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Essential Oil from *Nigella Sativa* Seed Differentially Ameliorates Steroid Genesis, Cellular ATP and Prostate Functions in Anti-Psychotic Drug-Induced Testicular Damage of Rats

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Abstract

Haloperidol is basically employed in combating mental disorder but its usage is controlled because of its adverse effects in other tissues. This study investigates the differential effects of phenolic compounds from black seed oil on key markers linked to testicular dysfunctions induced by Haloperidol in rat model. The animals were divided into six groups (n=10). Group I was given distilled water while Group II received 15 mg/kg body weight of haloperidol *via* oral route. Group III, IV and V were pre, co and post-treated with the oil, respectively at therapeutic dose of 150 mg/kg body weight. Lastly, Group VI was treated with black seed oil at dose of 150 mg/kg body weight; for 14 days. The results revealed an increase in 5^{l}-nucleotidase and PACP activities with concomitant decrease of Δ^5-3β-HSD, Δ^5-17β-HSD and LDH activities in rats induced with haloperidol, followed by systemic oxidative damage and adverse histopathological changes in germinal epithelial cells. Co-treatment is most efficacious in preventing haloperidol-induced testicular damage in rat; followed by post and pre-treatment, respectively.

Keywords: Haloperidol; Steroidogenicity; ATP; Mixed-phenolics; Prostate cancer; Differential treatment

Introduction

The anti-psychotic drugs are commonly used in psychiatry, obstetrics, and anesthesiology [1,2]. Prior studies had also shown that neuroleptic drugs decreased the permeability of biological membranes for various inorganic and organic molecules [3,4]. They act as potent membrane permeability blockers and dopamine antagonist [5]. Thus, HAL binds to the dopamine D2 receptors for its action to be exerted in the brain [6,7], NADPH dependent and implicated in male reproductive damage [8].

Black seeds had been extensively studied, both phytochemically and pharmacologically. Prior phytochemical examination revealed amino acids, proteins, carbohydrates, quinones, unsaturated fatty acids, traces of alkaloids and terpenoids, fixed and volatile oils [9] while thymoquinone (TQ) was the main active constituent of the volatile oil [10]. The seeds have been used by patients to suppress coughs [11], slow down the carcinogenic process [12], anti-inflammatory [13] and antioxidant effects [14]. In addition, the crude oil prepared from the seeds produce a variety of pharmacological actions [15,16]. For this reason, the biosynthetic enzymes linked to key indicators of targeting infertility, testicular disorders, prostate cancer and high testosterone level which connote normal spermatogenesis [17-19] were examined in this study. Although, few studies had examined the pharmacological actions of black seed oil on male reproductive functioning [20-22] but the differential treatment (pre, co and post) of black seed oil against anti-psychotic induced damage drugs was scant. Besides, the specific underlying biochemical mechanisms by which its actions are elicited in targeting male reproductive challenges are poorly elucidated [23,24]. Thus, the aim of this study was to evaluate the differential defensive effect of black seed (*Nigella sativa*) oil in haloperidol-induced testicular dysfunctions using rat model.

Introduction

Chemicals and reagents

Haloperidol, substrates AMP, Deoxyribose, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), di-hydroxylepiandrosterone (DHEA), testosterone, nicotinamide adenosine dinucleotide (NAD^+), Bovine Serum Albumin (BSA), GSH, hydrogen peroxide, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). All the kits used for the bioassay were sourced from Randox Laboratories Ltd. (Crumlin, Dublin, Northern Ireland, UK). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Sample collection and characterization

The fresh seeds of black seeds (*Nigella sativa*) were obtained from Al-Medinat ventures, Kwara State, Ilorin, Nigeria. Authentication of the plants was carried out at the Department of Botany, University of Ilorin, Nigeria. The seeds were sorted out to remove all the possible stones and dirty materials and grounded into powder to enhance the efficiency of extraction of the active component(s). Quantification of phenolic compounds by HPLC-DAD reverse-phase chromatographic analyses was carried out under gradient conditions (as shown in Table 1).

Extraction of the essential oil

Pulverized ten (10 g) grams of the black seed was extracted by steeping in 100 mL of methanol overnight, for 24 h at 25°C. Thereafter, the mixture was filtered through Whatman No.1 filter paper. The filtrate, subsequently known as oil was concentrated and stored at −4°C for further analysis. About 5 ml of the oil was obtained after methanol removal.

Animal handling

Adult male Wistar rats (150-200 g) from the Animal House of the University of Ibadan, Nigeria were used in this experiment. They were housed in the metallic cage at the central Animal House of Kwara State University, Malete. The animals were maintained at a constant temperature (30-32°C) on a 12 h light/dark cycle with free access to food and water. The animals were used according to the institutional guidelines of Nigeria Academy and are in accordance with international guidelines.

