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*Research Article*

# Orange peel extract corrected lipid dysmetabolism and pro-inflammation, but not deranged antioxidant and hormonal status in orchidectomised rats

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*Orange peel extract, Oxidative stress, Inflammation, Lipid profile; Hormone*

**ABSTRACT**

**Background:** Testosterone is a metabolic hormone; therefore, its absence would affect food metabolism, and subsequently a wide array of associated endogenous processes, including oxidative and inflammatory events. Contrarily, orange peel is known to be rich in flavonoids, which have strong antioxidant and anti-inflammatory properties, asides from their modulatory roles on lipolysis and lipogenesis. Hence, we investigated the effects of ethanolic extract of orange peel (EEOP) on antioxidant, inflammatory, and lipid and reproductive hormonal profiles in experimental animal. **Methods:** The rats were divided into four groups (N=10), which included: Control (Sham orchidectomised) (group 1); Orchidectomised (Orchid) (group 2); Orchidectomised + Low dose of orange peel (Orchid + LDOP) (group 3); and Orchidectomised + High dose of orange peel (Orchid + HDOP) (group 4). EEOP was administered at a low and high dose of 200 and 600 mg/kg BW, *p.o.* respectively; however, normal saline (vehicle) was administered at 1 ml/kg BW, *p.o.* to groups 1 and 2 throughout the four weeks duration of the experiment. **Results:** Castration was accompanied by dsylipidaemia, without alteration of oxidative, inflammatory, and reproductive hormonal status. Although EEOP reversed alterations in lipid metabolism back to the baseline, it neither showed significant effects on oxidative markers (SOD, catalase, total antioxidant capacity and malondialdehyde) nor reproductive hormone (testosterone, FSH and LH) profile, even though it significantly reduced uric acid. The effects of EEOP were not dose-graded, except in the MDA result, which was significantly higher in group 3, relative to group 4. **Conclusion:** EEOP corrected lipid dysmetabolism and pro-inflammation, but not deranged antioxidant and hormonal status in a dose-independent manner in orchidectomised rats.



## INTRODUCTION

Surgical removal of the testes in human male sex is an ancient practice in medical science, which is carried out for various purposes, among which are management of prostate and testicular tumours, religious purification and self-cleansing, punishment of sex offenders, or sexual reassignment surgery (Tsai, 1996; Jenkins, 1998; Scott and Holmberg, 2003). However, in veterinary science, testicular extirpation is intended to instigate certain behaviour, reduce aggressiveness in animal, enhance physical development, or prevent unwanted pregnancies, which could cause overpopulation (Burciage *et al.,* 2006).Several longitudinal studies proved that low circulating level of testosterone independently predict the future development of metabolic syndrome, insulin resistance, diabetes, cardiovascular disease, obesity and dsylipidaemia (Kupelian *et al.,* 2006, Selvin *et al.,* 2007, Haring *et al.,* 2009; Christoffersen *et al.,* 2010; Ullah *et al.,* 2011). Moreover, two systematic reviews and meta-analyses showed that endogenous levels of total and free testosterone were lower in individuals with metabolic syndrome compared to those without the condition (Brand *et al.,* 2010; Corona *et al.,* 2011). More specifically, castration-induced sex hormone deficiency precipitates pro-inflammatory events (Zha *et al.,* 2013) and oxidative stress (Mogheiseh, 2019), among other physiological imbalances.

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Most researches on castration are conducted without the use of pharmacological interventions. These studies are designed to unravel various pathophysiological conditions linked with the testicular removal and the resulting deficiency in testosterone in the systemic circulation. Nevertheless, few studies reported on the effects of therapeutic substances, such as melatonin, citrus peel extract and exogenous testosterone (Klapcinska *et al.*, 2008; Mohamed *et al.*, 2014; Mogheiseh *et al.*, 2019), with a focus on re-establishing physiological state following castration.

Mohamed et al. (2014) had reported on the ameliorative effects of orange peel extract (OPE) on castration-induced oxidative stress, with an emphasis on liver and kidney tissues. However, in the present study, we are interested in the systemic effects of the extract following castration. Therefore, we investigated the dose-graded effects of ethanolic extract of orange peel (EEOP) on antioxidant, inflammatory, lipid and reproductive hormonal profiles in orchidectomised male Wistar rats.

