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Original Reserach Article

Antioxidant Role of Alcoholic Extract of Cinnamon (*Cinnamomum zeylanicum*) on Hormonal, Histological and Molecular Parameters in Male Wistar Rats Treated with Etoposide

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Abstract: ***Background:*** Etoposide is a popular chemotherapeutic agent that causes a massive oxidative stress and reproductive toxicity effect in male patients. ***Objective*****:** Examined the protective properties of cinnamon alcoholic extract (*Cinnamomum zeylanicum*) to etoposide-induced testicular damage in male Wistar rats. ***Methodology*****:** Forty adult male rats were randomly separated into four groups namely control, cinnamon extract, etoposide extract, and etoposide extract combined with cinnamon extract. The parameters of the hormones studied were testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), testicular tissue histology, and molecular evidence of oxidative stress. ***Results*****:** Findings revealed that cinnamon extract had an important ameliorative effect against etoposide induced hormonal imbalances, maintained testicular histoarchitecture, mitigated oxidative stress indicators as well as augmented antioxidant enzyme functions. Such findings imply cinnamon alcoholic extract has strong antioxidant and protective effect on chemotherapy-induced reproductive toxicity, and can be used as a potential therapy to preserve male fertility during cancer therapy. ***Conclusion*****:** The alcoholic extract of cinnamon (*Cinnamomum zeylanicum*) offers great protection against the etoposide induced testicular damage in the male Wistar rats through intensive antioxidant action. These results propose cinnamon extract as a promising, safe adjuvant treatment in preserving male fertility in cases of chemotherapy.

Keywords: Cinnamomum Zeylanicum, Etoposide, FSH, LH, Wistar Rats.

INTRODUCTION

Cancer is still ranked among the top causes of death across the globe [1], and chemotherapy has been one of the main modalities of treatment [2]. Nevertheless, chemotherapeutic agents have serious side effect especially on reproductive system [3]. A topoisomerase II inhibitor, etoposide is widely applied in the treatment of different malignancies such as testicular cancer, lung cancer and lymphomas [4]. Etoposide, a semisynthetic derivative of podophyllotoxin, has cytotoxic activity through inhibition of the topoisomerase II which is necessary in the replication and repair of DNA, therefore affecting rapidly dividing cells. Cancer cells and also harmful proliferative normal tissues such as testicular germinal epithelium [5]. Reproductive toxicity caused by chemotherapy has been widely clinically documented and experimentally, which is characterized by severe nitro-oxidative stress, inflammation, and apoptosis as seen by Moradi *et al*., in BEP therapy with high levels of MDA and nitric oxide and low levels of antioxidant enzymes [1]. Molecular mechanisms encompass mitochondria-derived ROS which cause cellular dysfunction at doses below lethal and alterations in a variety of signaling pathways including metastasis-associated protein 1, hormonal control, cell assignment [6].

The natural antioxidants have been considered promising agents in reversing the toxicities resulting to

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chemotherapy because they are able to counteract the free radicals, stimulate the endogenous antioxidant mechanisms, and adjust the inflammatory cascades [7], natural antioxidants in female infertility (could be applied to male), Nigella sativa oil benefits in oxidative stress and testicular histology [8], Cleistanthus collinus effects in male rats [9], and many other studies point at the potential of plant-based antioxidants in reducing reproductive damage caused by chemotherapy. Cinnamon (*Cinnamomum zeylanicum*), a part of cinnamon trees, is a compound that has centuries-old traditional medicinal application and has a variety of pharmacological applications [10]. Current scientific studies have shown that cinnamon has effective antioxidant, antimicrobial, anti-inflammatory and metabolic regulatory properties [11]. Cinnamon bioactive compounds, especially polyphenols (cinnamaldehyde, eugenol and cinnamic acid) have great free radical scavenging activities and are able to activate cellular antioxidants defense system [12]. A number of studies have reported the positive action of cinnamon extracts in a number of disease models such as diabetes, cardiovascular diseases and neuro degenerative diseases [13]. Such antioxidant properties of cinnamon are explained by the high concentration of the phenolic compounds that can directly scavenge the ROS, chelate metal ion, and prevent lipid peroxidation [14]. Also, it has been demonstrated that cinnamon extracts can increase the activities and expression of the endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [15]. This is a combination of mechanisms that lead to the cytoprotective properties of cinnamon against oxidative damage [16]. Although there is an increasing amount of literature on the antioxidant capacity of cinnamon, few studies have been done to explore its protective role in the face of reproductive toxicity caused by chemotherapy.

