



Preventive Role of Propolis and L-Arginine[®] Supplement on Dna Damage for Sperm and Male Reproductive Hormones Values in Selenium Nanoparticles-Remedied Rats

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Abstract | Rapid growth of uses of selenium nanoparticles (SeNPs) in our environment made the evaluation of its toxicity an urgent requirement. This study designed to investigate the impact of Green synthesized of SeNps and prophylactic effects of L-Arginine and propolis for male reproductive system. The animals evenly partitioned to four groups; (G1) group: were received distilled water. (G2) group: were intubated 200mg/kg B.W of Black Tea Selenium Nanoparticles (BTSe), (G3) group: were intubated 200 mg/kg of (BTSe) + 200 mg/kg of L-Arginine, (G4) group: were intubated 200 mg/kg of BTSe+ 200 mg/kg of propolis. The samples of blood had gathered from rats each three weeks for assessed the concentration value of FSH, LH, testosterone, and inhibin B hormone for each of groups above. At the termination of experiment were taken in from them specimen of testes for a purpose pathohistology study and assessed the percentage of DNA fragmentation. The current study observed damage in this sperms DNA and alteration of histopathological section. In addition reduction the levels of testosterone, inhibin b, FSH and LH hormones in group that ingestion BTSeNPs comparing the remnant groups, also been noted this reduction in above hormones getting worse with time progresses of experimental. In group4 show the propolis was enhancer factor for adverse effect of BTSe where FSH, LH, testosterone and inhibin b hormones values are elevated than remnant groups, as well as elevated the percentage integrity sperm DNA comparing with remnant groups. Concluded, the current experience revealed SeNPs have oxidative stress on reproductive system at high concentration, as well as the propolis and L-Arginine and has prophylactic effects on testes against the effect of SeNPs, regarded to his the antioxidant impact.

Keywords | L-Arginine, Selenium nanoparticles, Reproductive hormones, Genotoxic, Spermatogonia, Propolis.

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INTRODUCTION

Great interest was directed for Selenium nanoparticles (SeNPs) regarded to their physical properties and antimicrobial activity where entered different biomedical application and consumable products like food additives (Wang et al., 2018; Zambonino et al., 2023), topical antibacterial formulations (Sahoo et al., 2023), coatings for footwear (Abou Elmaaty et al., 2022) and paper towels

(Hadimani et al., 2023). Where entered Se-NPs in a great diversity ranging of dental implementations from remedying the variation types of diseases like oral cancer to delivery of drugs (Yazdi et al., 2015), in addition of SeNPs is targeted to avert colonization of bacteria above them in addition enhanced oral health (Ahmed et al., 2021). Nevertheless, SeNPs had suggested as protective the cardiovascular from disease by (Wang et al., 2018). SeNPs acts vital impacts on biological system and act as a cofactor