## METHODS

*Chemicals and drugs*

Sodium pentobarbital was obtained from Taj Pharmaceuticals, Maharashtra, India, while diagnostic kits for the determination of catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), c - reactive protein (CRP), uric acid (UA) and malondialdehyde (MDA) were obtained from Fortress Diagnostics Limited, United Kingdom. However, analytic kits for the determination of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), testosterone (Tt), follicle stimulation hormone (FSH) and luteinising hormone were obtained from Elabscience Biotechnology Company, Ltd., Wuhan, Hubei, China.

*Experimental animals and care*

Forty (40) adult (11-13 weeks old; weight: 210-230 g) male Wistar rats were used for this study. They were purchased from the Animal House of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria. Afterwards, the rats were kept in plastic cages with wood shavings at room temperature of 23 – 25.5$^{O}$ C and photo-periodicity of 12 hrs light/12 hrs dark in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin. The animals were acclimatised for two weeks prior to random assignment to separate groups. They were administered rodent pellet and water *ad libitum* daily, and weighed weekly. The Ethical Committee of the University of Ilorin approved (no ethical number was assigned) the experimental procedures that were followed in this study, which were also in accordance with the specifications in the 'Guide for the Care and Use of Laboratory Animals' documented by the National Academy of Sciences (National Academy of Sciences, 2011).

*Experimental design*

The rats were randomly divided into four (4) groups, which included: Control (Sham orchidectomised) (group 1); Orchidectomised (Orchid) (group 2); Orchidectomised + Low dose orange peel (Orchid + LDOP) (group 3); and Orchidectomised + High dose orange peel (Orchid + HDOP) (group 4). The EEOP was administered at a low and high dose of 200 and 600 mg/kg BW, *p.o.* respectively (Mohamed *et al.*, 2014), while groups 1 and 2 were administered normal saline (vehicle) at 1 ml/kg BW, throughout the 4 weeks duration of the experiment.

*Orange peel extraction*

The oranges used for the study were bought from the Kwara State Ministry of Agriculture and Natural Resources, Ilorin, Nigeria. They were washed before the peel was separated from the edible parts of the fruit. Thereafter, the peels were air dried for 4 weeks and then powdered by blending. The resultant (about 500 g) was subjected to cold extraction with 95 % ethanol (4.5 litres) for two days. The extract was filtered through Whatman No. 1 filter paper (Tokyo, Japan), and the extraction solvent was removed with an evaporator (Eyela N-1000, Tokyo Rikakikai Co., Tokyo, Japan) at 40$^{O}$ C. The dry extract was redissolved in normal saline to a concentration of 50 mg/mL and kept at 20$^{O}$ C until use.

*Surgical procedure for castration*

Twenty minutes prior to the extirpation of the testes, the rats were administered an anaesthesia (sodium pentobarbital at 40 mg/kg, *i.p.*; manufacturer: Nicholas Piramal Limited, London, UK) (Adeyemi *et al.*, 2020, 2020a, 2020b) and then an analgesia (sodium diclofenac at 10 mg/kg, *i.m.*; manufacturer: Wuhan Grand Pharmaceutical Company, Wuhan, Hubei, China) (Adeyemi and Olayaki, 2018). After the animals had lost reflexes, the hair over the scrotum was removed using a shaving stick, and the skin surface was disinfected with 70 % alcohol, followed by 2 % chlorhexidine solution. The rats were then placed on dorso-recumbent position and a mid-line ventral skin incision of about 1 cm in length was made in the scrotum in order to expose the tunica. Then, another mid-line incision was done in the tunica, slightly smaller than the incision in the skin, so as to allow the passage of testis out of the tunica. The

