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Morphological Alterations of the Rat Testicles Following Administration of Graded Doses of Leaves of Guava (*Psidium guajava* Linn.) Aqueous Extract**Kom-Abasi Daniel Etim¹, *Ubi Essien Isaac¹, Anozeng Oyono Igiri²**¹Department of Human Anatomy, Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku Campus, P.M.B. 1123, Calabar, Nigeria.²Department of Human Anatomy, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria. *E-mail: isaacubireal@gmail.com

Abstract: The current study design to assess the dose-response effect of administering an aqueous extract of guava leaves on the microstructure of the testicles due to the growing use of this plant in complementary and alternative medicine practice. Twenty-four male albino Wistar rats with an average body weight of 160 g apportion to four groups (n=6 each). Group, I served as the control and received distilled water; group II gave a lower dose of 500 mg/kg body weight of guava leaf extract; group III received a medium dose of 750 mg/kg; while group IV administer a higher amount of 1000 mg/kg per day. Treatments were given once daily by gavage and lasted for 14 days, while the stock solution prepares by dissolving 50 g of the extract in 30 ml of water. The cervical dislocation method used to euthanize the rats, dissect the abdominopelvic region to obtain tissue specimens from the testes for histological processing. The outcome exhibited the extract to cause moderate to severe (dose-dependent) morphological alterations with the seminiferous tubules' fibrotic appearance, strict spermatogenic arrest, and necrosis of both the interstitial cells of Leydig and the Sertoli cells. The ingestion of guava leaves at the investigated concentration and doses is harmful to the testicle, the primary reproductive organ in males. It should therefore be consumed with caution when being used primarily in folklore-traditional medicine.

Keywords: *Psidium guajava*; alteration; morphology; testis; folk medicine

INTRODUCTION

Guava, binomially known as *Psidium guajava* Linn., is an evergreen shrub or small tree belonging to the myrtle family (Myrtaceae) and native to the Caribbean, Central America, and South America. It widely cultivates in tropical and subtropical regions worldwide. Its fruit is oval and can range from as small as an apricot to as large as a grapefruit. Various cultivars have white, pink, or red flesh, and a few also feature red (instead of green or yellow) skin^{1,2}. The leaves are oval or elliptical, smooth on the upper surface, hairy on the lower body, and contained the flavonol morin, morin-3-O glycoside, morin-3-O- arabinoside, quercetin, and quercetin-3-O- arabinoside³.

It has been used in traditional medicine in many cultures throughout Central America, the Caribbean, Africa, and Asia, for inflammation, diabetes, hypertension, caries, wounds, pain relief, fever, diarrhea, rheumatism, lung diseases, and ulcers⁴.

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Guava is an edible fruit and can be eaten raw or cooked. The fruits' processing yields by-products can be livestock, and the leaves can also use as fodder⁵.

Traditionally, preparations of the leaves are used in folk medicine in several countries, mainly anti-diarrheal remedies⁴. More so, several other uses have been described elsewhere on all continents except for Europe. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, and cough in India, China, Pakistan, and Bangladesh^{4,6,7,8}; while in southeast Asia, the decoction is used as a gargle for mouth ulcers and as an anti-bactericidal in Nigeria^{7,8}. In recent years, guava leaves tea and some complimentary guava products are available in several shops in Japan as well as on the internet⁹ because guava leaf phenolic compounds have claimed to be food for specified health use (FOSHU) since they have beneficial health effects related to the modulation of blood-sugar level¹⁰.

As the global use of herbal medicinal products continues to grow, and more new products introduce into the market, public health issues and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely use, many remain scientifically untested, and their use not monitor. This result makes knowledge of their potential adverse effects very limited and identifies the safest and most effective therapies and the promotion of their rational use more difficult¹¹. The ethanol extract of *Psidium guajava* Linn has reported enhancing sperm output in healthy Wistar rats, as the study posited its beneficial effects on sperm production and quality, therefore suggesting the plant had the potentials to improve the sperm parameters of infertile males with oligospermia and nonobstructive azoospermia¹². However, on highlighting the relationship of guava leaves with male reproduction or fertility, a similar study reported that a fraction of red guava (*Psidium guajava* L.) leaves could reduce the weight of the testes, the diameter of the seminiferous tubules, the epithelial seminiferous tubules and the number of Leydig cells¹³.