Experimental protocol

The rats were acclimatized for two weeks and randomly divided into six groups of ten animals each (n=10). Group 1 (Control) was given distilled water only *via* oral route; Group 2 (Induced) was given therapeutic dose (15 mg/kg body weight) of Haloperidol of for 7 days. 15 mg/kg body weight is the conventional therapeutic dose for patients; Group 3 (Pre-treatment) was given 150 mg/kg body weight of black seed oil (BSO before) for 7 days plus therapeutic dose (15 mg/kg body weight) of Haloperidol (HAL after) for 7 days; Group 4 (Co-treatment) was given therapeutic dose (15 mg/kg body weight) of Haloperidol (HAL) plus 150 mg/kg body weight of black seed oil (BSO) for 7 days. Group 5 (Post-treatment) was given therapeutic dose (15 mg/kg body weight) of Haloperidol for 7 days (HAL before) plus 150 mg/kg body weight of black seed oil for 7 days (BSO after); Group 6 (Oil only) was given 150 mg/kg body weight of black seed oil (BSO only) for 7 days. The rats were fed with the same standard food and had free access to drinking water throughout the entire experiment. The duration for the experiment was two weeks (14 days). The rats were fed with the same standard food and had free access to drinking water throughout the entire experiment. These rats were euthanized 24 h after the last treatment session. The dose levels, durations of administration, and study endpoints were selected in accordance with conventional therapeutic dose, sub-acute exposure and bio-markers of reproductive dysfunctions, respectively. Also, previous studies had adopted this procedure [25].

Testicular homogenate preparation

After the treatment, the animals were submitted to euthanasia being previously anesthetized with ethyl acetate and testes were removed for testicular homogenate preparation. The right testes were homogenized in 4 volumes of an ice cold medium, consisting of 1.15% potassium chloride and 50 mM Tris-HCl buffer with a pH 7.4 in a motor driven Teflon-glass homogenizer at 10,000 rpm. The supernatants were isolated at 4°C and used for biochemistry and enzymatic assays.

Biochemical enzymatic antioxidants assay

The testicular supernatant collected was used for the estimation of CAT activity using hydrogen peroxide as substrate according to the method of Clairborne [26]. SOD activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30 ± 1°C according to Misra and Fridovich [27]. Protein concentration was determined by the method of Lowry, et al. [28]. GST was assayed using 1-chloro-2, 4-dinitrobenzene as the substrate according to the method of Habit [29] and expressed as mole CDNB-GSH complex formed/min/mg protein.

GSH assay

Reduced GSH was determined at 412 nm using the method described by Jollow et al. [30].

Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa et al. [31] and expressed as µmoles/mg protein.

Lactate dehydrogenase (LDH) assay

The liver homogenate was assayed for lactate dehydrogenase (LDH) activity using commercially available kit (Randox Laboratories UK). Assay was carried out according to the manufacturer's instructions [32].

5^{I}-Nucleotidase assay

The 5^{I}-nucleotidase activity was determined essentially by the method of Heymann et al. [33] in a reaction medium containing 100 µl of 10 mM $MgCI_2$ and 100 µl of 5 mM Tris–KCl buffer, pH 7.6 to final volume of 500 µl. The reaction was initiated by the addition of 150 µl 10 mM AMP. 150 µl of the homogenate was added to the reaction mixture and incubated at 37°C for 20 min. In all cases, reaction was stopped by the addition of 500 µl of 10% trichloroacetic acid (TCA) and the protein precipitated was removed by centrifugation for 10 min. 500 µl of the supernatant was added to 500 µl of 1.6% ammonium molybdate, then 200 µl of sodium acetate and 800 µl of 10% ferrous sulphate. The released inorganic phosphate (Pi) was assayed at 700 nm using colorimetric reagent and KH_2PO_4 as standard. Controls were carried out by adding brain homogenate fraction after TCA addition to correct for non-enzymatic nucleotide hydrolysis. Enzyme activities are reported as mmol Pi released/min/mg of protein.

Assay of prostatic acid phosphatase (PACP)

For a quantification determination of the PACP activity, the plasma supernatant was used. The acid phosphatase activity was measured in an acetate buffer at pH 4.5 using PNPP as a substrate [34]. Plasma PACP was measured using the same buffer, with the addition of a 0.1-ml plasma substrate [35].

