Original Research Article

Attenuation of Potassium Bromate-induced Infertility by African Locust Bean (*Parkia Biglobosa*)Seed

ABSTRACT

Background: *Parkia biglobosa* seed has been reported to enhance sperm quality. Thus, this study sought to assess its ability in attenuating $KBrO_3$-induced sperm abnormalities.

Methodology: *P. biglobosa* was extracted with soxhlet extractor with ethanol as the solvent. Twenty-four adult male Wistar rats were acclimatized under laboratory conditions and were randomly grouped into A, B, C and D. Group A was given distilled water orally. Animals in groups B, C and D were administered 100 mg/kg body weight of potassium bromate, but groups C and D were also treated with 100 and 200 mg/kg body weight of *P. biglobosa* respectively. Both potassium bromate and *P. biglobosa* were freshly prepared on daily basis and administered to rats by oral gavage. After 28 days of treatment, the animals were sacrificed under mild diethyl ether anaesthetization 24 hours after cessation of last treatment. The cauda epididymis were separated from both testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. This suspension was used for the determination of the sperm parameters.

Results: Exposure of animals to 100 mg/kg body weight of $KBrO_3$ significantly reduced sperm count, sperm motility, sperm viabilityand seminal pH but elevated sperm morphology when compared with animals in the control group at $p<0.05$. These perturbations were attenuated by 100 and 200mg/kg body weight of *P. biglobosa*seed extract in a dose-dependent manner.

Conclusion: The result of this study showed that potassium bromate induced abnormalities in sperm cells of treated animals, and seed extract of *P. biglobosa* attenuated these abnormalities in a dose-dependent manner. This pharmacological study is a useful tool for further drug development from the natural plant products.

Keywords: Fertility; *Parkia biglobosa* seed; potassium bromate; sperm qualities

1. INTRODUCTION

In many nations around the world, male infertility is steadily rising [1,2]. Infertility among men has reportedly increased to 12% in the USA, according to Louis et al. [3]. Numerous studies have linked exposure to endocrine disrupting chemicals (EDCs), particularly chlorinated compounds that are thought to cause poor sperm quality in males, to the fall in male fertility [4,5]. The EDCs are exogenous substances that disrupt the body's normal processes for maintaining homeostasis, reproduction, and development by interfering with blood hormone synthesis, secretion, transport, metabolism, binding, and removal [6]. The nuclear and non-nuclear hormone receptors for androgen, estrogen, progesterone, thyroid, and retinoid as well as for nuclear & non-nuclear and neurotransmitter receptors are also thought to be affected by the EDCs [5].

Sequel to digitalization, men now spend a lot of time using laptops in their jobs and are exposed to radiation from these devices, both of which may have an adverse influence on their ability to conceive [7,8]. The ability to have a fulfilling and safe sexual life as well as reproduce, and the freedom to choose whether and how frequently to do so are all dependent on good reproductive health among people [9].

The halide family of elements, which also contains fluorine, chlorine, and iodine, includes bromides as a member. It is well known that the bromides bind to the same receptors utilized to absorb iodine and interfere with the activity of thyroid hormones, resulting in a low thyroid status [6]. Therefore, bromides can be categorized as an EDC. Due to its oxidizing qualities, potassium bromate ($KBrO_3$), a member of the bromides group, is employed in the maturation of flour and has been utilized as a dough conditioner in the bread-making process for more than 50 years. For the time being, the WHO, Joint FAO/WHO Expert Committee on Food Additives has suggested a maximum level of 75 ppm of KBrO3 for treating flour, provided that baking items made from such treated flour have minimal residues of KBrO3 [10]. According to the same guidelines as for WHO, the level in Japan has been set at 30 ppm. Although $KBrO_3$ was removed from the list of approved food additives in Canada in 1994, it continued to be identified as an impurity in food packaging sheets [11]. In analytical chemistry, KBrO3 has also been introduced as an oxidizing agent, a main standard, and a brominating agent. Additionally, the sectors of barley, cosmetics, and water filtration employ $KBrO_3$. $KBrO_3$ has been labeled as potentially carcinogenic in numerous animal experiment studies. Unfortunately, $KBrO_3$ is carelessly utilized as a bread conditioner in less developed nations like Nigeria to increase bakeries' profitability [12,13].