several antioxidant enzymes like thioredoxin reductase and glutathione peroxidase, that scavengers the free radicals from the cell (Caglayan et al., 2019). Selenium (Se) entered in composition of least 25 from proteins of animals body that called selenoproteins, where carry out numerous vital functions such as anticancer agents, anti-inflammatory and antiviral agents (Minich, 2022), as well, selenium is suppressed the mutagenic factors from raiding DNA and thence can repressing tumor growth (Shrivastava et al., 2019; Al-Otaibi et al., 2022). Which raises the attention, that growing the studies spotlight on the manufacture of selenium nanoparticles, owing to less toxicity, biological activity at (vitro and vivo) and outstanding bioavailability (Adadi et al., 2019). The selenium nanomaterial (SeNps) quickly growing used within biological and material science, made a pressing requirement for evaluated the toxicity of SeNps consequences of exposure for it (Chaudhary et al., 2018). The propolis contain on over 300 phytochemical ingredients, mostly it classify as phenolic acid, terpene and flavonoid compounds families (Behl et al., 2021), these phytochemical ingredients gave propolis a number of biological properties, inclusive anti-inflammatory activities, antifungal, antioxidant and antibacterial further to other benefits (Zulhendri et al., 2022). The propolis according to recent researches were suggests it may be nominee to remediation of Inflammatory Bowel Disease because has able to potential for enhanced immune response, gut microbiome and inflammatory pathways (Al-Hariri, 2019; Xue et al., 2019), in addition was nominee mitigated cisplatin adverse effects on activity of spermatogenic, apoptosis and antioxidant situation in rats (Seven et al., 2021). The amino acid L-arginine (LA) had vital roles in each of disease and health (Wu et al., 2021). There are number of studies conducted on experimental animal and human, those have documented the LA ingestion as supplement dietary can produces several advantageous effects. Its metabolism for participant in synthesis of broad assortment of active biochemically compounds inclusive creatine who is considered an energy source, polyamines and agmatine that upholding the functions and proliferation of neuron and glutamate is a neurotransmitter that causes excitability. Moreover, from the metabolites of LA is nitric oxide which reinforce best blood flow for cerebral, led to enhanced memory processes, supports long-term potentiation (LTP) further, sleep-wake cycle arrangement (d'Unienville et al., 2021; Wu et al., 2022), several studies have documented the efficiently relieves unevenness between oxidation-antioxidant status in developing animals after intake L-Arg as dietary supplement (Bogdański et al., 2015; Akinrinde et al., 2021). In addition to, the nitric oxide (NO) had synthesized from Arg, where the nitric oxide has many vital roles in animals reproductive male system performance, immune function, and digestive tract function (Wu et al., 2021). This study designed and described to investigated

the impact of Green synthesized selenium nanoparticles upon male reproductive system and assess the prophylactic effects of LA and propolis for male reproductive system.

MATERIAL AND METHODS

ANIMALS

Before outset of experiment the 40 wistar rats have weight and age (250 ± 50 gm, < 3 month) were stay for 10 days in animal experiment house in College of Veterinary Medicine, Al-Qadisiyah University.

PREPARATION OF BLACK TEA SELENIUM NANOPARTICLES

Aqueous Black Tea (*Camellia Sinensis*) were prepared and utilized for Selenium Nanoparticles production as explained by Jayavarsha (Jayavarsha et al., 2023).

EXPERIMENTAL DESIGN

The forty animals weighed (250 ± 50 g) were partitioned to four groups and remedied by intubated orally for (63) days as follow;

Control (G1) group: rats of this group were received distilled water.

Black Tea Selenium Nanoparticles (BTSe) (G2) group: animals in this group were intubated 200mg/kg body weight of BTSe.

Black Tea selenium nanoparticles (BTSe) + L-Arginine (G3) group: animals in this group were intubated 200 mg/kg body weight of (BTSe) + 200 mg/kg body weight of L-Arginine.

Black Tea selenium nanoparticles (BTSe) and propolis (G4) group: animals in this group were intubated 200 mg/kg body weight of BTSe + 200 mg/kg body weight of propolis.

LABORATORY ANALYSIS

In this experience, have been anesthetized the rats by injected with ketamine and xylazine intramuscular (40mg/kg and 90mg/kg respectively), were gathered the samples of blood from rats by orbital sinus technique. each three weeks of experimental, the male reproductive system biomarkers concentration are assessed in serum of males were include follicle-stimulating hormone (FSH) kit (Uotila et al., 1981), luteinizing hormone (LH) kit (Uotila et al., 1981), testosterone kit (Tietz et al., 1995), and inhibin B hormone kit (Kricka, 2000) for each of groups above. At the termination of experiment, the rats anesthetized and sacrificed for taken in from them specimen of testes in order to pathohistology study according to way of Luna (Luna, 1968). In addition, In addition to that for assessed of percentage of DNA fragmentation via comet assay kits for sperm in seminiferous tubules (De Boeck et al., 2000).

Result of current experiment were examined at Level of $P < 0.05$ via two way ANOVA and utilized the least significant differences (LSD) for contrasted between means value (Snedecor George and Cochran, 1973).