Assay of testicular Δ^5-3β-HSD activity

To study the testicular Δ^5-3β-HSD activity, the left testes from each animal of each group was homogenized separately, maintaining chilling conditions (4°C) in 20% spectroscopic-grade glycerol containing 5 mM of potassium phosphate and 1 mM of ethylene di-amine tetra-acetic acid (EDTA) at a tissue concentration of 100 mg/ml homogenizing mixture in a homogenizer (Remi RQ-127A; REMI Laboratory Instruments, Mumbai, India). This mixture was centrifuged at 10,000 g for 30 min at 4°C in a cold centrifuge (Avanti™ 30; Beckman, USA). Δ^5-3β-HSD was measured by mixing 250 µl of the

supernatant with 250 µl of 100 µM sodium pyrophosphate buffer, pH 8.9, 10 µl ethanol containing 30 µg of dehydroepiandrosterone (Sigma, St. Louis, MO, USA) and 240 µl of 25 mg % bovine serum albumin (BSA) (Bangalore Genei, Bangalore, India). Δ5-3β-HSD activity was measured after the addition of 50 µl of 0.5 µM nicotinamide adenine dinucleotide (NAD^+) to the tissue supernatant mixture in a spectrophotometer (U-2001; Hitachi, Japan) at 340 nm against a blank (without NAD^+) [35]. One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

Assay of testicular Δ^5-17β-HSD activity

To study the testicular Δ^5-17β-HSD activity, the left testes from each of animal of each group was homogenized separately, maintaining chilling conditions (4°C) in 20% spectroscopic-grade glycerol containing 5 mM of potassium phosphate and 1 mM of EDTA at a tissue concentration of 100 mg/mL homogenizing mixture in a homogenizer (Remi RQ-127A, REMI Laboratory Instruments). This mixture was centrifuged at 10,000 g for 30 min at 4°C in a cold centrifuge (Avanti™ 30; Beckman). Δ5-17β-HSD was measured by mixing 250 µl of the supernatant with 250 µl of 440 µM sodium pyrophosphate buffers, pH 10.2,10 µl ethanol containing 0.3 µM testosterone (Sigma) and 240 µl of 25 mg% BSA (Bangalore Genei). Δ^5-17β-HSD activity was measured after the addition of 50 µl of 0.5 µM NAD^+ to the tissue supernatant mixture in a spectrophotometer (U-2001; Hitachi) at 340 nm against a blank (without NAD) [36]. One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

Histopathological examination

The testes were fixed in Bouin's fluid for 24 h, before they were cut longitudinally into 2 equal halves and again post-fixed in fresh Bouin's fluid for next 24 h. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene. Infiltrated and embedded in paraffin wax, the tissue blocks were made, cut into 5 mm thick sections using rotatory microtome. The sections were mounted on albumenized glass slides and stained with eosin and hematoxylin. Morphological study of testes was done with the help of ocular micrometer scale under light microscope.

Statistical analysis

The results of the replicates were pooled and expressed as mean ± standard deviation. A one way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was used for the post hoc [37]. Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis and the least significance difference (LSD) was accepted at P<0.05.

Results and Discussion

Previous phytochemical analysis have discovered several active components in the black seed which include amino acids, proteins, carbohydrates, quinones, unsaturated fatty acids, traces of alkaloids and terpenoids and fixed and volatile oils. Essentially, thymoquinone (TQ) and dithymoquinone (DTQ) were investigated as the main active constituents of the black seed volatile oil [38-40]. Additionally, former analysis of phenolic compounds by HPLC-DAD Reverse phase chromatographic analyses from our laboratory was carried out under gradient conditions [38,39]. The result indicated the presence of gallic acid, catechin, chlorogenic, caffeic acids, orientin, quercetin, quercitrin, rutin and luteolin [38].

