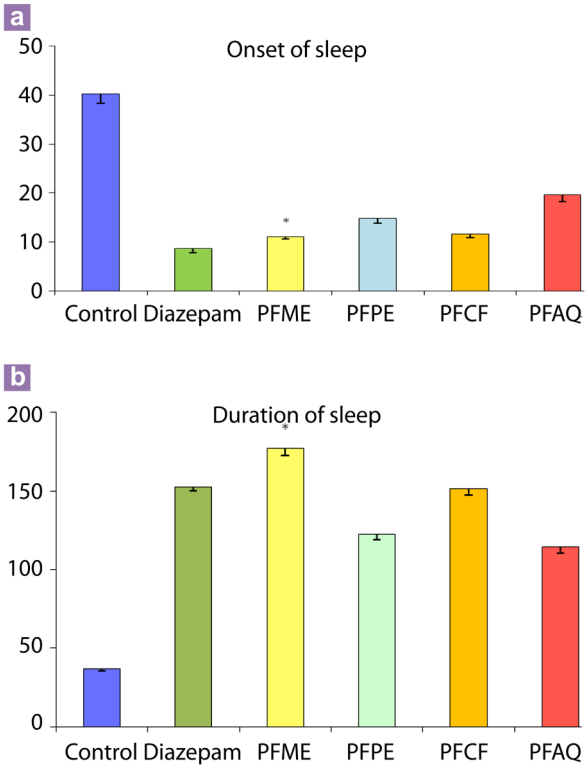

Figure 3: CNS depressant activity of *P. foetidus* on Thiopental sodium induced sleeping time test in mice.

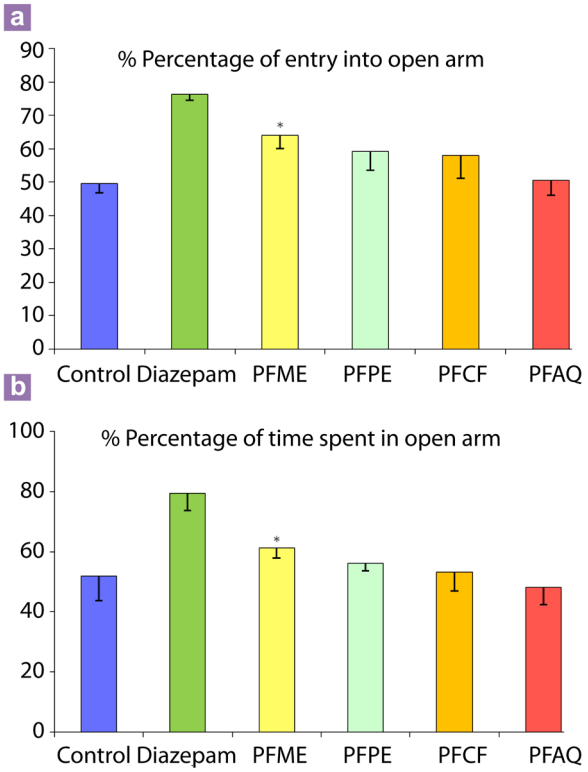

Figure 4: CNS depressant activity of different fractions of *P. foetidus* leaf extract on Elevated Plus Maze (EPM) test in mice.

Tail immersion method

In the tail immersion method, the different fractions of *P. foetidus* leaf extract exhibited a significant ($P < 0.001$) effect against conduction of mice analgesia induced by heat. The chloroform fraction produced the maximum response compared with the standard, nalbuphine, at 10 mg/kg (Figure 6).

Discussion

In this study, the effects of methanol, petroleum ether, chloroform, and aqueous fractions of *P. foetidus* leaf extracts were studied in various neuro-pharmacological models, and the open field and hole-cross tests were used to assess the effects of the locomotor function of the fractions. The results of the study showed that the methanol, petroleum ether and chloroform extracts of the *P. foetidus* leaf had a very good type of CNS depressant activity, as indicated by the decrease in the locomotor activity in mice. An increase in the locomotor function is considered an index of alertness, and the converse is an indication of sedative activity. The marked sedative effect of the extracts was found by a reduction in sleeping latency and an increase in the sleeping time induced by thiopental sodium (17,18).

The elevated plus-maze is a promising behavioural test for evaluating anxiety in animal models. A natural or spontaneous aversive stimulus, such as an unprotected opening, height or novelty, is involved in this test (13,19,20). Plant extracts that are known to diminish or reduce anxiety show an increase in the exploration of open arms in the elevated plus-maze test, in which much of the allotted time for the mice is preferably spent in the closed arms. As a result, an aversion toward the open arms is created, which generates a fear of open spaces. However, anxiolytic drugs produce an increase in the exploration of open arms, which is reversed for anxiogenic drugs (13). Drugs that cause an increase in the exploration of open arms are considered to be anxiolytic; the drugs that cause the reverse effects are anxiogenic (13). The methanol and petroleum ether fractions of the plant extract in mice maximally increased the entries into the open arms. These fractions increased the time spent in the open arms more