Cinnamomum zeylanicum has (and continues to be attracted many scientists) due to its (intrinsically) high content of bioactive compounds with numerous health-related effects, primarily related to the (significant) antioxidant and anti-inflammatory properties of these compounds [17]. These compounds appear to have diverse physiologic benefits relative to managing metabolic syndrome by (helping to better maintain) glycemic dysregulation, lipid metabolism, blood pressure and inflammation [18]. The cinnamon leaf oil contains significant levels of several important phytochemicals (cinnamaldehyde, eugenol, phenolic acids) capable of providing antioxidant, anticholinergic, and antidiabetic effects, based on advanced profiling methods used to identify these compounds [19]. While resveratrol has been studied as a potential product of cinnamon through its potential ability to protect testicular function, the resveratrol's ability to protect testicular function could be attributed to both antioxidant action (via upregulation of enzyme activity) and prevention of apoptosis; therefore, this information may provide insights into how the polyphenolic compounds found in cinnamon's bark and leaf provide similar protection against oxidative stress in reproductive tissues [20]. The current study will examine the ameliorative properties of cinnamon alcohol extract on hormonal, histological, and molecular changes of the testicular tissue in male Wistar rats induced by etoposide.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The Ceylon cinnamon (*Cinnamomum zeylanicum*) is a bark that was obtained in authenticated sources and confirmed by botanical specialists. Bark samples were cleaned thoroughly, dried at room temperature over a period of two weeks and ground into fine powder using a mechanical grinder. Ethanol was used as the solvent to carry out the alcoholic extraction in 70% ethanol. The cinnamon powder weighing one kilogram was placed in a glass container that had 5liters of 70% ethanol and allowed to stand at room temperature; the powder undergoes repeated shaking every 12 hours to ensure that the bioactive compounds have been completely extracted. Solid particles in the mixture were filtered using Whatman No. 1 filter paper. The concentrate was then dried by rotary evaporator at a low pressure at 45°C until all the ethanol was evaporated, to produce a dark brown semi-solid extract. The concentrated extract was again dried using the desiccator to extract the final dried alcoholic extract that was kept in the amber bottles at 4°C before use. The extraction value was estimated at around 18% w/w on the weight of dried powder [21].

Experimental Animals and Study Design

Adult male albino (200-250 g, 8-10 weeks) Wistar rats were maintained in the controlled conditions (22±2°C, 55±5% humidity, 12-hour light-dark cycle) with the commercial pellet diet and ad libitum water. After acclimatization, the groups (n=10 each) were assigned randomly: Group I (normal control): rats were treated with distilled water vehicle; Group II: rats were treated with cinnamon alcoholic extract (200 mg/kg/day orally) for 28 days; Group III: rats were treated with etoposide (5mg/kg/week through the intraperitoneal route); Group IV: rats were treated with etoposide and cinnamon extract with the same doses and duration. Doses were chosen by the previous literature due to effective antioxidant activity (cinnamon) and reproducible gonadotoxicity (etoposide) and daily toxicity, abnormal behavior, or death monitored during the study [22].

Sample Collection and Preparation

On the night before the treatment, twenty-four hours after the final treatment all the animals were starved with free access to water. Ketamine (90 mg/kg) and xylazine (10 mg/kg) were administered intraperitoneally to put anesthesia on the rats. The blood samples were taken by cardiac puncture in plain tubes and clotted at room temperature and 30 minutes. The

serum was centrifuged at 3000 rpm and 15 minutes at 4°C and put on -80°C until assay of hormones. After blood was taken, animals were deep anesthetized and cervically dislocated. The testes were excised immediately, the tissues that were adhering removed, and the tests weighed. Each animal was fixed in 10 % neutral buffered formalin and the right testis of the right testes was subjected to histological examination. The left testis was snap-frozen in liquid nitrogen and kept at -80 °C in biochemical tests involving oxidative stress indicators and antioxidant enzyme activities [23].

Hormonal Assays

According to the instructions of the manufacturer, commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). All the samples were to be run twice and the means calculated. Intra-assay and inter-assay coefficients of variation had values of less than 10 % in all hormonal assays. Absorbance was obtained at 450 nm using a microplate reader and the concentration of the hormone calculated using standard curves prepared with known concentrations of each hormone [24].