Figure 1 shows the levels of MDA, a maker of lipid peroxidation–*in vitro*. MDA was significantly (P<0.05) decreased in rat testes treated with black seed oil (BSO) *ex-vivo* in a dose-dependent manner. Similarly, MDA content as shown in Figure 2 was increased in animals sub-acutely treated with HAL *in vivo* by 68% when compared with the control. The increased MDA levels were markedly (P<0.05) prevented by pre and co-treated with black seed oil by 56% and 65%, respectively while, post-treated group significantly (p<0.05) reversed MDA level by 89%. Apparently, it was demonstrated from the present study that sub-acute administration of HAL in male rats induced testicular and/or reproductive alterations. As observed, there was a significant rise in MDA content after treatment with HAL *via* oral route. The increase was attributed to its interaction (halogen moieties) with cellular membrane thereby impairing testicular cell membrane. This result is in agreement with previously described studies where HAL was able to injure cell membrane and decreased sexual ability in males [41]. This outcome occurs due to the capability of HAL to facilitate the production of hypochlorous acid (HOCl) or hypoflorous acid (HOF) [42,43]. Alternatively, its toxic effects could be connected to the fact that the CYP 3A metabolized HAL into its pyridinium metabolite (HPP^+) *via* the specific isozymes CYP 450 3A4 [42,43]. However, combined therapies of pre, co and post administration with black seed oil caused a significant reduction of MDA content in HAL-induced rats. This agrees the aforementioned studies that black seed oil could initiated low serum MDA level in male rats [42,43]. The observed fall is in line with the conventional use of black seeds as a hyper-spermatogenic and reproductive mediator [42,43]. Nevertheless, the synergy of the phenolic compounds present in the black seed oil was responsible for the reduction of malondialdehyde level. This was corroborated by recent studies that poly-unsaturated fatty acids (PUFAs) and well known phenolic compounds such as gallic acid, chlorogenic acid and caffeic acid exhibited hyper-spermatogenic potentials in rats [44,45]. However, in a medical perception, treatment with oil from *Nigella sativa* should have only been applied after HAL administration, but also our approach gives additional information on all the possible treatments (pre, co and post) of the black seed oil.

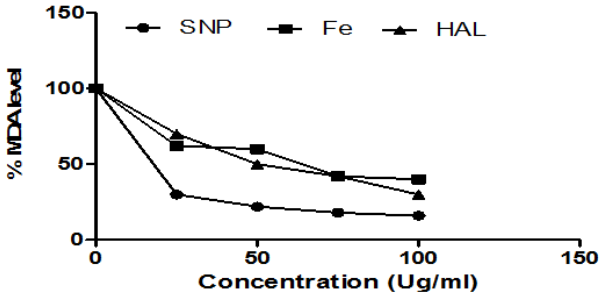

Figure 1: Lipid peroxidation in vitro; Oil from Black seed (OBS) inhibits sulphur nitroprusside (SNP), Iron sulphate ($FeS0_4$) and haloperidol (HAL). These pro-oxidants induced lipid peroxidation dose-dependently in rat testes. The percentage inhibition of MDA (Malondialdehyde) production was expressed in 100%. Significance was accepted at P <0.05.

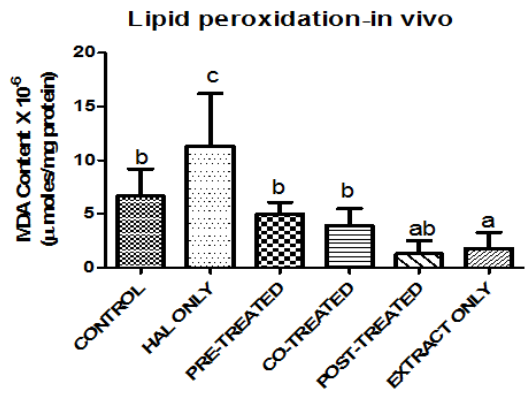

Figure 2: Malondialdehyde (MDA) level from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