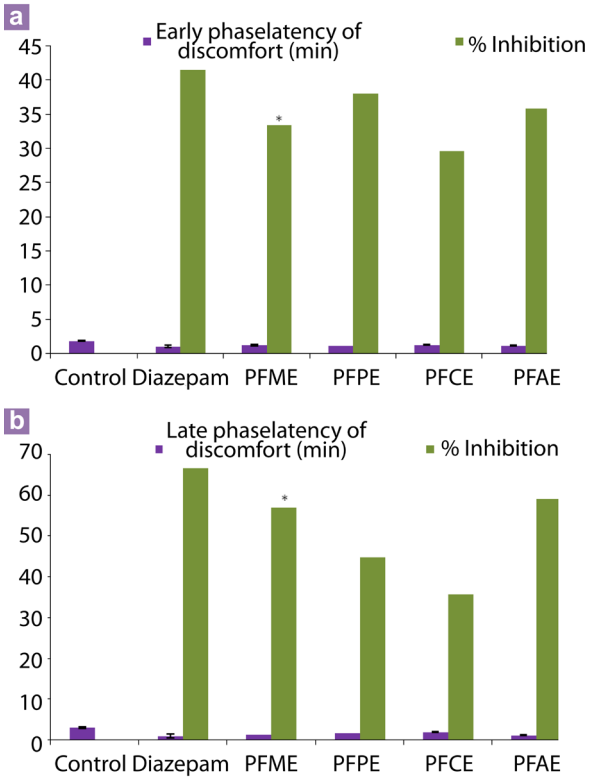

Figure 5: Antinociceptive activity of *P. foetidus* on Hindpaw licking in formalin test in mice.

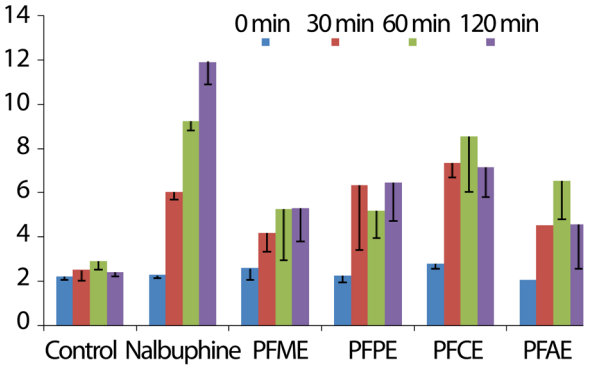

Figure 6: Antinociceptive activity of *P. foetidus* on tail immersion test in mice.

than the chloroform and aqueous fractions did.

Thiopental, a barbiturate, produced a sedative-hypnotic effect at a specific dose because of its interaction with the gamma amino butyric acid (GABA)A receptors, which enhances GABAergic transmission. It potentiates the GABA activity, thereby allowing chloride to enter the neuron by prolonging the duration of the chloride-channel opening. Thiopental could block the excitatory glutamate receptors. These molecular activities lead to decreased neuronal activity, which supports the findings obtained for the reference drug diazepam, which is a CNS depressant drug that decreases the time of the onset of sleep or prolongs the length of sleep or both (21). The CNS depressant effect of P. foetida could be because of the presence of secondary metabolites, which are capable of binding to the CNS receptors, and many of the metabolites from certain plant extracts have been documented to be appropriate components for such effects. A previous phytochemical analysis of P. foetida revealed the presence of flavonoids, alkaloids, steroids, saponins, and tannins, which is consistent with the established hypothesis (22–24).

The four fractions were evaluated in the formalin-induced pain and tail immersion tests for their analgesic effect. The formalin test is better correlated with clinical pain (25) and with elucidated central and peripheral activities. The response in the early phase typically represents a direct chemical stimulation of pain created by formalin because of its irritant effect on sensory C fibres (25). The late response in late phase is secondary to the development of an inflammatory response and the release of allergic mediators (25–28). The inhibition of the licking response in both phases indicates the significant analgesic action of *P. foetidus* extract in the formalin-induced assay.

P. foetidus fractions, in the tail immersion test, produced promising action after oral dosing of extracts at 30 min intervals. The results showed significant analgesic capacity against noxious thermal stimuli. The extent of the activity shown by the fractions is less than that of the standard drug, nalbuphine, and many times more than that of the control group, which justifies its effect as an antinociceptive agent.

Conclusion

The findings of this study suggest that the methanol, petroleum ether, chloroform and aqueous fractions of the *P. foetidus* leaf extract have strong anxiolytic and sedative properties in behavioural models. These fractions could be used as potent therapeutic agents to treat anxiety and relevant neuropsychiatric abnormalities. The fractions evidently have potent therapeutic utility as an analgesic agent against different stimuli. Future investigations are justified to discover the rudimentary mechanisms of the CNS depressant action and of the analgesic activity to identify

the active phytochemical ingredients for noted bioactivities in animal models.

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Conflict of Interest

None.

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Authors' Contributions

Conception and design, administrative, technical or logistic support: MMR
Analysis and interpretation of the data: MEU, MAUC
Drafting of the article: MEU
Critical revision of the article for the important intellectual content, final approval of the article, statistical expertise: MAR
Provision of study materials or patient: AMTI

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