Histological Examination

The formalin-fixed testicular tissues were dried using graded alcohol series, cleared in xylene and embedded in paraffin wax. Thickness of tissue sections were 5 micrometers, and the rotary microtome and glass slides were used to cut the tissue into these sections. Routine histological examination of the sections was done by staining them with hematoxylin and eosin (H&E). A pathologist who reviewed the slides blinded to the treatment groups used a light microscope to observe the slides under different magnifications. Some of the histological parameters assessed were: seminiferous tubule diameter, epithelial height, germinal epithelial integrity, presence of mature spermatozoa in the lumen, interstitial cell morphology and pathological changes (vacuolization, degeneration, or necrosis). Semi-quantitative scoring was done to determine the level of histological damage with a scale of 0 to 4 (normal and severe damage). The number of seminiferous tubules in at least 100 per animal was studied and the average score was obtained [25].

Oxidative Stress Markers

A 1: 10 (w/v) homogenization of frozen testicular tissues was done in cold phosphate buffer saline (pH 7.4) using a glass homogenizer. Further the homogenate was centrifuged 20 minutes at 4°C at a rate of 10,000 rpm and the supernatant was collected to undergo biochemical tests. The concentration of proteins in tissue homogenate was measured by Bradford method in which the standard was the bovine serum albumin.

Table 1: The hormonal (serum) level in the various experiment groups

Parameter	Control Group	Cinnamon Group	Etoposide Group	Etoposide + Cinnamon Group
Testosterone (ng/mL)	6.82 ± 0.54	7.15 ± 0.61	2.34 ± 0.38	5.12 ± 0.47
LH (mIU/mL)	3.25 ± 0.32	3.18 ± 0.29	7.89 ± 0.76	4.23 ± 0.41
FSH (mIU/mL)	4.67 ± 0.41	4.52 ± 0.38	9.34 ± 0.82	5.98 ± 0.53

Lipid peroxidation was determined by the level of malondialdehyde (MDA) using thiobarbituric acid reactive substances (TBARS) method. In a nutshell 0.5 mL of tissue homogenate was combined with 2 mL of TBARS reagent which entailed thiobarbituric acid, trichloroacetic acid and hydrochloric acid. The mixture was boiled in a water bath of boiled water after which it was cooled on ice followed by centrifugation. The supernatant was also measured at 532 nm. The determination of MDA concentration was carried out using a standard curve and the result was given in nmol/mg protein. The 2,4-dinitrophenylhydrazine (DNPH) method was used to measure the protein oxidation which caused protein carbonyl content. DNPH solution was added to tissue homogenate, precipitation was done using trichloroacetic acid, ethanol-ethyl acetate mixture was used as a wash solution, and dissolution of guanidine hydrochloride was performed. At 370 nm, the absorbance was recorded and protein carbonyl level was given as nmol/mg protein. The amount of nitric oxide was estimated by measuring the levels of total nitrite and nitrate using the Griess reagent. Griess reagent was added to the samples and the absorbance was measured at 540 nm. A sodium nitrite standard curve was used to compute the concentration of nitric oxide and it was expressed using μmol/mg protein [22].

Antioxidant Enzyme Activities

The activity of Superoxide dismutase (SOD) was determined on the basis of the inhibition of Pyrogallol autoxidation. The mixture was a reaction of a tissue homogenate, pyrogallol, and Tris-HCl buffer. It was observed that the change in absorbance followed the 4-minute time frame at 420 nm. The level of enzyme which inhibited pyrogallol autoxidation by 50 % was considered to be one unit of SOD activity, and expressed in U/mg of protein. The activity of catalase (CAT) was determined by the degradation of hydrogen peroxide. The hydrogen peroxide solution was put in the tissue homogenate and the absorbance was measured at 240 nm and the value was recorded after 3 minutes. CAT activity was determined by the use of the extinction coefficient of hydrogen peroxide and is given as 2 umol H_2O_2 broken/min/mg protein. The coupled enzyme assay system was used to determine the activity of glutathione peroxidase (GPx). The reaction mixture was constituted of tissue homogenate, glutathione, glutathione reductase, NADPH and hydrogen peroxide. NADPH oxidation was

observed using 340 nm and GPx activity was reported in terms of nmol NADPH oxidized/min/mg protein. The lower level of glutathione (GSH) was estimated with the aid of Ellman reagent (5,5'-dithiobis-2- nitrobenzoic acid). The deproteinization of the tissue homogenate was performed using metaphosphoric acid, centrifuged and supernatant reacted with Ellman reagent. The resulting yellow color was taken at 412 nm and the concentration of GSH was determined using a standard curve and was estimated in µmol/mg protein [22].