Structures within the testes are essential for the production of sperm and a hormone called testosterone, which is responsible for sex drive, fertility, and the development of muscle mass and bone mass¹⁴. Infertility and problems of impaired fecundity have been a concern for ages and are also a significant clinical problem today, affecting 8-12% of couples worldwide. Of all infertility cases, approximately 40-50% is due to "malefactor" infertility. Many as 2% of all men will exhibit sub-optimal sperm parameters, which may be one or a combination of low sperm concentration, poor sperm motility, or abnormal morphology¹⁵. While male infertility can have a substantial impact on a couple's psychology and physiology, it can be due to several reasons. Analysis of retrospective data indicates that sperm quality/counts may have declined in some parts of the world, but there seem to be geographical variations in the semen quality^{16,17,18}. The reason for geographic variations in semen characteristics is not exact, but it may be due to environmental, nutritional, socioeconomic, or other unknown causes¹⁹.

In some related investigations using a histopathological method to study liver tissue samples from an experimental model administered 450 mg/kg body weight of *P. guajava* for seven days, the results revealed striking edema and mild periportal mononuclear cell infiltration of the hepatic cords²⁰. Moreover, the histopathological determinations in another study on the liver and spleen of three treatment groups that received guava extract at the respective doses of 0.2, 2.0, and 20.0 g/kg/day for six

months, showed hydronephrosis in male rats. Still, nephrocalcinosis and chronic pyelonephritis observed in some guava-treated female rats, thus suggesting that for the safe use of guava leaves for herbal treatment, the recommended dose and duration of therapy should not be exceeded²¹. While the justification of this research may lead to how several studies point to the health benefits of *Psidium guajava* Linn without taking into account the therapeutic dose, there are still some public health concerns about its safety when ingested in an uncontrolled manner in the treatment or management of acute and chronic health disorder.

MATERIALS AND METHODS

Ethical Consideration

The necessary approval/consent to conduct this research was given by the Research/Ethics Committee of the Department of Anatomy in the Faculty of Basic Medical Sciences of Cross River University of Technology (CRUTECH), Okuku Campus, Nigeria. Collection of Plant Leaves/Preparation of Extract

Fresh leaves collected from guava trees grown in Okuku, a remote town located in Cross River State, Nigeria. After authenticating at the Botany unit of CRUTECH, the leaves air-dried at room temperature for 14 days, after which a mechanical blender use for grinding them into a fine powder. The maceration method to use to extract the ground leaf substance with 100 g of the sample being soak in 1000 ml of water. The mixture was decanted and filtered using sterile Whatman paper, and the filtrate was evaporated to dryness to yield aqueous residue²². The semi-solid extract obtains in syrup form store in a refrigerator for further use.

Materials/Tools

These included protective clothing (lab coat), rat cages, hand gloves, permanent marker, electronic weighing balance for measuring the test samples/checking the bodyweight of animals (Brand: M/s Vijay Scales & Sons, Guwahati, Kamrup, Assam), syringes for measuring the doses (BD Emerald 2ml syringe with 22Gx1), medical dissecting kit, chemical reagents such as formalin (for fixation of tissue specimens), alcohol (for dehydration), xylene (for clearing or removal of alcohol), hematoxylin and eosin (for staining of tissues), specimen collection containers, pulverizer for grinding materials, graduated cylinder used to measure the volume of liquids (Brand: Nalgene), paraffin wax for embedding tissue, embedding mold for making blocks, rotary microtome for cutting thin sections of tissue, water bath used for relaxing and smoothing out of tissue before mounting on a glass slide, microscope slides used for holding the objects (i.e., tissue sections) for examination under a microscope, and a light microscope for viewing thin slices of tissue (Brand: MT5 Histology Pathology Lab Microscope).

Assessment of Lethal Dose (LD50) of the Extract

The acute oral toxicity of guava leaf aqueous extract was determined using Lorke's method, which has two phases: 1 and 2, respectively. Phase 1 required the use of nine animals divided into three groups of three rats each and administered different doses (10, 100, and 1000 mg/kg b.w.) of the test substance. The animals were then placed under observation for 24 hours to monitor their behavior and ascertain if mortality will occur. Phase 2 involved the use of three animals, which share out into three groups of one animal each and administered higher doses (1600, 2900, and 5000 mg/kg b.w.). They also observed for 24 hours for behavior as well as mortality²³. The mean lethal dose

calculated using the formula $LD50 = \sqrt{(D0 \times D100)}$, where D0 = highest dose gave no mortality, while D100 = lowest amount produced mortality.

Experimental Animals and Administration of Treatment

Twenty-four male Wistar rats weighing around 160 g were procured and maintained in the right housing conditions without restriction to feed and water and with strict adherence to the guidelines for the care and use of experimental animals. After acclimatizing for two weeks, the rats distribute into four groups, with each group consisting of six animal subjects. Group I take as the control, group II designed low dose treatment with 500 mg/kg/day of *P. guajava* leaf extract; group III regarded as the median/medium dose administered 750 mg/kg; while group IV received 1000 mg/kg and designated high amount. The stock solution prepares by dissolving 10 g of the leaf extract in 30 ml of water (10÷30) to yield a stock concentration of 0.33 g/ml. We give all treatments daily using a syringe with the attached cannula for fourteen days, after which the animals euthanized using the cervical dislocation method²⁴. An incision was made along the central aspect of the rat's anterior body wall to dissect/expose the abdominopelvic cavity and obtain tissue specimens from the testicles for standard histological processing and light microscopic evaluation.

Histological Procedures

The tissue samples were preserved in 10% formal saline in a container with tight-fitting lids for three days to prevent autolysis, improve staining quality, aid optical differentiation of cells, and after that, dehydrated in different grades of alcohol before clearing in xylene. This process was followed by infiltration with molten paraffin wax to remove the clearing agent and embed it in paraffin wax. The tissue was mounted on wooden blocks to enable sectioning with a rotary microtome. The cut sections at 5-micron meters (μm) floated in a warm water bath to allow mounting on microscope slides and after that stained with hematoxylin and eosin dyes. At the same time, DPX (Distyrene Plasticizer Xylene) promptly add to preserve the stain. The tissue slides were then allowed to dry for micrographing and interpretation²⁵.

RESULTS AND DISCUSSION

Acute Oral Toxicity (LD50) Report

Administration of guava leaf aqueous extract on albino Wistar rats did not show any toxic effect. Moreover, neither lethality nor mortality was observed or recorded during the acute toxicity test, even when the animals give a benchmark dose of 5000 mg/kg.

Physical Observation

Two animals were discovered dead during the main experiment; one of the rats in group II that give a lower dose (500 mg/kg) died two days after administration of treatment commenced, and another rat from group IV that received a high amount (1000 mg/kg) also experienced mortality three days after treatment.

Histomorphological Findings

The following micrographic plates are the results of histological processing of testicular tissue samples:

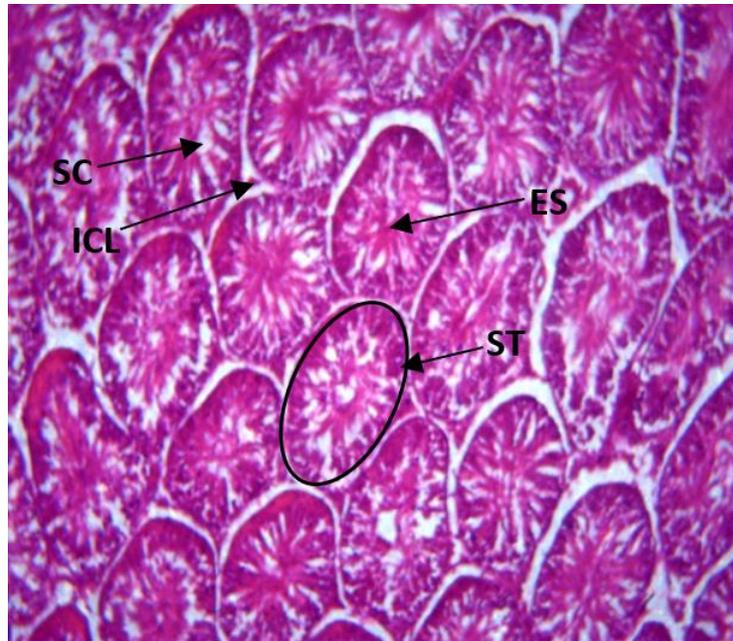


Figure 1: Photomicrograph of group I; control section of the testicle (x400)(H/E), shows normal morphology with seminiferous tubules (ST) consisting of Sertoli cells (SC), enhanced spermatogenesis (ES) and a lining of interstitial cells of Leydig (ICL).