The development and documentation of helpful medicinal plants have been facilitated by the use of herbs and spices in culinary practices [14,15]. The use of herbal remedies and natural products in healthcare has regained popularity across the globe [16]. Numerous studies on the efficacy of herbal extracts in the treatment of human ailments have been conducted in Africa and approved for usage [16,17]. This interest in medicinal plants may be explained by the known side effects of some conventional medications, their high cost, and the introduction of less expensive traditional medicine or alternative medicine therapies in undeveloped and developing nations [18]. While residents of big African towns engage in the use of both modern medicine and herbs, rural dwellers in some African communities rely primarily on herbs for the treatment of various illnesses [19]. African locust beans are one of these frequently utilized herbs (*Parkia biglobosa*). *P. biglobosa* is a plant from the Leguminosae family that may be found in West Africa's tropical and semi-arid regions, mainly in Nigeria's middle belt and southwest [20]. Due to its high protein, lipid, vitamin, tannin, and mineral content, *P. biglobosa* contains leguminous pods with a tough pericarp; the seeds are used as a food condiment and as an alternative to meat [21]. *P. biglobosa* pods can be harvested and processed into a fermented food item called in the Yoruba, Hausa, and Igbo languages as "iru," "dawadawa," and "ogili," respectively. The fermented pods can be used as a protein supplement because they contain about 40% protein, 32% fat, 24% carbohydrate, and vitamins [22]. There have been suggestions that *P. biglobosa* can improve fertility by improving the quality of male sperm due to its rich nature and popular use in Nigeria [23]. It is possible that this hypothetical study on *P. biglobosa*'s sperm profile effectiveness contributed to perceptions of its rising patronage in Nigeria. A recent study by Obeten et al. [24] showed that *P. biglobosa* seed enhanced sperm quality. Thus, this study sought to assess its ability in attenuating $KBrO_3$-induced sperm quality destruction.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of *Parkia biglobosa*

P. biglobosa (African locust bean) seed was purchased from a local market at Orita-Challenge area of Ibadan, Nigeria and were identified by a botanist. They were sun dried, then a mechanical blender (Moulinex) was used to grind them into powder. According to the procedures outlined by Airaodion et al. [25,26], the extraction was carried out using a soxhlet device with ethanol as the solvent.

2.2 Animal Treatment

The experiment involved twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160 g. Before the experiment began, they were acclimated for seven (7) days in a laboratory setting. The rats were kept in wire-mesh cages with unlimited access to water and commercial rat food. The animals were housed in conventional temperature and humidity settings with 12-hour light/dark cycles. The Declaration of Helsinki and the regulations set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals were followed in the conduct of this investigation. Additionally, animal experiments were conducted in compliance with NIH protocol [27]. They were assigned to groups A, B, C, and D at random. Oral distilled water was administered to group A as the normal control. Animals in groups B, C and D were administered 100 mg/kg body weight of potassium bromate, but groups C and D were also treated with 100 and 200 mg/kg body weight of *P. biglobosa* respectively. Rats received daily doses of freshly produced potassium bromate and *P. biglobosa* by oral gavage for 28 days. Twenty-four hours after the last treatment, the animals were sacrificed while being lightly sedated with diethyl ether. The cauda epididymis were separated from both testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. This suspension was used for the determination of the sperm parameters.

2.3 Analyses of Epididymal Sperm Characteristics

2.3.1 Sperm Motility and Count

According to the procedures described by Ogbuagu et al. [28], spermatozoa were retrieved by making small cuts in the cauda epididymis and placed in 0.05 M phosphate buffered saline (pH 7.4). Sperm content and sperm motility were assessed in sperm suspension. Any type of flagellar or head movement, as well as the slow and quick movements of spermatozoa (also known as progressive movements), were used to calculate the percentage of mobility. A Neubauer hemocytometer was utilized to calculate the sperm count.

2.3.2 Sperm Viability

Eosin/nigrosin staining was utilized for the determination of sperm viability. On a microscope slide, the staining was examined using a ratio of one drop of fresh semen to two drops of staining agent. On a different slide, a smear was applied and allowed to dry. Spermatozoa with damaged membranes and unstained spermatozoa were counted at a magnification of 3100[29].