RESULTS

Effects on Body and Organ Weights

All animals survived and none of them died during the period of the experiment. The control and cinnamon extract alone groups exhibited normal weight gain phenotypes that were in line with the normal rats of this age. The systemic toxicity of chemotherapy was observed by the animals in the etoposide-treated group having much lower body weight gain than the controls. This loss of weight was however inhibited in part by concurrent administration of cinnamon extract and this may indicate a protective effect on etoposide-induced cachexia. The weights of the testes were significantly lower in etoposide group than the controls which indicated testicular atrophy caused by the chemotherapeutic agent. Combined treatment with cinnamon extract was able to preserve testicular weight significantly, and testicular damage induced by etoposide was blocked. The group that was treated only with cinnamon extract exhibited no significant differences regarding body or testicular weights with the control group and this confirms the safety of the extract at the dose that was being given.

Hormonal Parameters

Etoposide treatment on the subjects showed significant changes in the reproductive endocrine axis by hormonal examination. Table 1 revealed that the serum testosterone level in the etoposide group was significantly lower and it was found to be reduced by about 65% of the level in control animals. This drastic reduction of testosterone is an indicator of poor testicular steroidogenic effects due to. Oxidative cell damage of Leydig cells. The levels of LH and FSH were found to increase in the etoposide group in a compensatory manner, which proved the disruption of the feedback mechanism of hypothalamic-pituitary-gonadal axis. The high level of gonadotropin is simply an effort to encourage testicular activity by the pituitary due to the decreased production of testosterone and the ability to generate sperms.