RESULTS

The values of follicle-stimulating hormone (FSH) in serum elucidated in Figure 1A, where revealed significant ($P \leq 0.05$) increment in FSH concentration in serum remedied with BTSe nanoparticles + L-Arginine where compared to males cured with BTSe nanoparticles only and control. Likewise also revealed the group cured with BTSe nanoparticles and propolis has significant raised ($P \leq 0.05$) than group was remedied BTSe nanoparticles and L-Arginine. The Figure 1B illustrated the impact of Black Tea selenium nanoparticles on luteinizing hormone concentration (LH), where showed diminished in value of LH in serum of rat males comparing the rest groups. In other side showed LH concentration after three weeks same the concentration after nine weeks within group in G3 but the graduated increment in LH concentration in G4 where enhance the value with time. The result elaborates in Figure 1C exposes that testosterone concentration was raised significantly ($P < 0.05$) in serum of males were cured with BTSe nanoparticles + propolis and BTSe nanoparticles + L-Arginine from what it is in group1 and group 2. testosterone value in group 3 after three weeks more than it value in group 4 for the same time whereas data were reversed at the terminated of study where the value in group BTSe + propolis raised significantly ($P < 0.05$) than the group BTSe + L-Arginine. Revealed the result in Figure (1-D) displays that inhibin B significantly ($P < 0.05$) lifted in serum of males rats cured with BTSe + propolis and BTSe + L-Arginine (group3 and group4) when comparability with group cured with BTSe only and control. group 2 was value of inhibin B lowered significantly ($P < 0.05$) from rest groups through all duration, whereas value of inhibin B was significantly raised in group 3 than rest groups after first duration but after third duration was significantly raised ($P > 0.05$) in group 4. The result revealed in Figure (1-D) displays that inhibin B significantly ($P < 0.05$) lifted in serum of males rats cured with BTSe + propolis and BTSe + L-Arginine when comparability with group cured with BTSe and control. As for group BTSe has lowered significantly ($P < 0.05$) inhibin B value than another groups through all duration, whereas value of inhibin B was significantly raised in group 3 than rest groups after first duration but after third duration was significantly raised ($P > 0.05$) in group 4. The DNA damage percentage of sperms was clarified in Figure (1.E). DNA damage percentage was categorized for high, medium in addition low. The result reveals that group BTSe have leasted of low DNA damage percentage contrasting

with sperms in male in control and male cured with BTSe + propolis and BTSe + L-Arginine. As well as the data of comet assay illustrated high DNA damage percentage in group orally ingested with BTSe + propolis no difference significantly than high DNA damage percentage in control group. Figure 1F and a Fluorescent Microscope Figures (2-5) have presented a characteristics of comet assay to sperms, where observance a significant lifts ($P < 0.05$) at tail length, tail moment, DNA % in tail and head diameter, however show significant minimized in the head DNA percentage within group had remedied with BTSe (G2) comparing to outcomes in another cured groups in current study. The outcomes of all criteria that have been mentioned formerly have enhanced in the group cured with BTSe + propolis.