testis was raised in order to expose the underlying blood vessels and tubules. Afterwards, the spermatic blood vessels and vas deferens were cauterised, and then the testis and associated tissues were severed below the cauterisation. A sterile cotton-tipped swab was used to gently return any remaining tissues into the scrotum. Thereafter, the procedure was repeated on the other testis. It should be noted that during the course of the surgical operation, ophthalmic ointment (Lacri-Lube; manufacturer: Allergan Ltd., Marlow International, The Parkway, Marlow, Bucks, SL7 1YL, UK) was applied intermittently to both eyes of the rats, in order to prevent corneal desiccation. A topical analgesic (lidocaine: 1-2 % at 2-4 mg/kg; manufacturer: Hubei Shinrezing *Pharmaceutical* Technology Co., *Ltd.*, Hubei, China) (University of British Columbia IACUC, 2010) was applied locally to the incision before suture. After suturing, the skin surface was clean with the disinfectants, and the castrated rats were allowed to recover in clean cages, during which they were monitored intermittently until they fully recover. After the castration of each animal, the surgical instruments were rid of blood and debris, and then disinfected by dipping them in a hot glass bead steriliser for approximately 30 seconds, after which they were allowed to cool completely.

For the rats in the control group, a sham operation was performed. The procedure described above was followed, but, in this case, the testes, spermatic blood vessels, vas deferens, and other associated tissues were returned into the scrotal sac after they were initially brought out through an incision made into the scrotum and tunica. Afterward, the cut in the scrotal sac was sutured as described above. In order to prevent post-surgical infection, the rats received an antibiotic (Amoxicillin; manufacturer: SmithKline Beecham, Puteaux, France, at 50 mg/kg, *p.o.*) during the first 7 days after testicular extirpation, about 8 hours post-administration of EEOP.

*Biochemical assays*

Twelve hours post-administration on the last day of the 10 weeks duration of the experiment, the rats were anaesthetised with sodium pentobarbital (40 mg/kg, *i.m.*, manufacturer: Nicholas Piramal Limited, London, UK) (Adeyemi and Olayaki 2018a; Adeyemi *et al.*, 2020) and then dissected in order to collect blood by cardiac puncture. The whole blood was collected into heparinised sample bottles prior to centrifugation at 1372 x g for 15 min, at – 4$^{o}$ C (Adeyemi *et al.*, 2020c; Olayaki *et al.*, 2020) using a cold centrifuge (Centurion Scientific Ltd., Chichester, West Sussex, UK). The resultant supernatant plasma samples were pipetted into separate plain bottles, and the biochemical assays were done immediately according to the manufacturers' instruction.

*Data analysis*

Data were analysed using Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, Illinois, USA). Statistical evaluations of the differences between the group mean values were tested by one-way analysis of variance (ANOVA) and then least significant difference *post - hoc* test for multiple comparisons. The results were expressed as mean ± standard error of mean (SEM) and statistical significance was considered at $p \leq 0.05$.

## RESULTS

*Effects of ethanolic orange peel extract (EEOP) on oxidative stress and inflammatory markers and reproductive hormones in orchidectomised rats*

There was no significant difference in SOD, CAT, CRP, FSH and LH when comparisons were made across the groups (Table 1.0). However, compared to the control group, we recorded significant decreases in TAC and Tt in groups 2-4 ($p$ – 0.00). Moreover, relative to the former, there were significant elevations in MDA level in groups 2 and 3 ($p$ – 0.02 and 0.00 respectively). In comparison to group 2, we documented significant decreases in UA level in groups 3 and 4 ($p$ – 0.04 and 0.02 respectively), although a significant increase in MDA in group 3 ($p$ – 0.05). Compared to group 4, there was a significant elevation in MDA level in group 3 ($p$ – 0.02) (Table 1.0).

*Effects of EEOP on lipid profile in orchidectomised rats*

There was no significant difference in HDL-C levels when comparisons were made across the groups (Table 2.0). Nevertheless, relative to the control group, there were significant increases in TC, TG, LDL-C and VLDL-C in group 2 ($p$ – 0.00). Compared to the latter, significant diminutions were recorded in TC, TG, LDL-C and VLDL-C in groups 3 and 4 ($p$ – 0.00). As regards the derivatives of the traditional lipid profile, there were significant elevations in LDL-C/HDL-C and TG/HDL-C ratios in group 2, compared to the control group ($p$ – 0.00 and 0.01 respectively). However, relative to the former, we observed significant decreases in TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios in groups 3 and 4 ($p$ – 0.00) (Table 2.0).