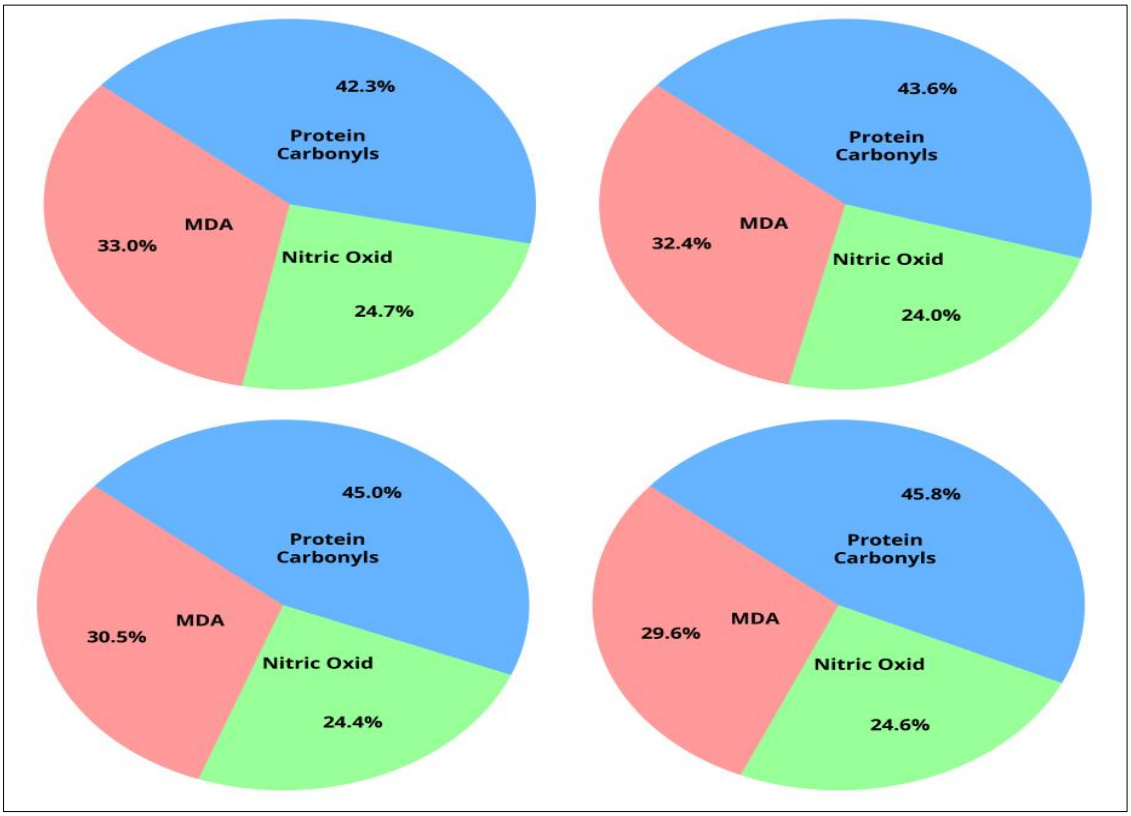

Figure 1: Oxidative stress markers distribution between treatment groups

Cinnamon extract treatment has improved these hormonal imbalances to a great extent. Etoposide plus cinnamon group had a considerable increase in testosterone levels to about 75 % of control levels which was a great improvement compared to the etoposide alone group. Equally, the co-treated group had a high level of LH and FSH than that of etoposide alone though it was still slightly high compared to controls. These results suggest that cinnamon extract is a good protective agent of testicular endocrine activity against. Etoposide-

induced damage. The group of cinnamon extract alone demonstrated similar levels of hormones to controls, and a non-significant positive change in testosterone, which indicated that the extract does not have any adverse effect on the reproductive endocrine system at the given dosage.

Histopathological Findings

In both the control and cinnamon-alone groups, microscopic analysis of testicular sections revealed normal architecture with well-organized seminiferous tubules, intact germinal epithelium (810 cell layers), full germination series of spermatogonia to mature spermatozoa, and abundant luminal spermatozoa, regular basement membrane and healthy interstitial Leydig cells which did not show any inflammation or fibrosis and thus validated the safety of cinnamon extract. Conversely, the etoposide-treated animals showed extreme testicular injury, such as disturbed tubule architecture, loss of tubule diameter and epithelial height, extensive loss of germ cell, vacationolization, Sertoli-cell-only tubules, thickened basement membrane, paucity of mature spermatozoa, interstitial oedema, inflammatory infiltration, degeneration of Leydig cell, haemorrhage, and considerably high histological damage grades.

Table 2: Oxidative stress markers testicular tissue

Parameter	Control Group	Cinnamon Group	Etoposide Group	Etoposide + Cinnamon Group
MDA (nmol/mg protein)	2.34 ± 0.28	2.12 ± 0.24	8.76 ± 0.89	4.21 ± 0.51
Protein Carbonyls (nmol/mg protein)	3.45 ± 0.37	3.28 ± 0.32	11.23 ± 1.15	5.67 ± 0.63
Nitric Oxide (µmol/mg protein)	1.87 ± 0.21	1.76 ± 0.19	6.54 ± 0.72	3.12 ± 0.38

Cinnamon extract administration together with etoposide led to impressive histological protection. In the co-treated, there was a significant testicular architecture preservation than in the etoposide alone group. Majority of the seminiferous tubules were of normal diameter and epithelial height and had structured spermatogenic layers of cells. Although a certain level of vacuolization was also observed, it was significantly lower than in etoposide group. Spermatogenic development was relatively normal and spermatogonia, spermatocytes, spermatids and mature spermatozoa were observed in the right place. The amount of spermatozoa in tubule lumens was medium spermatozoa demonstrating functional spermatogenesis. Interstitial spaces were normal and there were little inflammatory alterations and healthy Leydig cells. The histological damage score was significantly less in the group that was co-treated than etoposide but still higher than the controls, which suggests that there was considerable but incomplete protection. These results create a definitive morphological data to support the idea that cinnamon extract can prevent the structural damage of the testicular tissue caused by etoposide.

Oxidative Stress Markers

Measuring oxidative stress indicators in testicular tissues indicated severe oxidative stress generated by the treatment with etoposide. The levels of malondialdehyde as the initial indicator of lipid peroxidation as it was presented in Table 2 were significantly higher in the etoposide group with the value of 3.7 times higher than the controls. Such an enormous rise in MDA is a sign of severe oxidative damage on cellular membranes that can affect membrane integrity and functionality of cellular organelles. The content of protein carbonyl as an indication of oxidative modification of proteins was also increased in the etoposide group with a 3.3-fold increase of the carbonyl content in the etoposide group being higher than control values. Higher protein carbonyls are signs of oxidative damage to cellular proteins, which causes the enzyme activities and structural protein dysfunction. The etoposide group also exhibited a great upsurge in nitric oxide levels, with the upsurge being approximately 3.5-fold. Although nitric oxide plays significant physiological roles, over production of nitric oxide causes nitrosative stress and peroxynitrite, a strong oxidant, which causes damage to lipids, proteins, and DNA.