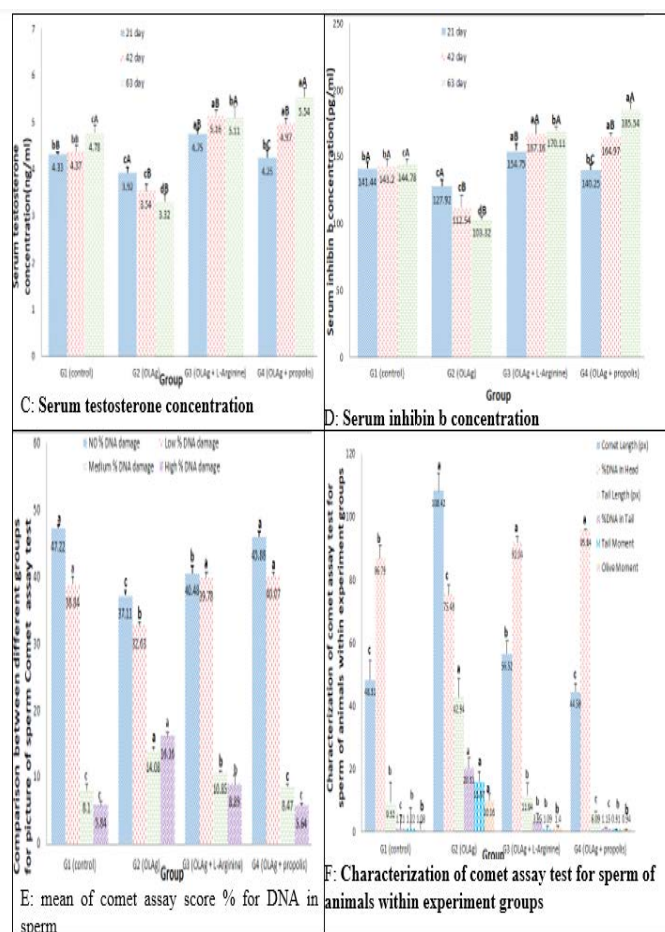


Figure 1: Impact of Black Tea selenium nanoparticles (BTSe), L-Arginine and propolis were taken it orally on serum FSH, LH, testosterone and inhibin B for triple times (after 3, 6 and 9 week) to each experimental group and mean score of DNA damage and the value of comet assay criteria % to sperm for male rats. Numbers represent mean \pm standard error. Different capital letters signify to significant difference ($P < 0.05$) between periods in same group. Different small letters signify significant difference ($P < 0.05$) between periods all groups. Control (G1) group: rats of this group were received

distilled water. Black Tea selenium nanoparticles (BTSe) (G2) group: animals in this group were intubated 200mg/kg body weight of BTSe.

Black Tea selenium nanoparticles (BTSe) + L-Arginine (G3) group: animals in this group were intubated 200 mg/kg body weight of (BTSe) + 200 mg/kg body weight of propolis.

Black Tea selenium nanoparticles (BTSe) and propolis (G4) group: animals in this group were intubated 200 mg/kg body weight of BTSe + 200 mg/kg body weight of L-Arginine.