According to Eliasson's research the proportion of undamaged cells correlated with sperm viability.

2.3.3 Sperm Morphology

The procedure outlined by Airaodion et al. [30] was used to determine the morphology of the sperm. To determine the anomalies in spermatozoa, sperm suspension was stained with eosin, and smears were produced on slides, dried by air, and made permanent. With an oil immersion objective and x100 magnification, the slides were examined under the microscope. Spermatozoa that were morphologically aberrant were enumerated, and their proportion was calculated.

2.3.4 Determination of Seminal pH

According to the procedure outlined by Airaodion et al. [31], the pH of the seminal solution was measured using pH paper with a range of 6.0 to 10.0. A drop of the material was uniformly distributed on the pH paper after being

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fully mixed. After roughly 30 seconds, the impregnated zone's color became consistent, and the color was compared to the calibration strip to determine the pH, with the matching value being recorded.

2.4 Statistical Analysis

The results are presented as the mean ± standard deviation. One-way Analysis of Variance (ANOVA) and Tukey's test were used to determine the degree of homogeneity among the groups. P values 0.05 were taken into consideration as statistically significant for all analyses, which were performed using Graph Pad Prism Software.

3. RESULTS

Exposure of animals to 100 mg/kg body weight of $KBrO_3$ significantly reduced sperm count (fig. 1), sperm motility (fig. 2), sperm viability (fig. 3) and seminal pH (fig. 5)but elevated sperm morphology (fig. 4) when compared with animals in the control group at $p<0.05$. These perturbations were attenuated by 100 and 200mg/kg body weight of *P. biglobosa*seed extract in a dose-dependent manner.

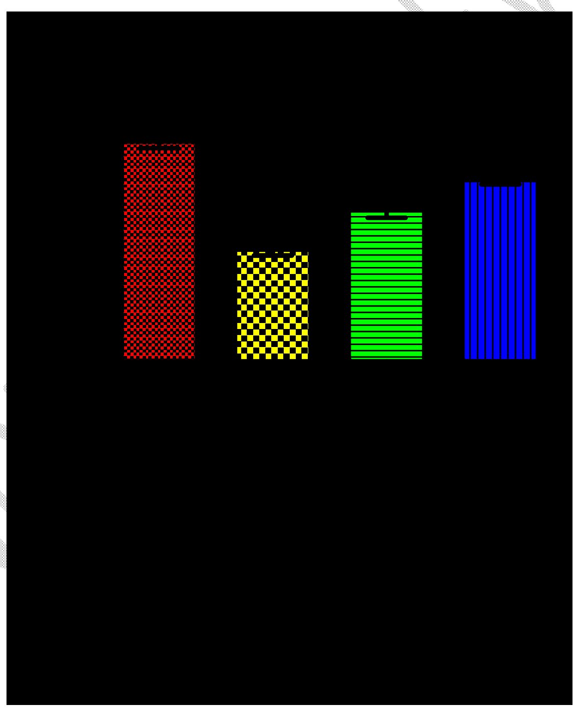

Fig. 1: Effect of *Parkiabiglobosa* seed extract on the Sperm Count of Potassium bromate-induced Male Rats

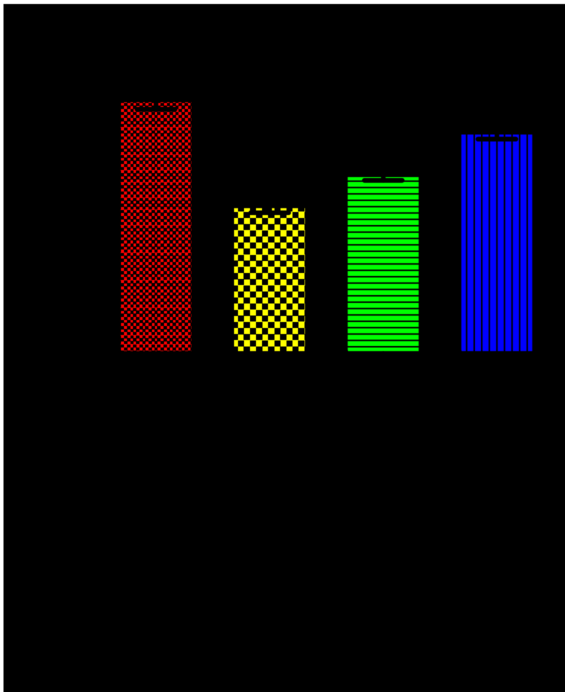

Fig. 2: Effect of *Parkiabiglobosa* seed extract on the Sperm Motility of Potassium bromate-induced Male Rats