Combination of cinnamon extract immensely reduced oxidative stress indicators. MDA was significantly lower in the etoposide plus cinnamon group than in the etoposide alone group and this showed that the group provided significant protection against lipid peroxidation. Protein carbonyl contents were also found to be significantly decreased (almost by 49%) which indicates that it protects against protein oxidation. The concentration of nitric oxide was found to decrease about 52 % in the co-treated group than in etoposide mono-treated group, which implies that nitrosative stress is also modulated. Although these markers were still slightly higher as compared to controls, the radical decreases indicate. good antioxidant protection by cinnamon extract. The cinnamon extract alone group did not have any significant difference in oxidative stress marker levels, which were similar to or a bit lower than controls which confirms that the extract does not cause oxidative stress and may even improve basal antioxidant status.

Antioxidant Enzyme Activities

Assay of antioxidant defense mechanisms showed that enzymatic antioxidants were severely depleted after the administration of etoposide as indicated in Table 3. The etoposide group had significant reduction in the superoxide dismutase activity which was used to destroy the superoxide radicals; the reduction was about 58% greater in the etoposide group than the

controls. This suppression of SOD activity curtails the initial defense against the effect of the superoxide radical which results in their build-up and consequently the formation of the other reactive species. The activity of catalase that breaks down hydrogen peroxide into water and oxygen was also reduced by about 62% in the etoposide group. The loss of CAT activity causes the build-up of hydrogen peroxide that is capable of creating hydroxyl radicals during Fenton reactions and causing extensive cellular damage.

Table 3: Antioxidant enzyme testicular tissue activities

Parameter	Control Group	Cinnamon Group	Etoposide Group	Etoposide + Cinnamon Group
SOD (U/mg protein)	18.45 ± 1.82	21.23 ± 2.05	7.67 ± 0.89*	14.32 ± 1.45
CAT (µmol/min/mg protein)	42.67 ± 4.12	46.89 ± 4.56	16.23 ± 2.15*	33.45 ± 3.67
GPx (nmol/min/mg protein)	56.34 ± 5.23	61.78 ± 5.89	22.45 ± 2.87*	42.67 ± 4.34
GSH (µmol/mg protein)	8.92 ± 0.87	9.67 ± 0.95	3.45 ± 0.42*	6.78 ± 0.71

The level of glutathione peroxidase activity that lowers the lipid hydroperoxides and hydrogen peroxide with the help of glutathione as a substrate was reduced by about 60 % in the etoposide group. Reduced GPx activity impairs the glutathione-dependent antioxidant system permitting. Unsaturated lipid peroxidation to continue. It was found that the reduced glutathione content which is the major non-enzymatic antioxidant in cells was significantly depleted with an approximate of 61% decrease in the etoposide group relative to controls. Depletion of GSH shows the use of this important antioxidant in the effort to counteract undue levels of ROS and represents extreme oxidative stress.