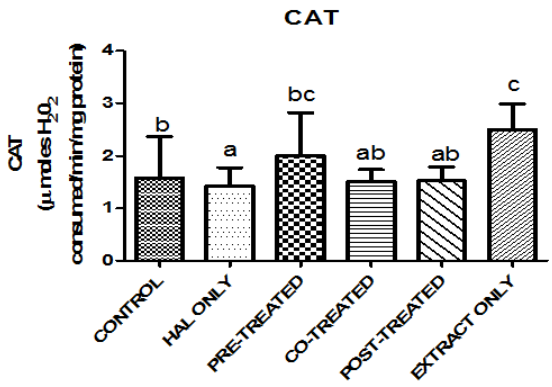

Figure 3: Catalase (CAT) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

Several endogenous metabolites have the capacity to cause an increase in the concentration of electrophiles which are radicals [46]. They inherently react with oxygen to produce ROS. ROS are essentially formed in metabolic processes of living cells particularly when they interact with xenobiotic and immunosuppressive agents [46]. In this study, Figures 3-6 show the antioxidant levels and markers of oxidative stress. Figure 3 shows the activity of CAT in the post-mitochondrial fraction of rat testes. CAT activity was significantly ($P<0.05$) decreased by 10% in the animals treated with HAL when compared with the corresponding control animals. The decrease was prevented by 41% and 6%, respectively on pre and co-administration. Similarly, as shown in Figure 4, the activity of SOD was significantly ($P<0.05$) decreased by 54% in the animals administered with HAL when compared with the control group. Conversely, the decreased was prevented by 88% and 36%, respectively on pre and co-administration. However, the post-treated and BSO groups were upturned by 99% and 35%, respectively when compared with the control group. Also, Figure 5 shows sub-acute treatment with HAL caused a significant ($P<0.05$) decrease in testicular GSH, antioxidant protein by 49% in relation with the corresponding control animals. Pre, co and post treatment significantly ($P<0.05$) elevated the GSH level by 140%, 59% and 139%, respectively. Furthermore, Figure 6 shows significantly ($P<0.05$) depleted the activity of GST by 71% when compared with the control group. Animals that were pre, co and post treated resulted in significant ($p<0.05$) increase of GST activities by 24%, 275% and 233%, respectively. As observed in this study, antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) were depleted in the testes resulting into diminished of reduced glutathione (GSH) level following administration with anti-psychotic drug. Reduced alteration of HAL on both enzymatic and non-enzymatic antioxidants was well-suited with the results of previous studies [47,48]. While, protective effects of black seed oil on pre, co and post treated group was due to free radical scavenging ability [49] of the oil. Hence, these results established that oil from black seed can decrease the testicular toxicity *via* checking oxidative stress by eliciting pharmacological action as potent anti-oxidation in the testes.

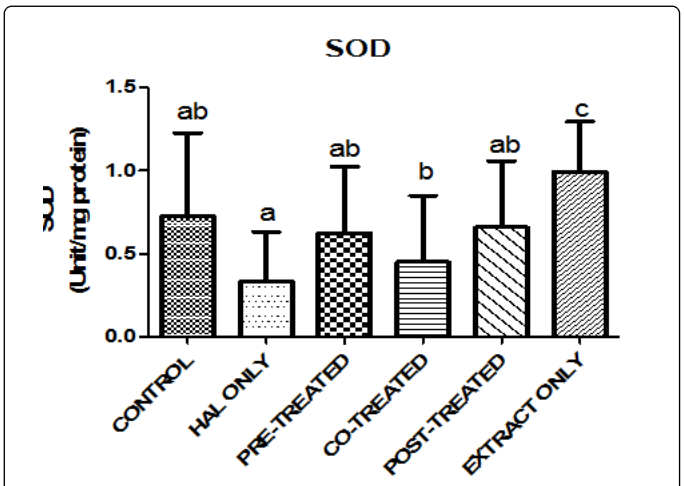

Figure 4: Superoxide dismutase (SOD) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

Deficiency of lactate dehydrogenase is a condition that affects how the testicular cells break down glucose for optimal use of energy in spermatogenic cells. Figure 7 shows that HAL-treated group depleted the activity of lactate dehydrogenase (LDH), key marker linked to the production of adenosine triphosphate (ATP) in the testes by 27%, when compared with the corresponding control group. The depleted activity of LDH was increased on pre, co and post-treatment by 87%, 55% and 18%, respectively. The present investigation showed low activity of LDH after therapeutic use of HAL in rat. This suggests that HAL can cause diminution of ATP in reproductive male cells if repeatedly used for longer periods (sub-chronic or chronic exposure). The observation was similar to previous studies which reported that continuous exposure to rhabdomyolysis showed low cellular ATP and experienced testicular fatigue [50] erectile dysfunctions [51], immature sperms and induction of sexual dysfunctions in patients during long term treatment [52]. Also, reduced activity of lactate dehydrogenase

following HAL administration was linked to the genetic mutation of the *LDHA* gene [53].

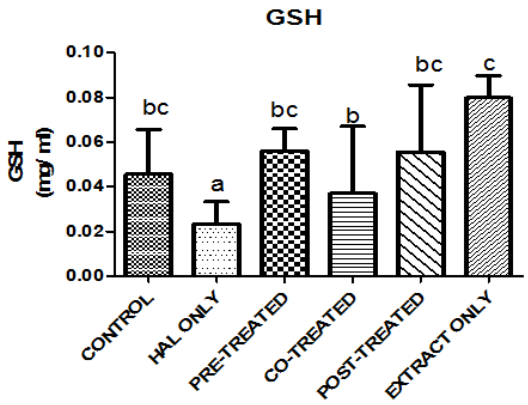

Figure 5: Reduced glutathione (GSH) level from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

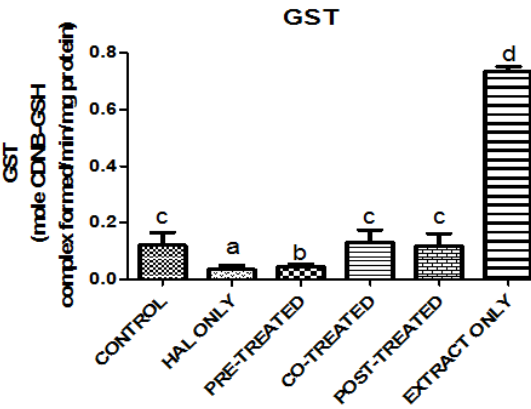

Figure 6: Glutathione-S-transferase (GST) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