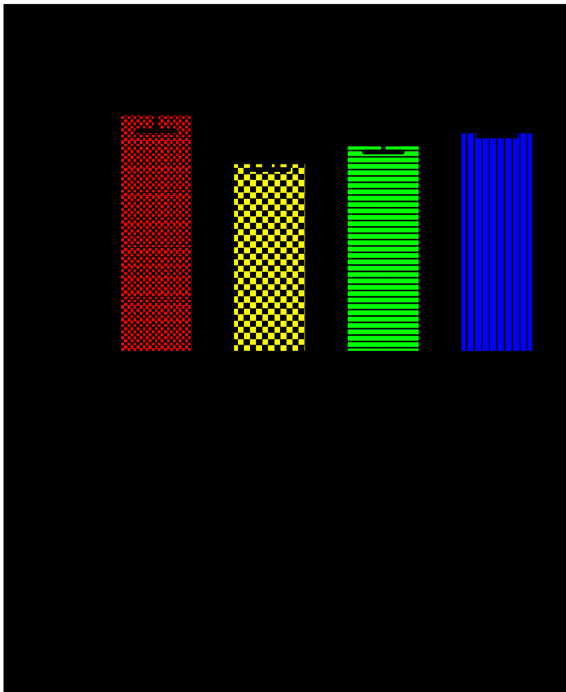

Fig. 3: Effect of *Parkiabiglobosa* seed extract on the Sperm Viability of Potassium bromate-induced Male Rats

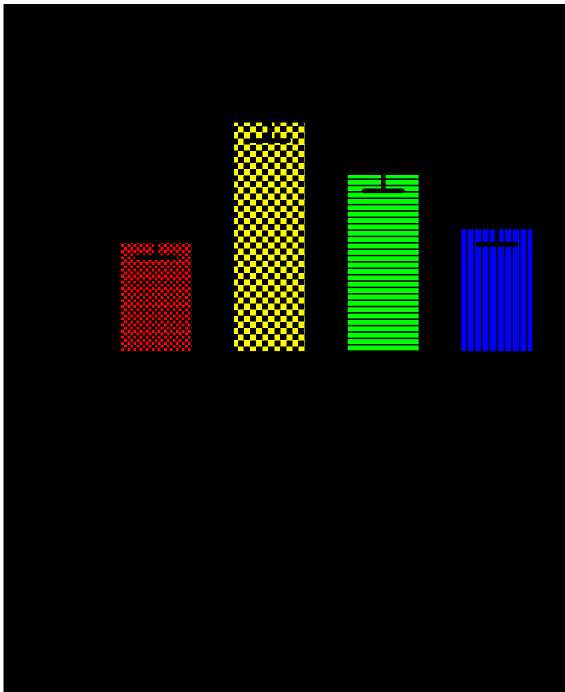

Fig. 4: Effect of *Parkiabiglobosa* seed extract on the Sperm Mofphology of Potassium bromate-induced Male Rats

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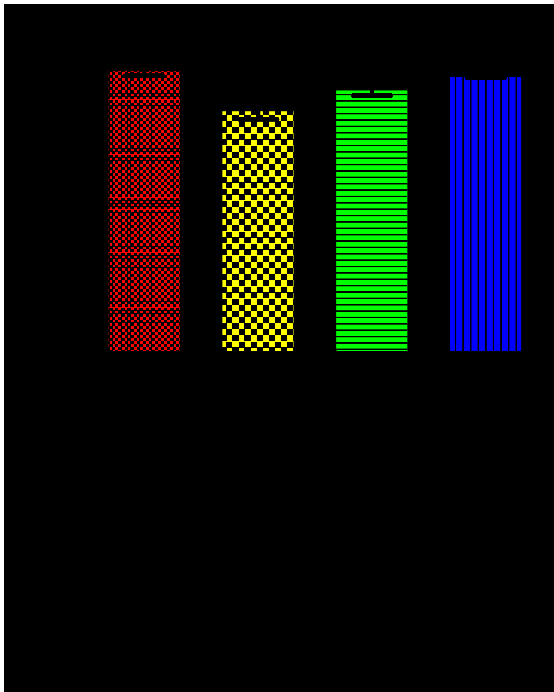