Table 1.0: Effects of ethanolic extract of orange peel (EEOP) on oxidative stress and inflammatory markers and reproductive hormones in orchidectomised rats

| Groups/Parameters | CAT (U/ml) | SOD (U/ml) | TAC (trollox equivalent (mM) | MDA (µM) | CRP (ng/ml) | UA (mg/dL) | LH (mIU/ml) | FSH (mIU/ml) | Tt (ng/ml) |
|---|---|---|---|---|---|---|---|---|---|
| 1. Control | 1824.88±14.37 | 1.13±0.10 | 2.91±0.21 | 0.29±0.16 | 0.09±0.03 | 3.61±0.15 | 0.72±0.21 | 0.25±0.01 | 3.58±0.12 |
| 2. Orchid | 1911.00±12 7.85 | 1.24±0.13 | 1.57±0.17$^{*}$ | 0.97±0.20$^{*}$ | 0.13±0.07 | 4.03±0.20 | 0.60±0.22 | 0.25±0.01 | 0.15±0.00$^{*}$ |
| 3. Orchid + LDOP | 1926.43±10 5.05 | 1.08±0.12 | 1.63±0.17$^{*}$ | 1.47±0.18$^{*\#a}$ | 0.13±0.02 | 3.20±0.21$^{\#}$ | 1.07±0.07 | 0.23±0.01 | 0.16±0.00$^{*}$ |
| 4. Orchid + HDOP | 1990.09±74. 98 | 1.07±0.05 | 1.57±0.24$^{*}$ | 0.79±0.21 | 0.08±0.03 | 3.05±0.43$^{\#}$ | 1.06±0.23 | 0.24±0.01 | 0.22±0.07$^{*}$ |

Values are expressed as mean ± SEM. $^{*}p < 0.05$ is significant compared to control group; $^{\#}p < 0.05$ is significant compared to orchid group; $^{a}p < 0.05$ is significant – Orchid + LDOP *vs* Orchid + HDOP. NB: Orchid – Orchidectomized; LDOP – Low dose of orange peel extract; HDOP – High dose of orange peel extract; CAT – Catalase; SOD – Superoxide dismuatse; TAC – Total antioxidant capacity; MDA – Malondialdehyde; CRP – C - reactive protein; UA –Uric acid; LH – Luteinizing hormone; FSH - Follicle stimulating hormone; Tt – Testosterone.

Table 2.0: Effects of ethanolic extract of orange peel (EEOP) on lipid profile in orchidectomised rats

| Groups/Parameters | TC (mg/dL) | TG (mg/dL) | HDL-C (mg/dL) | LDL-C (mg/dL) | VLDL-C (mg/dL) | TC/ HDL-C | LDL-C/HDL-C | TG/ HDL-C |
|---|---|---|---|---|---|---|---|---|
| 1. Control | 84.99±3.03 | 68.47±2.92 | 18.96±1.55 | 41.84±2.22 | 13.69±0.58 | 4.64±0.50 | 2.23±0.08 | 3.67±0.20 |
| 2. Orchid | 103.28±2.39$^{*}$ | 85.40±3.79$^{*}$ | 19.43±0.96 | 58.50±2.40$^{*}$ | 17.08±0.76$^{*}$ | 5.35±0.17 | 3.02±0.10$^{*}$ | 4.42±0.20$^{*}$ |
| 3. Orchid + LDOP | 76.54±4.44$^{\#}$ | 63.57±3.32$^{\#}$ | 19.44±1.13 | 43.31±2.34$^{\#}$ | 12.71±0.66$^{\#}$ | 4.00±0.36$^{\#}$ | 2.26±0.16$^{\#}$ | 3.28±0.11$^{\#}$ |
| 4. Orchid + HDOP | 76.30±4.66$^{\#}$ | 63.14±1.59$^{\#}$ | 19.26±1.08 | 45.09±3.46$^{\#}$ | 12.63±0.32$^{\#}$ | 3.99±0.25$^{\#}$ | 2.35±0.14$^{\#}$ | 3.31±0.18$^{\#}$ |

Values are expressed as mean ± SEM. $^{*}p < 0.05$ is significant compared to control group; $^{\#}p < 0.05$ is significant compared to orchid group. NB: Orchid – Orchidectomised; LDOP – Low dose of orange peel extract; HDOP – High dose of orange peel extract; TC – Total cholesterol; TG - Triglyceride; HDL-C – High density lipoprotein cholesterol; LDL-C - Low density lipoprotein cholesterol; VLDL-C - Very low density lipoprotein cholesterol.