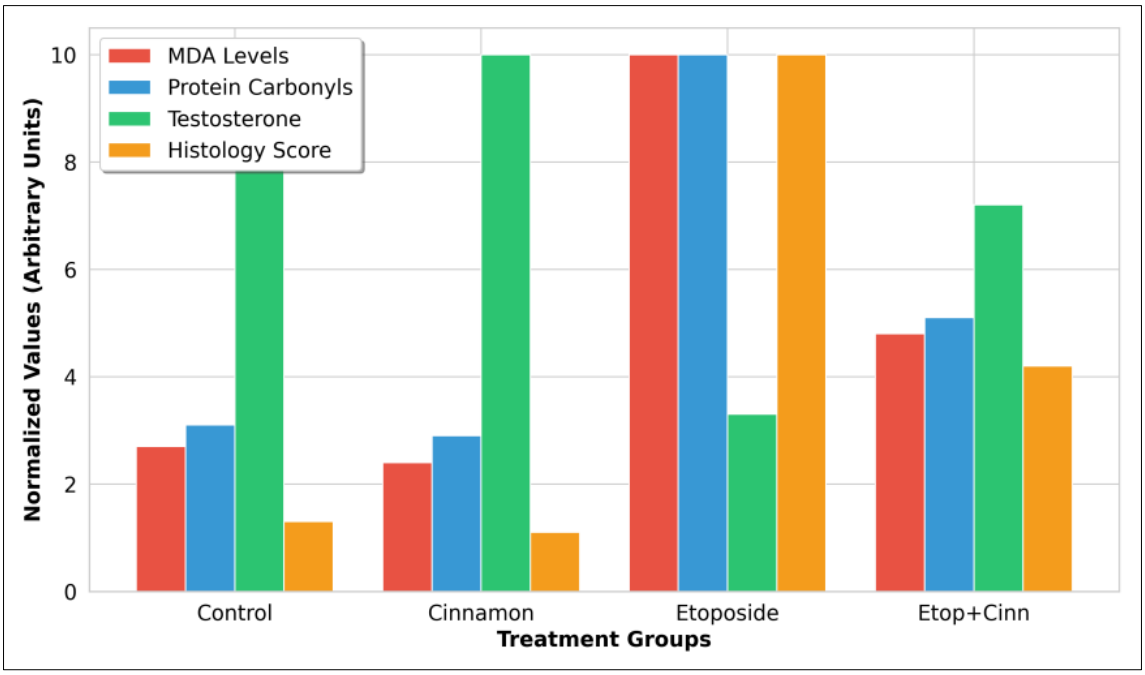

Figure 2: Comparative evaluation of markers of testicular damage

Cinnamon extract treatment showed a great deal of restoration of antioxidant enzyme activities. Approximately 87 % increase in SOD activity was observed in the co-treated group in comparison to the etoposide alone group which reached the values of approximately 78% of control. CAT activity was also demonstrated to be restored with nearly 106% increment above etoposide group with nearly 78% control activity being achieved. The co-treated (GPx increased by about 90% over etoposide versus alone) nothing less than about 76% control values were achieved. The GSH content was also restored substantially in the co-treated group with an estimated increase of about 97 % in relation to etoposide-alone with an estimated success of about 76 % of the control levels. These substantial enhancement of antioxidant defense mechanisms is a reason as to why cinnamon extract has a protective effect on oxidative damage.

Interestingly, the antioxidant enzyme activities were improved in interest groups (cinnamon extract alone) in comparison to controls (SOD increased by about 15%, CAT increased by about 10, GPx increased by about 10% and GSH increased by about 8). Although these were not necessarily statistically significant, they indicate a possibility of cinnamon extract improving basal antioxidant capacity without oxidative stress. This preconditioning effect can be one of the effects that help

the cinnamon extract to have the protective effect by boosting the antioxidant defense mechanisms prior to the oxidative damage.

Correlation Analysis

The results of the Pearson correlation analysis showed that there are significant associations between different parameters that were measured in this paper. Testosterone level exhibited positive and significant correlations with all antioxidant enzyme activities (SOD, CAT, GPx and GSH content) and negative correlations with oxidative stress measures (MDA, protein carbonyls, nitric oxide and histological damage scores). These correlations are indicative that oxidative stress and antioxidant status has a direct effect on testicular steroidogenic functioning. Testosterone had an inverse relationship with LH and FSH, and a positive relationship with oxidative stress markers, which confirms that oxidative damage destroys the hypothalamic-pituitary-gonadal feedback loop.