Fig. 5: Effect of *Parkiabiglobosa* seed extract on the Seminal pH of Potassium bromate-induced Male Rats

4. DISCUSSION

Food additives are one of the biggest issues facing the food business today [13]. The short- and long-term genetic and toxic consequences of all food additives should be tested because they have not yet been adequately controlled. These appear to be of crucial importance to human health as a result of the daily consumption of food additives through food, which can build in the body over the course of a lifetime due to the extremely lengthy exposure [32]. Numerous investigations have shown that $KBrO_3$ has genotoxic and carcinogenic effects in both experimental animals and human. A variety of short-term genotoxicity testing methods are currently being employed to assess the genotoxic harm caused by $KBrO_3$ [33]. Products from medical plants are abundant in secondary metabolites and therapeutic essential oils, which study the therapeutic effect of different medicinal plants as natural antioxidants to treat chronic diseases [34] as DNA damage, mutagenesis, and carcinogenesis. Semen analysis and testicular biopsies are standard laboratory procedures used to evaluate male fertility. Male infertility has been shown to be caused by semen motility, vitality, and morphology [35], in addition to other factors such as malnutrition, genetic abnormalities, and serious sickness, among others [36]. Another study found that most men are either too shy or ashamed to visit a clinic for proper medical consultations, so they instead turn to herbal or plant-based formulations for self-medication [37]. As a result, only a small percentage of men seek proper medical treatment to definitively resolve their infertility issues. The spermatozoa morphology

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Comment [H5]: Has been shown to be= is test is an accurate way to assess the genotoxicity and germ cell toxicity of various chemicals. In the current study, the impact of African locust bean on $KBrO_3$-induced sperm morphology mutation in male rats was evaluated. According to the study's findings, as shown in fig. 1 through 3, $KBrO_3$ significantly ($p<0.05$) decreased sperm count, motility, and viability when compared to the control group. According to reports, treating animals with potassium bromate caused a noticeably increased level of free radical activity in the testicles and other bodily tissues [38,39]. Carcinogenesis may result from the unchecked generation of free radicals [40,41]. Reduced sperm count, motility, and morphology are just a few examples of the reproductive dysfunction brought on by the alteration in sperm characteristics [42]. According to a related study, adding $KBrO_3$ to drinking water inhibited the growth of growing rats, which in turn led to lower testicular and epididymal weights [43]. However, these disturbances were alleviated when rats were given $KBrO_3$ and 100 mg/kg and 200 mg/kg of *P. biglobosa* at the same time. According to a recent study by Ezirim et al. [38], the antioxidant capacity of *P. biglobosa*seed extract allowed it to potentiate $KBrO_3$-induced testicular oxidative stress. Following the findings of another, sperm motility and viability increased at a dose of 300 mg/kg body weight of *P. biglobosa* extract, but decreased at a dose of 500 mg/kg [24]. This shows that a low dose of *P. biglobosa* may improve reproductive health by enhancing sperm motility. This outcome backs up Udo-Affah et al. [44]'s observation that mice subjected to *P. biglobosa*'s extract exhibited significantly improved sperm motility. This rise was noted, and it was linked to the extract's high iron concentration. Sperm vitality is the percentage of healthy, live sperm found in the semen [45].