This was in conformity with the previous discoveries that experimental animal model with low levels of ATP manifested sub-chronic reproductive problems with altered expression of LDHA protein followed by complicated erectile dysfunctions [53]. The differential treatment with black seed oil could be attributed to the high content of essential fatty acids in the oil. It is also because lipids are fundamentally known as key antecedent of spermatogenesis [54,55]. Additionally, the reduction on the ATP levels can impair processes like astenospermia (sperm motility) blocking conception in female subjects [56]. Also, as shown in Figure 8, 5^{I} nucloetidase, relevant marker of AMP and ATP hydrolysis was significantly (P<0.05) increased in HAL-treated group by 28% when compared with the control. Pre, co and post-treatment caused a significant (P<0.05) decrease by 48%, 50%, and 34%, respectively. The group treated with BSO only significantly (P<0.05) down-turned the activity of 5^{I} nucloetidase by 68% in ATP hydrolysis when compared with the control group. The activity of this enzyme is very essential because it is the regulator of testicular extracellular adenosine [57] and plays important biochemical roles, such as energy transfer [58]. Thus, the effect of administration of HAL on this enzyme leads to an increased removal of extracellular adenosine decreasing its (ATP) levels, which leads to testicular dysfunctions. These observations corroborated the recent findings which reported that the lowering of 5^{I}-nucleotidase (5NT) activity in the testes reduced flux of spermatogenic cells, resulting into loss of libido [58]. This is an indication of abnormal spermatogenesis [59,60]. Interestingly, pre, co and post treatment with the black seed oil prevented the alterations, thus, decreasing extracellular levels of adenosine during formation of germinal cells. This discovery was in line with recent inventions that the occurrence of *erectile dysfunction* and impotence among *patients* where cellular ATP *levels* were *low* was ameliorated by natural antioxidant drugs [61,62].

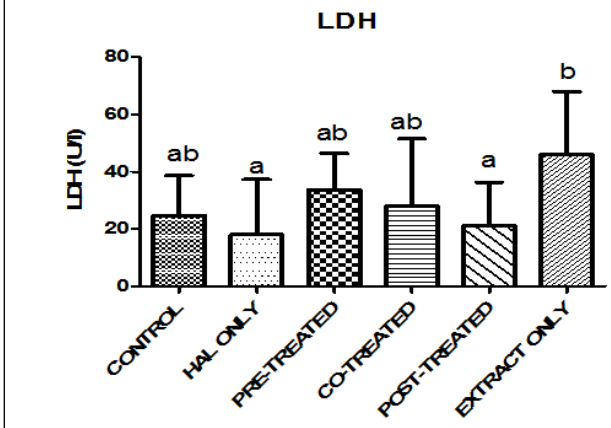

Figure 7: Lactate dehydrogenase (LDH) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

Prostatic acid phosphatase (PACP), also regarded as prostatic specific acid phosphatase (PSAP). It is an enzyme produced preponderantly in the prostate, responsible for its wellness and functionality. Its inhibition is the pharmacological basis for prostate cancer's [63]. Prostate cancer could lead to abnormal testicular functioning. Also, PACP inhibition is the most aimed target for anti-tumorigenic developed as an alternative to drugs [64]. Figure 9 shows the activity of prostatic acid phosphatase (PACP), marker of prostate cancer. PACP was significantly (P<0.05) increased by 79% when compared with the control group after sub-acute treatment with HAL. Pre, co and post-treatment caused a significant (P<0.05) decrease by 20%, 25% and 30%, respectively. As observed, oral administration of HAL resulted in the activation of prostatic oncogenic-system *via* increased PACP activity. This observation supports the previous study that men suffering from prostate cancer had increased amounts of PACP or PSAP [64].

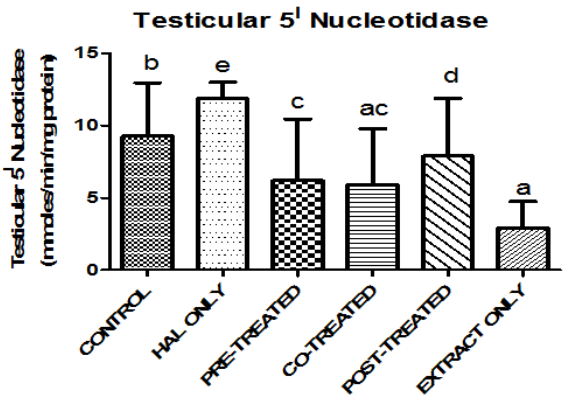

Figure 8: 5^{I}-nucleotidase activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically different significant.