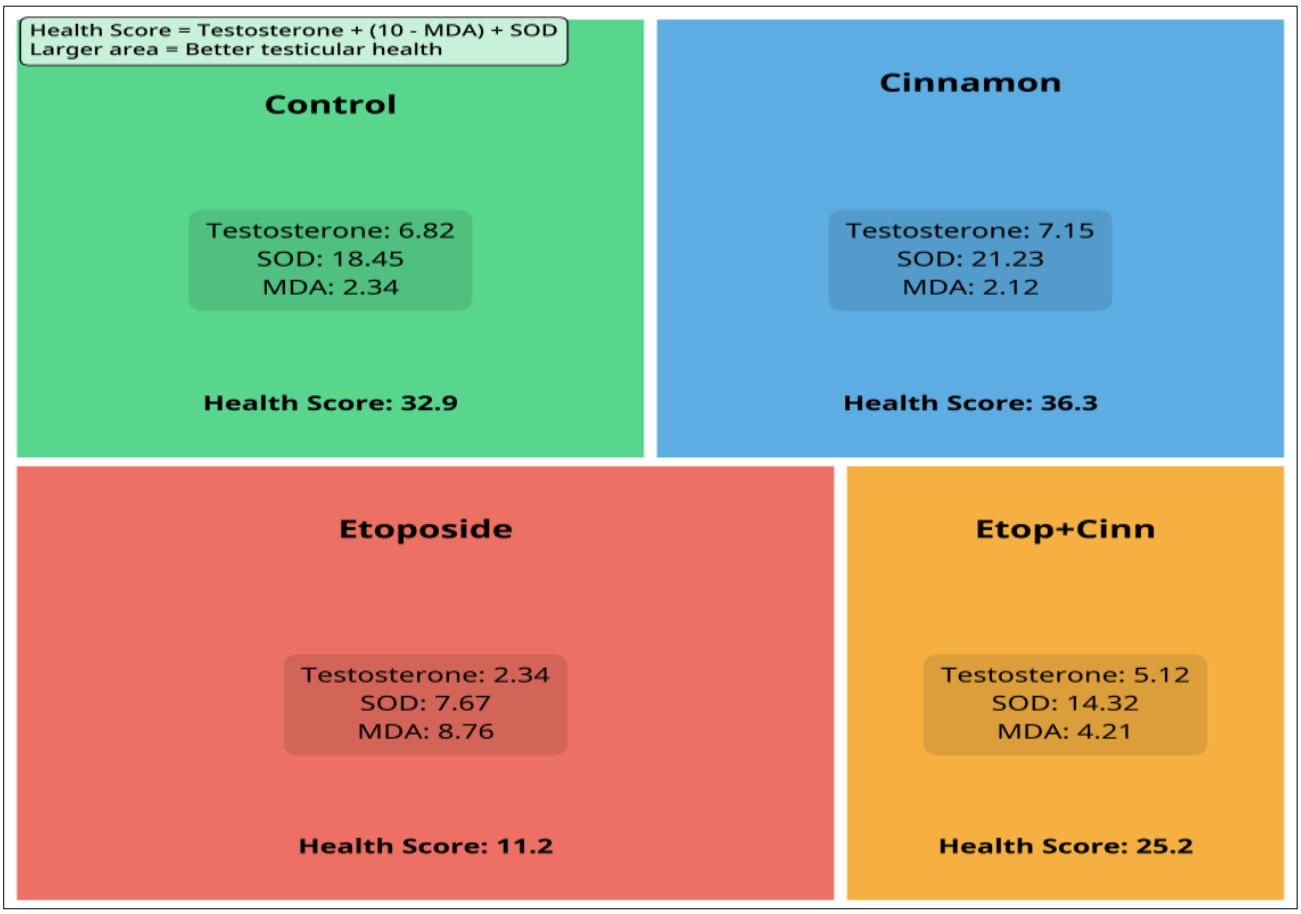

Figure 3: Correlation of the antioxidant status and reproductive parameters

Histological damage scores showed a high positive correlation with all the oxidative stress markers and high negative correlations with antioxidant parameters showing that the extent of structural damage is directly proportional to the oxidative-antioxidant balance. MDA levels were also significantly positively correlated with protein carbonyls and nitric oxide, which shows that various types of oxidative damage take place concomitantly. The antioxidant enzymes were highly positively interrelated with each other, which indicates that the antioxidant defense system is coordinated. These are correlation patterns that have statistical backing of the mechanistic relationships between oxidative stress, antioxidant defenses, and reproductive dysfunction which supports the idea that cinnamon extract would use the antioxidant mechanisms as the main mechanism of its protective effect.

DISCUSSION

This paper creates a comprehensive evidence that cinnamon alcoholic extract is an effective antioxidant that significantly prevents the testicular damage caused by etoposide due to its effective antioxidant effects. The endocrinological results show that etoposide has severe effects on reproductive endocrine axis resulting in an extraordinary decrease in testosterone and activating gonadotropin instead, which is typical of primary testicular failure. The cinnamon extract significantly replenished the testosterone levels and gonadotropins normalcy, proving to be an effective defender of testicular endocrine functions as Khamis *et al*., results indicate in antioxidant protection of the steroidogenesis-related genes [26]. Direct morphological protection evidence was shown by histopathological analysis, with the etoposide group having severe

structural damage with depleted germ cells and interrupted spermatogenesis, as was observed by Moradi *et al*., in BEP-treated rats [1]. The outstanding maintenance of seminiferous tubule structure by the use of cinnamon shows good cytoprotection by the antioxidant effect, anti-apoptotic effect, and maintenance of cellular homeostasis [15].

The presence of oxidative stress markers shows clearly that etoposide causes severe damage in numerous ways with the dramatic increase in MDA, protein carbonyls, and nitric oxide indicating the presence of lipid, protein damage, and the generation of oxidative stress, which are consistent with Ayad *et al*., on the central role of oxidative stress in infertility [27]. