

Metabolic and Reproductive Parameters in Young Female Rats Subjected to Feed Restriction and/or Ginger Oil Administration



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Abstract:

Background: It is well known that the medicinal plant ginger (*Zingiber officinale*) has anti-oxidative properties that shield tissues and organs against oxidative damage.

Aims: Forty-eight, 9-week-old Sprague Dawley female rats (159±13 g) were used to evaluate the effects of feed restriction and/or ginger oil supplementation on growth, reproductive parameters, and blood metabolites.

Methods: Animals were randomly divided into 4 treatment groups (12 rats/treatment) in 2 × 2 factorial arrangements: *ad libitum* feed (CON), 25% feed restriction (G2), *ad libitum* feeding with 0.2 mL/rat ginger oil (G3), and 25% feed restriction plus 0.2 mL/rat ginger oil (G4). Animals in groups G2 and G4 received 75% of their pre-treatment feed intake (25% restriction). Six rats from each group were sacrificed at the end of treatments for blood and tissue collection, while the remaining rats were mated with mature males.

Results: No ginger-by-feed restriction interactions were detected in any of the tested parameters. Body size and BW increased as the experiment advanced ($P < 0.01$). However, G2 had the lowest BW towards the end of the treatment. Glucose was greater ($P < 0.05$) in feed-restricted animals, while urea nitrogen was greater in animals on *ad libitum* feeding. Combined ovarian weights and reproductive tract weights were greater in ginger-supplemented than non-supplemented animals. Similarly, rats on *ad libitum* feeding had greater ovarian weights than those on restricted feeding.

Conclusion: Ginger oil supplementation appears to have favorable effects on reproductive tract development and ovarian weight regardless of feed restriction.

Keywords: Body weight, Litter size, Ovary, Reproduction, Ginger, Metabolic parameters.

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1. INTRODUCTION

The metabolic status of animals affects reproductive performance. Numerous studies demonstrated the effects of nutrition on many aspects of reproduction in several species [1, 2]. Undernutrition reduces body weight and body condition scores [3]. Such effects of undernutrition are manifested in reduced gonadotropin secretion, delayed puberty, reduced estrus expression, impaired follicular development, and delayed postpartum return to estrus. These effects of nutrition are to ensure that reproduction is supported by a plentiful food supply that will meet the high nutritional demands of gestation and lactation [4].

More research addresses the nutrition-reproduction interaction in females than in males. This is primarily because the consequences of undernutrition not only affect the female herself but also her offspring's growth and development. Thus, the female's "nutritional investment in reproduction" is much greater than that of the male [4]. This can be seen when maternal malnutrition during gestation influences the prenatal development of her offspring, resulting in lower fetal weights and delayed ossification [5]. In addition to the effects above, undernutrition can affect the level of tissue oxidation. Feed supplements (or additives) can be used to counteract the oxidative effects. A study by Guo *et al.* [6] reported that feed restriction during the luteal phase of the estrous cycle altered energy metabolism and increased oxidative stress of ovarian tissues in sheep. In the same study, arginine supplementation was effective in reducing the negative effects of feed restriction through altering gene expression, increasing resistance to oxidative stress, and reducing apoptosis.

Ginger (*Zingiber officinale* Roscoe), a medicinal plant anciently used in Asia, Europe, and the Middle East, is known to have anti-oxidative effects [7]. Ginger has been shown to protect tissues and organs against oxidative stress. It was able to reduce the induced oxidative effects of several stressors on various tissues [8-10]. Besides acting as an antioxidant, ginger supplementation appears to improve male reproductive parameters; rats [11] and mice [12] supplemented with ginger experienced increased sperm concentration, viability, motility, and serum total testosterone. Herbal medicines with antioxidant properties, such as ginger, can neutralize intermediate free radicals, halt oxidation chain reactions, and ultimately enhance specific sperm fertility indicators, thereby improving the fertility potential of sperm in roosters [13, 14]. Male fertility can be improved by using medicinal herbs with antioxidant properties. These herbs help reduce oxidative stress, enhance gonadal hormone levels, boost spermatogenesis, and ultimately increase male reproductive efficiency [14]. In terms of direct effects on reproduction, we could not find any literature on the effects of ginger on pubertal female rats. For this reason, this study was conducted to evaluate whether ginger oil supplementation can reduce the negative effects of feed restriction on metabolic and reproductive parameters in young female rats.

2. MATERIALS AND METHODS

2.1. Animal Care

This experiment was conducted at the Animal House, Faculty of Veterinary Medicine, Jordan University of Science and Technology (JUST). All experimental procedures were approved by the JUST Animal Care and Use Committee. Forty-eight, 9-week-old, albino Sprague Dawley female rats obtained from the Animal House, Faculty of Veterinary Medicine (159±13 g body weight) were used in this study [15]. Rats were caged individually and allowed 10 days of adaptation to the experimental conditions, during which the amounts of feed offered and refused were recorded for each animal to evaluate voluntary feed intake. Cages were located in rooms with constant temperature (22 °C) and photoperiod (12 h light/12 h dark).

2.2. Experimental Treatments

Following adaptation and intake assessment, rats were randomly assigned to one of four treatment groups (n=12) in 2 × 2 factorial arrangements: *ad libitum* feeding with no ginger oil supplementation (CON, G1), 25% feed restriction (based on the assessed intake; G2), *ad libitum* feeding with 0.2 mL/animal/d ginger oil (Hemani®, Pakistan; G3), and 25% feed restriction plus 0.2 mL/animal/d ginger oil (G4). The offered feed was a commercially pelleted mixture containing 16% crude protein. The treatments above lasted for 30 days, during which body weight and size (nose to tail) were recorded weekly for each rat. Rats in G3 and G4 received ginger oil daily (gavage), while G1 and G2 were gavaged with water. Drinking water was offered as a free choice to all animals.

At the end of the experimental period, 6 rats from each group were randomly selected to be bred with sexually mature males. Females were marked for identification and placed in pairs with one male for 10 days in a breeding cage. Litter data (birth date, size, and weight) were recorded. The remaining females were used for blood and tissue collection. Rats were anesthetized using intraperitoneal injection of Ketamine - Xylazine combination (using 1 ml syringe, 75-90 mg/kg ketamine, and 5-10 mg/kg xylazine in the same syringe). Blood was collected *via* cardiac puncture. Animals were later killed using an anesthetic overdose, after which tissues were harvested and weighed. All rats were dissected, and their internal organs (heart, liver, spleen, pituitary gland, kidneys, and whole reproductive tract with ovaries) were removed and weighed. Blood was collected into tubes, allowed to clot at room temperature, and then centrifuged (1500 x g) for 15 minutes. Serum was harvested and stored in vials at -20 °C until assayed for cortisol or metabolites.

2.3. Blood Analysis

Serum cortisol concentration was quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications (Monobind, Inc., Lake Forest, CA, USA). All samples were run in duplicates in a single assay (sensitivity = 0.25 µg/dL; CV = 3.85%).

3.2. Cortisol and Blood Metabolites

Cortisol concentration was not affected by treatment (Table 1). Similarly, serum cholesterol and triglycerides did not differ among groups (Table 1). Serum glucose (Fig.

2A) and urea nitrogen (Fig. 2B) were significantly influenced ($P < 0.05$) by feed restriction but not by ginger supplementation. Feed restriction resulted in increased serum glucose and reduced urea nitrogen in rats.

Table 1. Blood cholesterol, triglycerides, and cortisol in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

Blood Parameter	Treatment			
	Control (n=6)	Feed Restriction (n=6)	Ginger (n=6)	Ginger Plus feed Restriction (n=6)
Cholesterol (mg/dL)	55.3±5.2	56.9±4.8	57.5±4.8	61.5±4.8
Triglycerides (mg/dL)	117.4±18.6	97.0±16.6	98.7±16.6	101.6±16.6
Cortisol (µg/dL)	4.25±0.87	5.06±0.87	4.37±0.87	4.92±0.87

Note: ¹Ginger oil was supplemented at the rate of 0.2mL/rat/day.

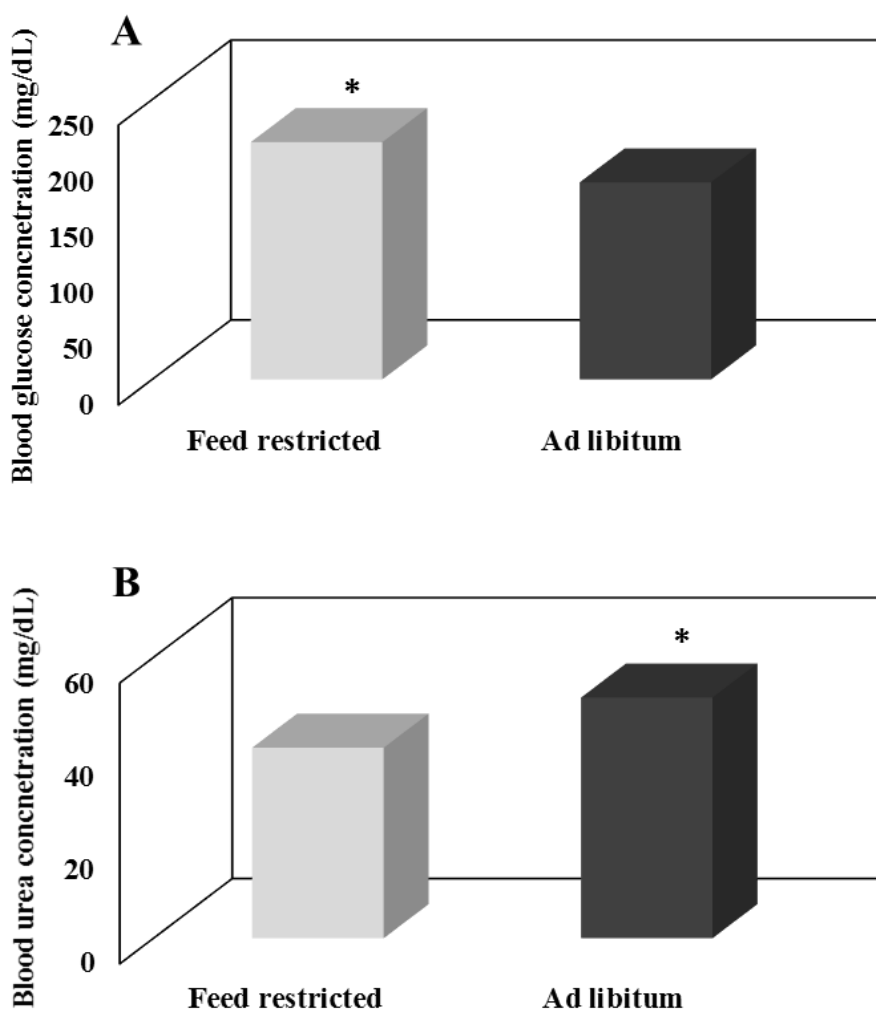


Fig. (2A,B). Blood glucose and urea nitrogen in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

* denotes significant difference between means ($p < 0.05$).

3.3. Organ Weights

Several organs were collected and weighed. The right and left ovaries and kidneys for each animal were weighed separately. There was no feed restriction by ginger supplementation interactions with respect to any of the organs measured. Kidneys, heart, liver, spleen, and pituitary gland were unaffected by treatment (Table 2). Right ovaries were affected ($P < 0.05$) by feed restriction and tended to be influenced ($P < 0.10$) by ginger supplementation but not their interaction (Fig. 3A and C). Left ovaries, however, were not significantly affected by

either treatment (Fig. 3B and D), although they were numerically heavier in the *ad libitum*-fed rats and in rats receiving ginger oil. The right ovaries of rats in the feed-restricted groups weighed less than those of animals receiving *ad libitum* feeding. Similarly, ginger supplementation tended to increase right ovarian weights compared to animals not receiving ginger regardless of feed restriction (Fig. 3B). Reproductive tract weights were also influenced by ginger supplementation ($P < 0.05$). Rats in the ginger-supplemented groups had heavier reproductive tracts than their non-supplemented counterparts (Fig. 4).

Table 2. Organ weights in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

Organ Weights	Treatment			
	Control (n=6)	Feed Restriction (n=6)	Ginger (n=6)	Ginger Plus Feed Restriction (n=6)
Left kidney (g)	0.85±0.05	0.83±0.05	0.84±0.05	0.90±0.05
Right kidney (g)	0.89±0.06	0.93±0.06	0.85±0.06	0.98±0.06
Heart (g)	0.77±0.04	0.78±0.04	0.83±0.04	0.74±0.04
Liver (g)	8.29±0.36	7.65±0.36	8.49±0.36	8.29±0.36
Spleen (g)	0.61±0.03	0.65±0.03	0.57±0.03	0.61±0.03
Pituitary gland (mg)	9.9±6	19.6±6	19.4±6	10.5±6

Note: ¹Ginger oil was supplemented at the rate of 0.2mL/rat/day.

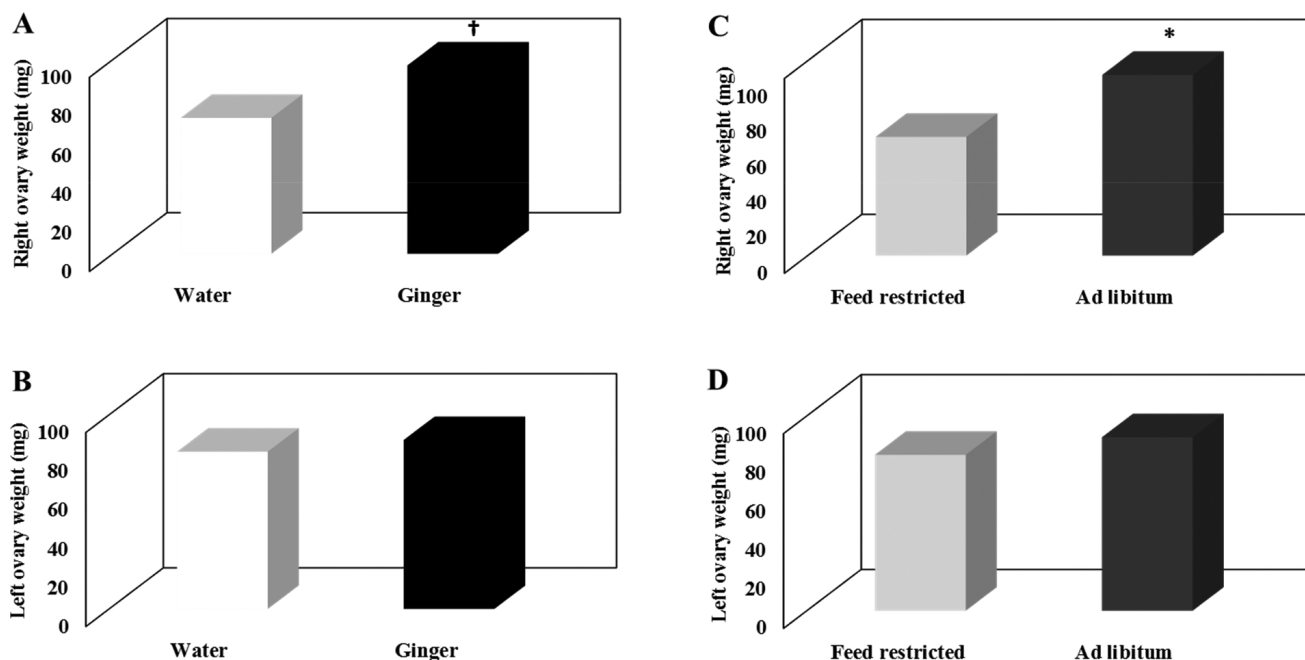


Fig. (3A-D). Right and left ovarian weights in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

* denotes significant difference ($p < 0.05$), while † denotes a tendency for means to differ ($p < 0.10$).

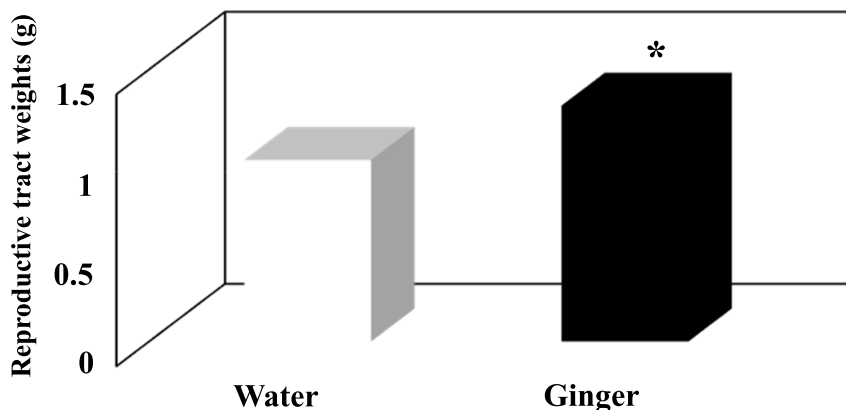


Fig. (4). Reproductive tract weights in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

* denotes significant difference between means ($p < 0.05$).

Table 3. Litter data in young female rats subjected to 25% feed restriction and/or ginger oil supplementation for 30 days¹.

Parameter	Treatment			
	Control (n=6)	Feed Restriction (n=6)	Ginger (n=6)	Ginger Plus Feed Restriction (n=6)
Dam birth weight (g)	237.3±6.6	234.6±6.6	235.4±7.4	224.6±6.1
Rats giving birth (Ratio)	5/6	5/6	4/6	6/6
Litter size (No.)	11.5±0.7	11.8±0.7	12.8±0.7	12.2±0.7
Litter weight (g)	61.7±9.1	78.5±9.1	75.2±9.1	70.5±9.1
Individual pup weight (g)	6.3±0.6	6.6±0.6	6.0±0.6	5.8±0.6
Days from male introduction to birth (d)	24.2±0.7	25.4±0.8	24.0±0.7	24.6±0.8

Note: ¹Ginger oil was supplemented at the rate of 0.2mL/rat/day.

3.4. Litter Data

Dam birth weight, parturition percentage, litter size, litter weight, individual pup weight, and the time from male introduction until parturition were unaffected by treatment (Table 3).

4. DISCUSSION

The strong link between nutrition and reproduction ensures that the female is able to sustain her own body before supporting another life (or lives). A low plane of nutrition can negatively influence reproduction; however, the magnitude of this depends on severity and duration. Feed restriction can increase the level of tissue oxidation, leading to apoptosis [6]. Ginger, an antioxidant, has been shown to protect against oxidative stress induced by many factors [16, 17]. This study evaluated the effects of supplementing ginger oil to feed-restricted female rats on metabolic and reproductive parameters.

Rats in the feed restriction group (G2) experienced a reduction in growth (body weight and size). This was

expected as these animals received 75% of the estimated intake for 30 days. The other feed restriction group (G4) did not experience any effects on body weight and size. Rats receiving a lower plane of nutrition experience reduced body weights in Sprague-Dawley male and female rats exposed to feed restriction. Ginger oil supplementation to G4 rats appears to be the main factor preventing the effects of the low plane of nutrition on growth. This may be due to the energy content in ginger oil; various varieties of ginger oil provide from 375 to 396 kcal/100g [19].

Rats receiving ginger oil appeared to be more docile and less stressed during the daily handling for treatment administration. Ginger supplementation has been reported to modulate stress response in fish [20]. The authors observed a lesser increase in cortisol concentrations following exposure to hypoxic conditions in the ginger-supplemented group compared with the controls. In this study, however, we detected similar cortisol concentrations among the four treatment groups.

In previous studies, ginger extract administration lowered blood cholesterol and triglycerides in rats [21] and humans [22]. In the present study, serum cholesterol and triglycerides were unaffected by ginger oil administration. Similar results were reported by Jeena *et al.* [23], whereby ginger oil administration did not induce a significant decrease in blood cholesterol and triglycerides. The discrepancy in findings may be due to the type of supplement (extract vs oil) and dose of ginger used in the studies.

Ginger supplementation had no effect on serum urea nitrogen and glucose. Serum urea nitrogen was lower in the feed-restricted groups, while serum glucose was greater in the feed-restricted groups compared to those receiving *ad-lib* feeding. The lower feed intake is the primary reason for such a decrease. Reduced total feed intake is associated with lower crude protein intake, which partially accounts for the decline in urea nitrogen. Additionally, the body's requirement for glucose may have activated gluconeogenesis, resulting in the utilization of amino acids for the production of glucose. This can also explain the greater glucose concentration in the feed-restricted rats. One additional factor contributing to this justification is the numerical increase in cortisol concentration in the feed-restricted groups (Table 1). Cortisol is a metabolic hormone known to induce glucose production through gluconeogenesis. Despite its effects on body weight, feed restriction did not affect organ weights, with the exception of the right ovaries, which weighed less in the feed-restricted rats than in those receiving *ad libitum* feeding. The lower plane of nutrition is probably the cause of reduced ovarian weights in feed-restricted rats, which also relates to the retarded growth observed in these animals. The current study might not have shown that a reduction in body weight has been severe enough to yield a measurable reduction in organ weight. Chapin *et al.* [18] reported lower liver, kidneys, and right ovarian weights in Sprague-Dawley female rats subjected to feed restriction, especially when those animals reached 70% of the control rats' body weights. The severity and duration of feed restriction in our project did not match that of the aforementioned study. Similar to feed restriction, ginger supplementation did not affect the non-reproductive organ weights. Similar findings were reported when ginger powder [22] or oil [23] was supplemented in rats. In either of the previous studies, organ weights were comparable among control and ginger-treated rats regardless of form or the administered dose.

The current study found positive effects of ginger oil supplementation on reproductive development without affecting litter data (litter size, pup weight, *etc.*). Ginger oil-supplemented rats had heavier reproductive tracts and tended to have heavier right ovaries than their non-supplemented counterparts did. Left ovaries, although numerically heavier, were unaffected by treatment. To our knowledge, no previous studies investigated the effects of ginger oil supplementation on reproductive development in feed-restricted female rats. Our results are similar to previous studies that found that ginger supplementation increased ovarian weight in rabbits [24]. The notable increase in ovarian weight observed in the experimental group compared to the control group suggests that the dose

might be an optimal level for enhancing ovarian function [24]. Feed restriction has recently been reported to alter energy metabolism and increase oxidative stress of ovarian tissue in sheep [6]. The positive effects of ginger oil reported in the current study may be due to its anti-oxidative properties. In previous studies, rats exposed to oxidative stress (treated with isoproterenol) expressed reduced tissue antioxidant levels [namely catalase, superoxide dismutase (SOD) and glutathione (GSH)] [10]. Treating these rats with ginger extract reversed the oxidative effects and returned the aforementioned antioxidants to their normal (catalase and SOD) or above-normal (GSH) levels. Devine *et al.* [25] reviewed the effects of oxidative stress on ovarian function. They concluded that depletion of GSH stimulates apoptosis in antral follicles and granulosa cells, while antioxidant supplementation inhibits such effects.

CONCLUSION

In summary, the current study evaluated the effects of feed restriction and ginger oil supplementation on growth, blood metabolites, and reproductive development in young female rats. There was no interaction between the two treatments with respect to any of the measured variables. Feed restriction resulted in a significant reduction in body weight accompanied by a reduction in the weight of the right ovary. On the other hand, ginger oil supplementation resulted in increased reproductive tract and right ovarian weights. Such effects may have occurred due to the anti-oxidative properties of ginger oil. Future research should address longer-term, more severe feed restrictions with ginger oil supplementation. Additionally, histological assessment of ovaries should shed some light on the impact of supplementation on follicular development.

AUTHORS' CONTRIBUTIONS

HSS, BSO, RTK, SMJ, and MDO contributed to the study's concept and design, HSS, RIQ, LBO, BSH, AI, WAL, and MM collected the data, HSS, BSO, and RTK performed the analysis and interpretation of the results, and HSS and RTK drafted the manuscript. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

SOD	=	Superoxide dismutase
GSH	=	Glutathione

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures were approved by the JUST Animal Care and Use Committee (Approval No.225/03/04/16B).

HUMAN AND ANIMAL RIGHTS

This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed to report experiments involving live animals and promote ethical research practices.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available upon reasonable request from the corresponding author [H.S.S].

CONFLICT OF INTEREST

The authors declare no conflict of interest with anyone regarding this work.

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