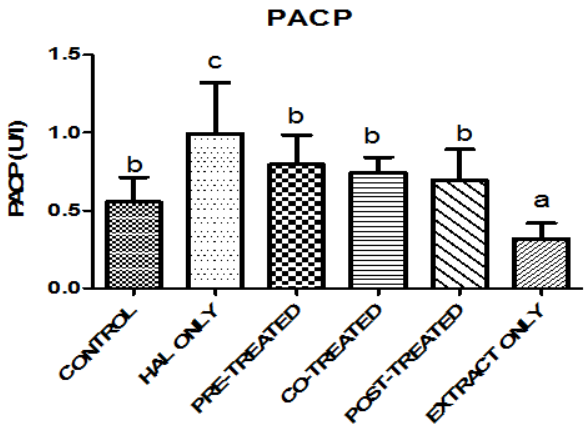

Figure 9: Prostatic acid phosphatase (PACP) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

More so, the highest levels of prostatic acid phosphatase had been found in metastasized prostate cancer [65]. Essentially, our results revealed that pre, co and post treatment with black seed oil caused a significant reduction in plasma PACP activity. The decrease in PACP activity was as a result of synergistic action of the phenolic compounds such as chlorogenic acid, orientin, rutin quercetin luteolin present in the oil [38]. Previous reports suggested that these phenolic compounds such as orientin, rutin and luteolin were accounted to inhibit PACP activity either as a single compound or in synergy with other compounds [65]. This approach is linked to various practices of traditional medicine where mixture of plant constituents was commonly approved for the treatment/management of several reproductive disorders [65].

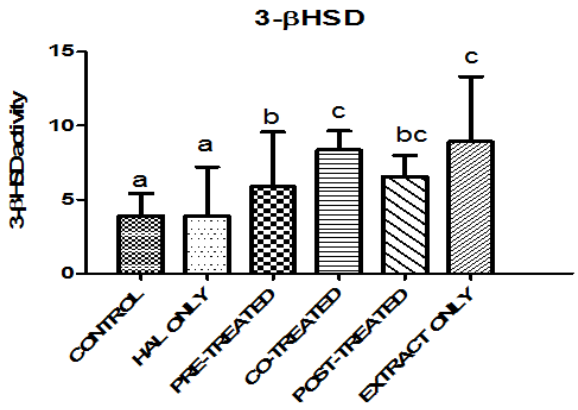

Figure 10: Δ^5-3β-hydroxy steroid dehydrogenase (Δ^5-3β-HSD) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

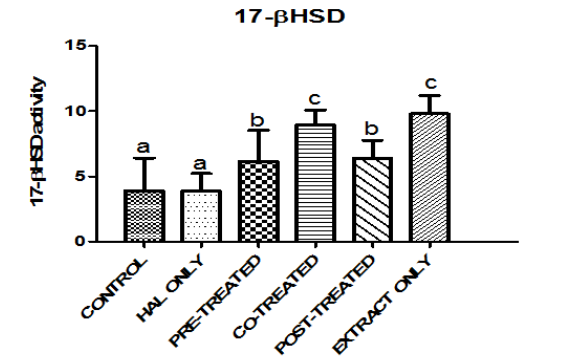

Figure 11: Δ^5-17β-hydroxy steroid dehydrogenase (Δ^5-17β-HSD) activity from germinal epithelial cells in haloperidol-induced testicular damage rats treated with oil from black seeds. Data are presented as mean ± SD (n=10). Bars with different letters are statistically significant.