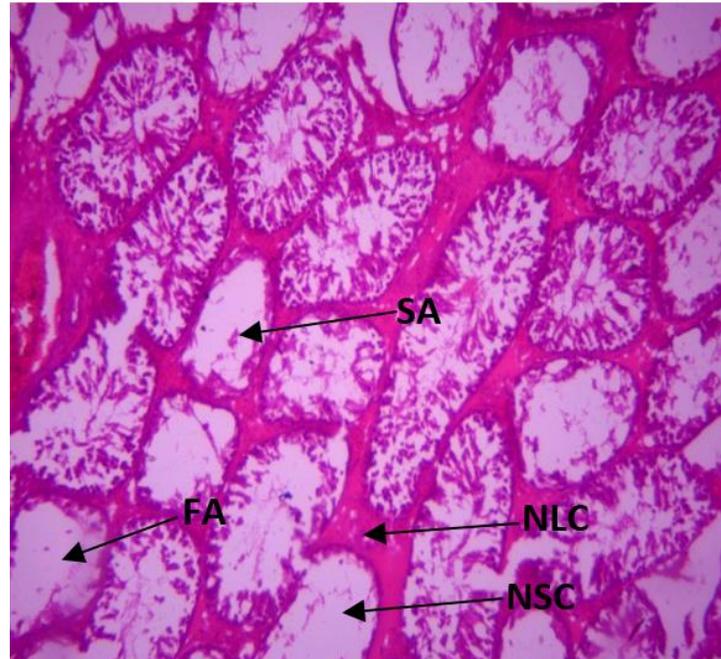


Figure 2: Photomicrograph of group II; section of the testicle administered lower dose of 500 mg/kg (x400)(H/E), shows moderate spermatogenic arrest (SA) fibrotic appearance (FA) of the seminiferous tubules, and necrosis of both the Leydig cells (NLC) and Sertoli cells (NSC).

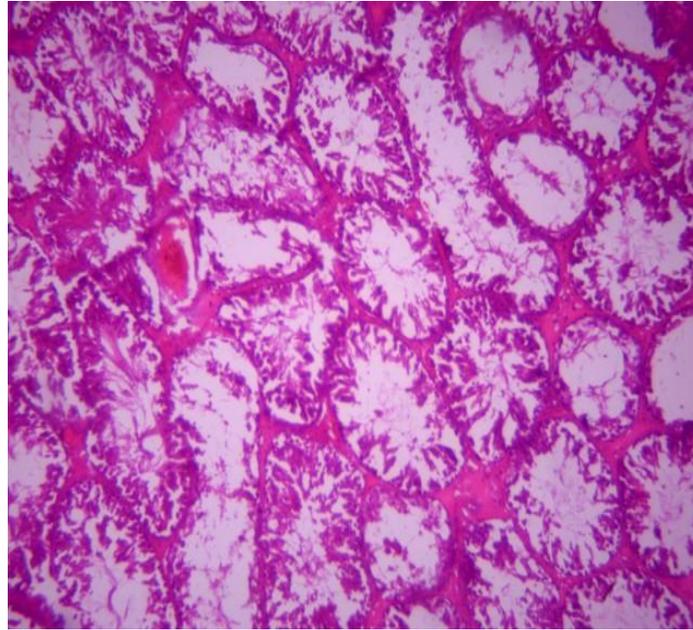


Figure 3: Photomicrograph of group III: section of the testicle administered median dose of 750 mg/kg (x400)(H/E) shows the moderate effect with spermatogenic arrest, the fibrotic appearance of seminiferous tubules, and necrosis of both interstitial cells of the Leydig and Sertoli cells.



Figure 4: Photomicrograph of group IV, the section of the testicle administered higher dose of 1000 mg/kg (x400)(H/E) shows the severe effect with spermatogenic arrest, the fibrotic appearance of seminiferous tubules, and severe necrosis (SN) of both interstitial cells of the Leydig and Sertoli cells.

The outcome of the assessment of acute oral toxicity, which meant to find out the amount of *P. guajava* leaf that can kill half (50%) of the animals in a group of the test population, showed that the median lethal dose (LD50) level of the aqueous extract is over 5000 mg/kg, as there was no record of mortality, fatality or lethality even when the rats dosed up to that amount. This finding is in alignment with a previous report of a toxicological evaluation of aqueous extract of different varieties of guava leaves in rats, which indicated that there were no deaths recorded during the study and that the NOAEL (no observed adverse effect level) estimate of white, red and pink guava leaves extract is 50 - 5000 mg/kg²⁶.

The microscopic evaluation of testicular morphology following administration of graded doses of guava leaf extract as evidenced in the photomicrographs of tissue samples from the various experimental groups, starting from the lower dose treatment with 500 mg/kg (figure 2) through the medium dose of 750 mg/kg (figure 3) and up to the higher amount of 1000 mg/kg (figure 4) all exhibited considerable dose-dependent alterations that could describe as being moderate and/or severe in effect, with visible histopathological features of spermatogenic arrest, the fibrotic appearance of the seminiferous tubules and severe necrosis of both the interstitial cells of Leydig and Sertoli cells. There is a shortage of published evidence on the effect of guava leaves on the testes' morphology, most of the available reports/databases on findings from physiological and biochemical parameters, and the use of guava leaf essential oils.

For instance, a comprehensive review of *P. guajava* Linn leaves' health effects in the last decade did not show any evidence of morphological findings. However, the study reported that ethnomedicine applications of guava had been verified by several researchers over the last decade against many disorders, demonstrating its potential in the treatment of the most common worldwide disease²⁷. Also, there have been reports that guava possesses anti-viral, anti-inflammatory, anti-plaque, and anti-mutagenic activities and that this plant's extract shows antinociceptive activity. Simultaneously, it is also useful in liver damage inflammation and serum production, with the further indication that its ethanolic extract can increase sperm quality and quantity and can use for the treatment of infertile males²⁸.

Furthermore, during a screening of medicinal plants to inhibit protein tyrosine phosphatase 1B (PTP1B), the antidiabetic effect of an extract from *P. guajava* leaves was evaluated on mice. Significant blood glucose-lowering effects were observed after intraperitoneal injection of the extract at a dose of 10 mg/kg in both one and 3-month old mice, leading to the suggestion that the extract possesses antidiabetic potentials against type 2 diabetic mice model and that this effect is at least in part, mediated via the inhibition of PTP1B²⁹. Also, a water extract of *P. guajava* leaves screen for hypoglycemic activity on alloxan-induced diabetic rats. In both acute and subacute tests, the water extract, at an oral dose of 250 mg/kg, is said to have shown statistically significant hypoglycemic activity³⁰. However, a careful review of most of the reports has demonstrated that lower doses (below 300 mg/kg) utilize in these studies.

Nevertheless, the present research outcome concerning the morphology of the testicle is in corroboration with some earlier reports from related studies. In one of those investigations, the effect of the aqueous extract of *P. guajava* leaves on erythromycin-induced liver damage in rats was assessed. The histopathological analysis of tissue samples from the experimental animal administered 450 mg/kg body weight of *P.*

guajava extract only, as well as those treated with 300 mg/kg of the extract and 100 mg/kg of erythromycin stearate for seven days revealed striking edema and mild periportal mononuclear cell infiltration of hepatic cords in the liver. It further reported that pretreatment with 150 mg/kg of the extract showed a slight degree of protection against the induced hepatic injury caused by 100 mg/kg of the said erythromycin drug. In comparison, biochemical analysis of the serum obtained also revealed a significant increase in serum levels of hepatic enzymes measured in the groups administered with 100 mg/kg of erythromycin stearate and 300/450 mg/kg of *P. guajava* extract compared to the control group samples and those pretreated with 150 mg/kg of the extract. The researchers thereby posited that the aqueous extract of *P. guajava* leaf possesses hepatoprotective property at a lower dose and a hepatotoxic property at a higher amount but recommended further studies with prolonged duration²⁰.

In another report from toxicity studies of *P. guajava* leaves, the histopathological determinations of the liver and spleen of the three treatment groups that received the extract at the respective doses of 0.2, 2.0, and 20.0 g/kg/day (equivalent to 1, 10 and 100 times of usual therapeutic dose for the treatment of diarrhea) for six months, showed a mild degree of fatty change and hydronephrosis in male rats. Still, nephrocalcinosis and chronic pyelonephritis observe in some guava-treated female rats. The results of that investigation, according to the researchers, suggested that for the safe use of guava leaves as an anti-diarrhoeal drug, the recommended dose and duration of treatment should not be exceeded²¹.

The testicle, which is the male primary reproductive structure/organ, comprises seminiferous tubules surrounded by connective tissue stroma that consists of testosterone-producing Leydig (or interstitial) cells. The tubules line with a seminiferous or germinal epithelium layer, which contains supporting Sertoli (sustentacular) cells, and spermatogenic cells, as demonstrated in the normal (control) section of the rat sample presented in figure 1. The spermatogenic cells continuously multiply and, through several phases of spermatogenesis, differentiate into mature sperm. Simultaneously, the Sertoli cells nourish them and mediate the effect of testosterone, which is indispensable for the maintenance of spermatogenesis. Consequently, the function of the Sertoli cells depends mostly on the operation of the Leydig cells, and a local control mechanism between the two cell systems assumed^{31,32}.

The resulting micro-anatomic distortions, due to fibrotic appearance of the seminiferous tubules and necrosis of both the interstitial cells of Leydig and Sertoli cells as observed in the present study is in line with the report that a fraction of red guava (*Psidium guajava* L.) leaves can reduce the weight of the testes, the diameter of the seminiferous tubules, the epithelial seminiferous tubules and the number of Leydig cells; adding that the chemical contents of red guava leaves are alkaloids, flavonoids, tannins, essential oils, and beta-sitosterol which thought to be antifertility¹³. Moreover, the histopathological effect on liver tissue samples of an experimental model administered 450 mg/kg body weight of the extract for seven days revealed striking edema and mild periportal mononuclear cell infiltration hepatic cords²⁰. Furthermore, the histopathological determinations in another study on the liver and spleen of the three treatment groups that received guava extract at the respective doses of 0.2, 2.0, and 20.0 g/kg/day for six months, showed hydronephrosis in male rats. Still, nephrocalcinosis and chronic pyelonephritis observed in some guava-treated female rats, thus suggesting that for the

safe use of guava leaves as herbal medicine, the recommended dose and duration of treatment should not be exceeded²¹.

While *P. guajava* may have been regarded as a plant of medicinal or therapeutic value, having report use in traditional systems of medicine for the treatment of various ailments including diabetes, diarrhea, wounds, rheumatism, anti-allergy, male infertility, lung problems, and ulcers^{4,8,27-30}. the rationale behind the current research design had taken into consideration the possibility that some unsuspecting individuals who employ herbal remedies in folk treatment and management of chronic health conditions, may not be circumspect about their dose-response effect on other life parameters such as the reproductive health, even when large amounts of these plant products required for use with a prolonged duration. Moreover, it would not have been presumptuous before now for such individuals to think that the ingestion of guava leaves even at high doses or amounts may be devastating to some life parameters, going by the myriads of reports regarding its health benefits, safety, and exhibition of low toxicity, even with an LD50 value of over 5000 mg/kg as already indicated in this research. About the limitations of this study, the histopathological method uses for the assessment of the dose-response effect on the male primary reproductive (testicular) morphology, thus making room for more investigations with various parameters to develop a clear hypothesis.

CONCLUSION

The aqueous extract of leaves of guava (*P. guajava* Linn.) at the investigated doses (500, 750, and 1000 mg/kg) and concentration (0.33g/ml) is detrimental to the morphology of the testicle and should therefore cautious ingested, especially if large amounts need in folk-traditional medicine for the treatment of acute and chronic health problems or diseases such as diabetes and other ailments. However, we recommend that further investigations identify and/or isolate the active substance(s) in the leaves that may be responsible for the dose-response alterations reported herein.

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CONFLICT OF INTEREST

None declared.

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