## DISCUSSION

The present study revealed that castration causes dsylipidaemia, without necessarily altering oxidative, inflammatory and reproductive hormonal status. Even though EEOP reversed alterations in lipid metabolism back to the physiological baseline, it neither showed significant effects on oxidative markers (SOD, CAT, TAC and MDA) nor reproductive hormone (Tt, FSH and LH), although it significantly reduced UA level. It is known that low circulating level of Tt exerts a stimulating effect on the anterior pituitary cells which secretes gonadotropes, i.e. FSH and LH. The recorded insignificant difference in FSH and LH levels across the groups could be secondary to diminished response or rather continuous stimulation-induced exhaustion and hence reduced secretory function of the adenohypohysial cells.

Mohamed *et al.* (2014) claimed that bilateral extirpation of testes results in an imbalance in the antioxidant system, based on their examination of antioxidant profile in the hepatic and renal tissues. However, previously we had reported that six weeks castration is not accompanied by significant alterations in the levels of MDA and SOD (unpublished). This finding was partially supported by the present study, which lasted for four weeks, the difference being likely caused by the duration of the studies. Deficit in Tt following orchidectomy did not disrupt all components of the enzymatic/non-enzymatic antioxidant system as there was no significant disparity in CAT and SOD activities across the groups. Ayodele *et al.* (2020) had also

reported that CAT activity is not disrupted following testicular removal. Nevertheless, we submitted that within four weeks the overall activity of the antioxidant system could be attenuated following deficit in Tt. This was demonstrated by the significant reduction in TAC in group 2, compared to control group. Compromised integrity of the free radical scavenging effects of the antioxidant system would escalate the level of reactive species, which could cause the peroxidation of lipids, among other lethal effects. Although we did not estimate the level of reactive radicals in this study, the secondary effects of these species were observed i.e. an increase in MDA level following castration. It is well-known that OPE is rich in flavonoids, which have strong antioxidant property (Chen *et al.*, 2017). The administration of OPE had been reported to scavenge nitric oxide, 2,2-diphenyl-1-pcrylhydrazyl, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), $22^{1}$-azobis-2-methyl-propanimidamide and dihydrochloride radicals (Chen *et al.,* 2017). While we could partially relate to the fact that EEOP did not increase SOD and CAT following testicular extirpation, due to the fact that the activities of these enzymes was no comromised in the midst of testosterone insufficiency, it was surprising that the extract did not appreciate the TAC, which was significantly reduced after orchidectomy. Strange as it maybe, this result is in agreement with the level of lipid peroxidation, which was marked by MDA. Therefore, it could not be said that the result we obtained is as a result of experimental error during the assay of TAC. We thought it would be sufficient for EEOP not to influence lipid peroxidation; however, we were taken aback by the significant increase in MDA in group 3 (Orchid + LDOP), relative to the control, untreated orchidectomised and Orchid + HDOP groups. These unexpected results generated a question: is the antioxidant potency of OPE dependent on the underlying pathological state? We could not answer this question at the moment. However, overtime, other studies could support these findings, from which we could make inference to explain our present result better in further research work.