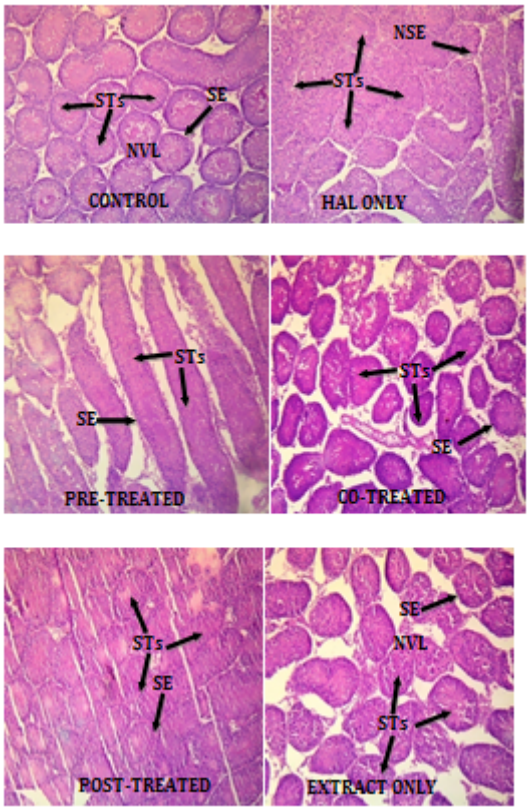

Figure 12: Microscopic findings of eosin and hematoxylin 5 µm thick stained section of rat testes administered with black seed oil in HAL-induced damage (x400): (NVL=No Visible Lesions; STs=Seminiferous Tubules, SE=Spermatogenic Epithelium, NSE=Necrosis of Spermatogenic Epithelium).

The quantitative ability of steroidogenic cells to biosynthesize the specific steroids is rate-determining step on the levels of steroidogenic enzymes [66]. The level of these enzymes was associated with their specific gene expression [67]. Figures 10 and 11 respectively show the activities of Δ^5-3β-HSD and Δ^5-17β-HSD, markers linked to testosterone production. The activities of Δ^5-3β-HSD and Δ^5-17β-HSD were inhibited when compared with the control group after sub-acute administration with HAL. The low activities of Δ^5-3β-HSD and Δ^5-17β-HSD were upturned on pre, co and post-treatment. The non-significant decrease in the activities of steroidogenic enzymes-Δ5 3β-hydroxy steroid dehydrogenase (Δ^5-3β-HSD) and Δ5-17β-hydroxy steroid dehydrogenase (Δ^5-17β-HSD) in rats treated with HAL may be linked to the sub-acute exposure of the anti-psychotic drug or perhaps the drug is not systemic to steroidogenic metabolism. This observation predicts mechanism by which HAL induces its anti-spermatogenic effects in rat peradventure its exposure period is elongated and they are used as prime enzymes in testicular androgenesis [68]. This was corroborated by recent study that elevated androgenic enzymes are major markers for testosterone production and hyper-spermatogenesis [69]. However, Pre, co and post-treatments with black seed oil prevented the decrease of steroidogenic enzymes activities in rats induced. Previous studies reported down-regulations on both Δ^5-3β-HSD and Δ^5-17β-HSD activities among males anguished with pathological conditions or reproductive dysfunctions [69]. Also, the increased activity caused by the black seed oil confirms its aphrodisiac potentials in traditional medicine. The observed chemoprevention could be attributed to synergistic, additive, collective or competitive interactions of the multiple phenolic compounds. Additionally, it could be linked to the interaction of the active component of the oil-thymoquinone with HAL to produced non-toxic metabolites [70].

Furthermore, Figure 12 shows microscopic findings of eosin and hematoxylin 5 µm thick stained sections of rat testes administered with black seed oil in HAL-induced damage. As depicted from the study, animal group that were therapeutically administered with haloperidol (HAL only) demonstrated numerous variably-sized seminiferous tubules (STs) with irregular outlines/elongation. There was also necrosis and disruption of spermatogenic epithelium with the formation of multinucleated germ cells. These irregularities were associated with prevalence of erectile dysfunctions and male infertility [70,71]. This detection is line with recent development which reported that the damage to spermatogenic epithelium cells have a fundamental role in male reproductive pathogenesis [70,71]. Also, a different work reported that considerable damage to the seminiferous tubules caused men to produce abnormal sperm morphology [71]. The group of animals pre and post treated with black seed oil portrayed numerous, uniformly-sized, closely-packed STs with regular outlines and slightly reduced height of spermatogenic epithelium with no visible lesions. Co-treated group shows numerous, variably-sized STs, while some of these STs have irregular outlines; slightly reduced height of spermatogenic epithelium. The failure of co-treatment to entirely prevent the toxic effects induced by HAL suggests its toxicities *via* another mechanistic-metabolic process. Generally, pre-treatment with oil is most efficacious (score is shown in Table 1) in reversing haloperidol-induced testicular damage in rat; followed by its prevention *via* co and post-treatment respectively.

Treatment	IESTS	NSE	MGC
Control	0	0	0
Hal only	2	2	2
Pre-treatment	0	0	1
Co-treatment	1	0	0
Post-treatment	0	1	0

Extract-oil only	0	0	0
IESTS: Irregular Elongation of Seminiferous Tubules; NSE: Necrosis of Spermatogenic Epithelium; MGC: Multinucleated Germ Cells. Key to scores: 0=No pathologic effect; 1=Mild effect, 2=Severe effect.			

Table 1: Scoring of HAL-induced histopathology in testes of male rats.

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