Sperm morphology is a significant and accurate predictor of the toxicity of chemicals on reproductive system cells [46]. They provide a clear indicator of the caliber of sperm production in chemically treated experimental animals since they can be utilized to assess spermatogenic damage, fertility, and heritable genetic abnormalities [47]. Genotoxic chemicals have the ability to affect the normal course of gametogenesis, which could result in changes to the shape of sperm cells. This process is not autonomous and is governed by intricate mechanisms that are polygenically controlled. A suitable indication of genetic damage may be any qualitative or quantitative change in the aforementioned trait that can be linked to dietary, pharmaceutical, or environmental alterations [48]. In the current study, the consequences of giving male rats dosages of potassium bromate, a dietary additive, were examined in terms of morphological changes in sperm cells. According to our findings, compared to the control group, potassium bromate supplementation significantly increases sperm morphological abnormalities (Fig. 4). These results corroborated those from Elhaddad et al. [32], who found that treatment of animals with $KBrO_3$ affected the number and motility of epididymis sperm and increased the incidence of defective sperm. This demonstrates how artificial food additives may have an impact on reproductive health. The impact of $KBrO_3$ on sperm morphology was lessened by administering *P. biglobosa* seed extract at doses of 100 and 200 mg/kg body weight.

Genetic damage may manifest as defective sperm or other phenotypic manifestations during the process of spermiogenesis if the pre-meiotic, meiotic, or post-meiotic spermatogenic cells are exposed to a mutagenic substance that interferes with DNA. A complicated array of morphological and metabolic processes is involved in the production of sperm heads. Sperm may grow abnormally during the spermiogenesis process. Small deletions or point mutations are the most plausible causes of sperm head defects [48,49]. Additionally, it was thought that an aberrant chromosome was the cause [50]. Two other investigations [51, 52] found that variations in testicular DNA or physiological, cytotoxic, or genetic causes might cause abnormalities in sperm heads, which would then impair the differentiation of spermatozoa [53].

The seminal pH represents the harmony between the alkaline seminal vesicular secretion and the acidic prostatic secretion, which make up the majority of the accessory gland secretions. When $KBrO_3$ treated animals in this study were compared to the control animals after 28 days of treatment, it was observed that seminal pH had decreased (Fig 5). This could be because the food additive changes the pH range that is typical for the treated animals. The medium of seminal plasma becomes acidic if the pH decreases, making sperms extremely fragile and raising the mortality rate [54,55]. The impact of $KBrO_3$-induced seminal acidity was ameliorated by the administration of various dosages of *P. biglobosa* extract.

The majority of medications containsplant extracts as their active components and iswidely used since they have few adverse effects. Because of this, herbal remedies are now more commonly used in traditional African homes than conventional medications. Plants have given mankind a wide range of effective medicines to lessen or eliminate infections and disease-related suffering. Some medications produced from plants continue to be significant and important despite advances in synthetic pharmaceuticals [56, 57]. Despite the progress made in contemporary medicine, there are still many illnesses or infections (diseases) for which effective medications have not yet been developed. In order to treat inflammatory disorders like diabetes, liver diseases, gastrointestinal disorders, and infertility, safer drugs from the environment are urgently needed [58, 59]. Research on herbal plants or medicines has been promoted by the interdisciplinary approach used to treat some disorders, and there appears to have been significant progress in recent years in the pharmacological evaluation of diverse plants employed in traditional systems of medicine [60].

The results of the semen parameters seen in this investigation support a study by Hussain et al. [61] that showed a polyherbal formulation could have a synergistic efficacy leading to an improvement in the key semen parameters in oligospermic males. The findings of the semen analysis indicate that the experimental animals' semen parameters were improved by our herbal formulation of *P. biglobosa* seed extract. Prior to this, medicinal plants were divided into two primary categories: dismutgencity, which refers to a plant's ability to work outside the cell membrane, and antimutagency, which refers to a plant's ability to act in numerous places [32]. Additionally, certain medicinal plants have the capacity to function both outside and inside cells. It's possible that our medicinal plant, *P. biglobosa,* has antimutagenic and disantimutagenic properties. These observations point to novel antimutagenic functions of *P. biglobosa* as well as mutagenic effects of potassium bromate.

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5. CONCLUSION

The result of this study showed that potassium bromate induced abnormalities in sperm cells of treated animals, and seed extract of *P. biglobosa* attenuated these abnormalities in a dose-dependent manner. This pharmacological study is a useful tool for further drug development from the natural plant products.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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