Oxidative stress and inflammatory processes are two endogenous events that complement each other (Adefisayo *et al.,* 2018; Adeyemi *et al.,* 2019). Hence, pro-oxidation is often accompanied by pro-inflammation (Adeyemi and Olayaki, 2018b; Olayaki *et al.,* 2018, 2019). The insignificant difference in CRP level across the group was not unexpected, as similar trend was recorded in the SOD and CAT activities. Even though the disruption of the antioxidant system (index by TAC) in the group 2 was not corroborated by the CRP level, there was an increase, although not significant, in UA in the untreated castrated group, relative to the control. There are claims in literature that flavonoids in OPE does not only have antioxidant but also anti-inflammatory action. It has been opined that the anti-inflammatory property of citrus flavonoids is related to individual flavonoid structure (Manthey *et al.,* 2001). For instance, flavones with $4^{l}$-OH groups or $3^{l}$,$4^{l}$-hydroxyl substituents on the B ring are selective inhibitors of lipoxygenase. However, flavones with 5 or more methoxy substituents have potent inhibitory activity for phosphodiesterase (Manthey *et al.,* 2001); while polymethoxylated flavone, such as nobiletin, is a strong inhibitor of lipopolysaccharide-induced TNF-α in human monocytes (Manthey *et al.,* 1999). Although EEOP was not observed in this study to have an antioxidant effect, but rather a pro-oxidative action, the administration of the extract was accompanied by significant reduction in UA level. Therefore, EEOP could be credited to have anti-inflammatory action. A positive and significant association was observed between UA and several inflammatory markers in a large population-based study (Ruggiero *et al.,* 2006). Experimental studies revealed that the product of metabolism of purine nucleotides i.e. UA stimulates the release of chemokine monocyte chemoattractant protein-1 (Kanellis *et al.,* 2003) and the syntheses of interleukin-1β, interleukin-6 and tumor necrosis factor-α (Johnson *et al.,* 2005).

Unlike the controversial effects of testicular extirpation on the antioxidant system, there are consistent reports that deficit in Tt instigates lipid dysmetabolism (Kupelian *et al.,* 2006; Yao *et al.,* 2011).Tt is not only being secreted by the testicular tissue, but also by the adrenal gland, although the former secretes the hormone more abundantly. Low level of Tt has been linked with an atherogenic lipoprotein profile, in particular, high LDL-C and TG levels (Wu and von Eckardstein, 2003). Researchers in cross-sectional studies had reported a negative correlation between serum levels of Tt and LDL-C (Simon *et al.,* 1997; Barud *et al.,* 2002). Contrarily, a positive correlation was documented to exist between serum Tt and HDL-C in both healthy and diabetic men (Van Pottelbergh *et al.,* 2003; Stanworth *et al.,* 2011). However, in the present study, we did not record any significant difference in the plasma level of HDL-C in the experimental groups, compared to the control. Nevertheless, significant elevations in the circulating levels of TC, TG, LDL-C and VLDL-C proved beyond reasonable doubt that there was an impaired lipid metabolism following bilateral testicular extirpation in the rats. The anti-dsylipidaemic effect of EEOP was clearly demonstrated in the treated groups, as the extract reversed the deranged lipid indices to their baseline levels i.e. to levels comparable to the control

group. Just as observed with the marker of inflammation (UA), the effects of EEOP on indices of lipid metabolism was not dose-dependent. There is dearth of information in the literature on the effects of OPE/flavonoids on lipid homeostasis. A preclinical study asserted that morin, a flavonoid, acts as an inhibitor of fatty acid synthase by regulating the sterol regulatory element-binding protein 1 (SREBP1-c), in addition to mediating elevated hepatic increase of carnitine palmitoyl transferase 1a (CPT1a) (Naowaboot and Piyabhan, 2016; Agardh *et al.*, 2017). In essence, the flavonoid has been appraised to inhibit lipolysis and lipogenesis. Derivatives of traditional lipid variables, such as TG/HDL-C, TC/HDL-C and LDL-C/HDL-C ratios are considered as predictors of ischaemic heart disease (Lemieux *et al.*, 2001). Moreover, outcome of prospective studies (Manninen *et al.*, 1992; Assmann *et al.*, 1998) proved that a high LDL-C/HDL-C ratio along side with high triglyceridaemia is associated with highest risk of coronary heart disease. Therefore, the recorded significant elevations in TG/HDL-C and LDL-C/HDL-C ratios in the untreated castrated group are indications that low level of Tt could precipitae cardiovasculaar diseases. EEOP did not only restore normal metabolism of lipids, it also reduced the risk of future cardiovascular events following testosterone insufficiency, although this action was noted to be dose-insensitive.

The non-assessment of other reproductive hormones and lipid indices such as inhibin, estrogen, gonadotrophins, phospholipids, free fatty acids, apolipoproteins A and B, among other, are considered as part of the limitations for this study. Hence, further research could be done to investigate these parameters using the same model.

## CONCLUSION

EEOP corrected lipid dysmetabolism and pro-inflammation, but not deranged antioxidant and hormonal status in a dose-independent manner in orchidectomised rats.

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