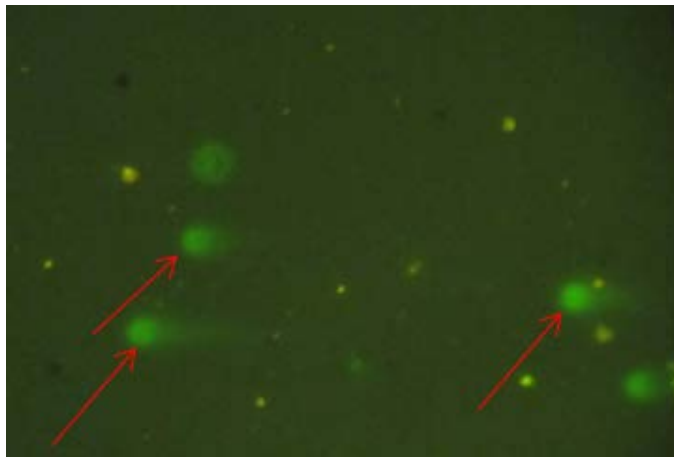


Figure 2: Comet assay for sperms of rats in G1

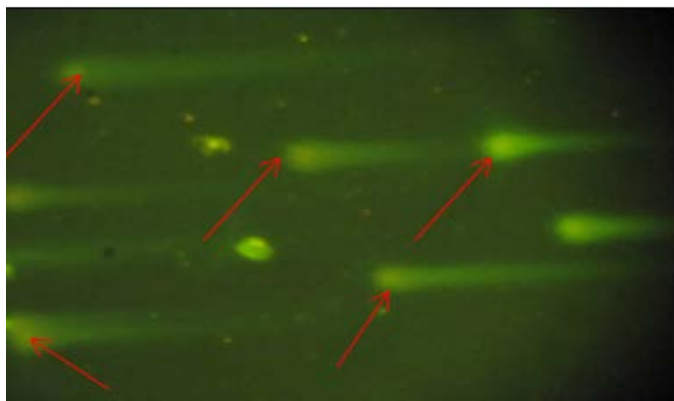


Figure 3: Comet assay for sperms of rats in G2

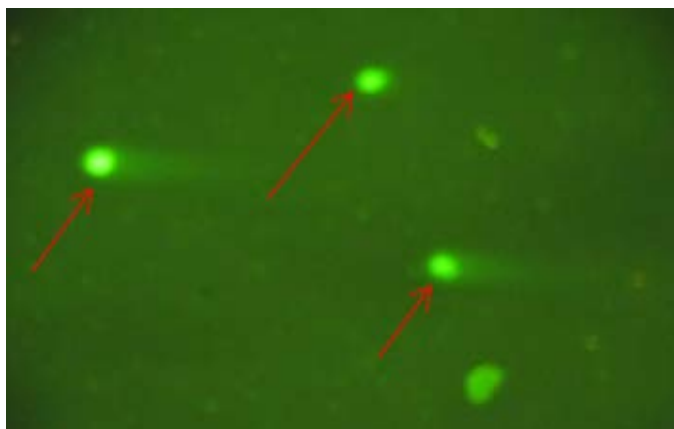


Figure 4: Comet assay for sperms of rats in G3

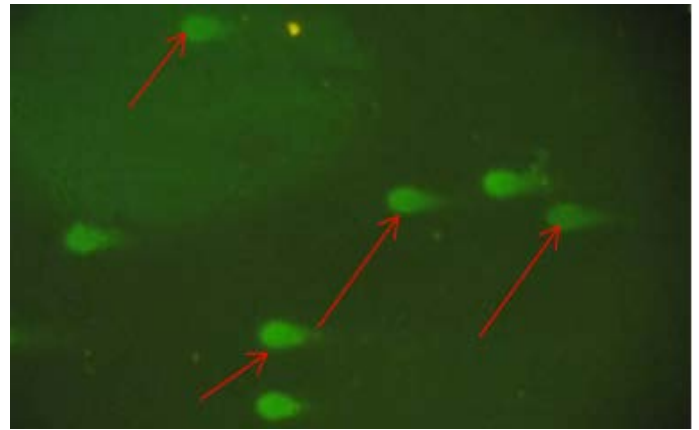


Figure 5: Comet assay for sperms of rats in G4

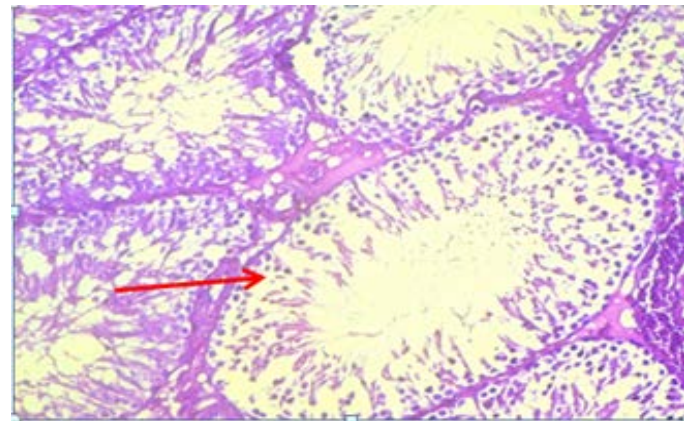


Figure 6: Histopathological section for rat testes of control group, no clear lesions was observed.

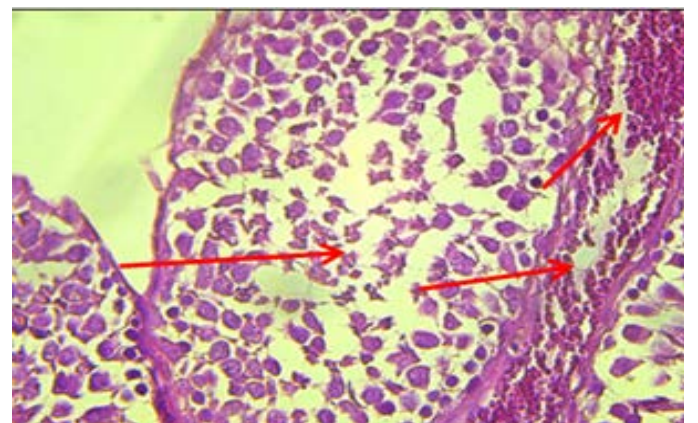


Figure 7: Histopathological section for rats testes of group 2, was observed congestion and inflammatory cell and showed germ cells degeneration and Leydig cells, and absence of spermatogenesis.

The study of histopathological for rats testes for group which intubated 200 mg/kg of BTSe + 200 mg/kg of L-Arginine and control group in Figures (6, 9) no clear lesion were observed, contrary to what exists in histopathological for rats testes in group intubated 200mg/kg body weight of BTSe in Figure (7) observed congestion and inflammatory cell and showed germ cells degeneration and Leydig cells, and absence of spermatogenesis. While histopathological of

rats testes in group which intubated with 200 mg/kg B.W of BTSe + 200 mg/kg B.W of L-Arginine in Figure (8) where noticed extension of interstitial space, cellular debris and derangement of spermatogonia anywhere in seminiferous tubule

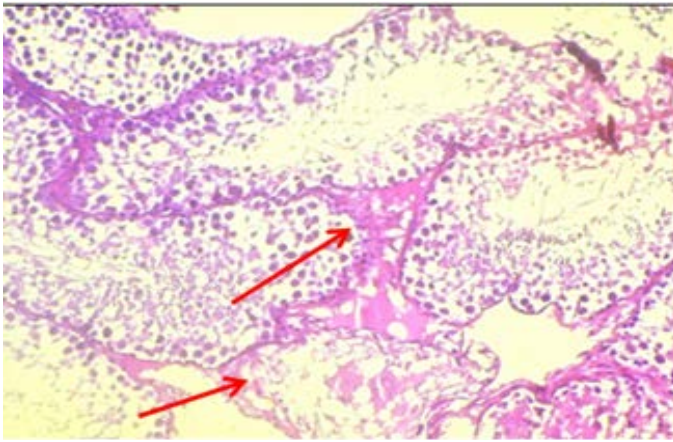


Figure 8: Histopathological section for rat testes of group 3, was observed extension of interstitial space, cellular debris and derangement of spermatogonia anywhere in seminiferous tubule lumen.

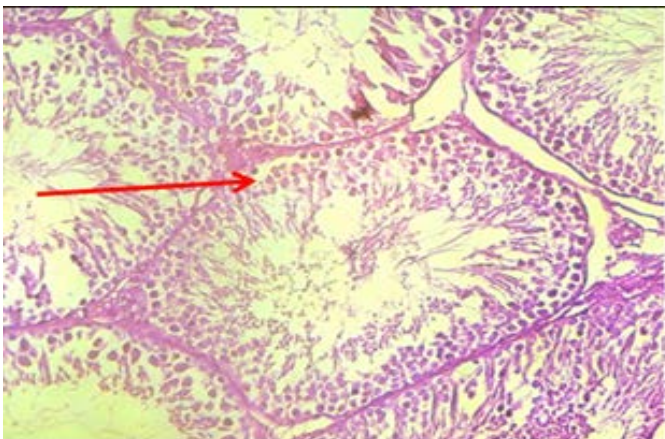


Figure 9: Histopathological section for rat testes of group 4, no clear lesions was observed.

DISCUSSION

Nanoparticles supplied versatile means to diminish the toxicity, improved bioactivity and enhance cells targeting. The toxicity and bioavailability or pro-oxidant and antioxidant impact of selenium rely on chemical form of it (Bhattacharjee et al., 2019). Selenium Nanoparticles (SeNPs) at low concentrations might not cause any toxic impact on spermatozoa (Seyedi et al., 2021). The current study observed damage in sperms DNA. Bhattacharjee and his colleagues record that all animals were remedied with selenium with different forms caused raised the activity of liver and kidney enzymes and considerably in form of Nano-Se. In addition Nano-Se caused bone marrow cell death and DNA damage but less than other forms (Bhattacharjee et

al., 2019), this is consistent with our results. In addition to the alteration in the architecture and congestion of the tissue, and inflammatory cells were observed, furthermore presence of degeneration in the germ and Leydig cells, and absence of spermatogenesis. This result is researchers agreed upon and buttressed with the well-known truth that the surface charge sign of nanoparticles and value have impact on their capacity on permeate within the cell (Shao et al., 2015). The surface charge of nanoparticles is negative, so that occur their mutual repulsion when it interact with membrane of cell. Nevertheless, *in vivo* the nanoparticles negatively charged in the body circulating, gradually tie up to plasma proteins as in consequence opsonization (Wani et al., 2020). The negative surface charge of nanoparticles had partially neutralizes by this interaction thus permitting to nanoparticles to inside the cell due to variant kinds of endocytosis such as clathrin-mediated and macropinocytosis (Zhao and Stenzel, 2018). The SeNPs opsonization and it penetration within the cell, that bring to direct sharing it in metabolism were fragmented through time, so that the biological impacts of SeNPs manifested gradually (Lesnichaya et al., 2021). The impact of a high SeNPs dose in this case by participated in metabolism during it interacted with Glutathione (GSH), that is escorted by Reactive Oxygen Species (ROS) production, that caused augments the Malondialdehyde (MDA) concentration content, that probably because a begin of Lipid peroxidation (LPO) operation within blood of animals, that caused extra tissue damage, which indicate the selenium nanoparticles at high dose bring to urinary system damage for animals (Lesnichaya et al., 2021). In other side, reduction the levels of FSH and LH hormones in group that ingestion BTSeNPs by comparing the remnant groups, resulting that lowering in testosterone and inhibin b, also been noted this reduction in above hormones getting worse with time progresses of experimental. This is what the researcher documented where testosterone has influential for proper evolution, maintenance on function and growth of genitourinary organs of animals (Gofur, 2019). Gonadotropins regulated through the axis hypothalamus-pituitary-gonad could stimulate testosterone excretion: LH motivate the Leydig cells and boosts testosterone synthesis by a chain of steroid synthases (Marques et al., 2018). The excess equipped with selenium nanoparticles is very probable caused damage of living system by the prolonged act for κCG-stabilized SeNPs that result cell damage, via the raised production of free radicals, are significant factors leading to injuries and disease (Lesnichaya et al., 2021). Where in group G3 has been observed alteration in levels of FSH and LH hormones comparing in group G2, in addition have raised in value of testosterone and inhibin b., the researchers reached a conclusion similar to what we have reached where Jia and his colleagues had demonstrated the nutrient supplement of L-Arg caused

notably elevated for serum LH and testosterone concentrations value, proposing that L-Arg induces secretion the LH from hypothalamus, so that alleviating the reduction in value concentrations of testosterone due to heat oxidative stress. Significantly elevation in Catalase (CAT) and Total superoxide dismutase (T-SOD) activity observed in animals ingested the L-Arg-supplemented thereby it has enhanced antioxidant situation in testis of animals (Jia et al., 2020). As for the fragmentation of sperm DNA in this group elevated the percentage integrity sperm DNA, in addition present alteration of histopathological section, was observed extension of interstitial space, cellular debris and derangement of spermatogonia the arginine ingested as dietary supplementation bring to significantly rising testicular weight, the altitude of the seminiferous epithelium and the spermatogonia number, this is consistent with our results. The value of follicle stimulating hormone (FSH) and luteinizing hormone (LH) hormone in serum have increased and this is confirmed by Lin and his colleagues, where have illustrated the molecular mechanisms for arginine for evolved of male testicular performance (Lin et al., 2020). As for in group G4 that ingestion BTSe and propolis show the propolis was enhancer factor for adverse effect of BTSe where the levels of FSH and LH hormones are elevated than remnant groups, as well as increased in concentration of testosterone and inhibin b. The researchers reached a conclusion similar to what we have reached in diabetic animals propolis enhances fertility potential through targeting steroidogenesis, mating behavior, testicular lactate metabolism and spermatogenesis. Nna and colleagues documented the Malaysian propolis have a promising supporting impacts with metformin in animals with diabetes mellitus induced improves subfertility/infertility (Nna et al., 2020). The fragmentation of sperm DNA in this group elevated the percentage integrity sperm DNA comparing with remnant groups. Propolis ingestion have improves the oxidative status for animals by lessening oxidation state, improves semen quality, maintaining cell energy and DNA damage. The propolis has anti-inflammatory impact of fundamentally mediated by hindering the passageways contributory in nuclear factor kappa B (NF- κ B), extracellular signal regulated kinases (ERK), Adenosine monophosphate-activated protein kinase (MAPK), Inhibitor kappa B-alpha (I κ B α), and c-Jun N-terminal kinase (JNK) activation (Zhang et al., 2014; Jung et al., 2014). Furthermore it, the current experience revealed rapprochement in architecture of testes for male group 1 and group 4 as observed in (Figure 9), this is what the researcher agreed upon. Effect of propolis has protective for testes against oxidative stress regarded to his the antioxidant impact to restoring architecture of testes and reproductive hormones levels. Many studies are documented that apoptosis of testicular germ cell incrementing in stressed animals, which happen as outcome to the in-

terplay among inflammation and oxidative stress. Where observed overexpressed of pro-apoptotic p53, caspase-9, caspase-8 and Bax/Bcl-2 ratio, are manifested to the participations each of the extrinsic and intrinsic signaling of apoptotic, both of them elicit to caspase-3 activation (Zhao et al., 2011; Maremanda et al., 2016; Zhao et al., 2017). Exciting for attention, the remediation with Propolis eliciting to lessening the testicular tissue apoptosis, as observed with the lessening in levels caspase-3 protein and its mRNA. in addition lessening apoptosis of testicular germ cell following to chemotherapy exposure (Sönmez et al., 2016). Protective effect had observed for Propolis, where It successfully lessen oxidative stress state and ameliorate antioxidants, destroyed tissues were substituted for had been healthy tissues, reduced TNF α , and rectification hormone levels (Nna et al., 2019).

CONCLUSIONS AND RECOMMENDATION

The current experience revealed the Selenium nanoparticles (SeNPs) have oxidative stress reproductive system at high concentration, as well as the propolis and L-Arginine and has prophylactic effects on testes against oxidative stress regarded to his the antioxidant impact to restoring architecture of testes and reproductive hormones levels. In this current study, we recommend that people who are exposed to high concentrations of selenium nanoparticles should take antioxidants such as propolis and L-Arginine to contribute to preventive the negative effects of Selenium nanoparticles at high concentrations, for it has an improved role on the oxidation state inside the animal's body.

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NOVELTY STATEMENT

This experiment has focused attention on preventive effect of using antioxidants like propolis as daily basis utility in supporting the oxidative status in blood and enhancer factor for adverse effect of high concentration BTSe on FSH, LH, testosterone and inhibin b hormones and the quality and integrity of the sperm.

All authors equally performed all the lab work as well animal handling and treatments. All Authors designed the entire work and worked simultaneously to collect data and statistically analyze it.

DATA AVAILABILITY

Authors will provide all data at the reasonable request. Conflict of interest The authors have declared no conflict of interest.

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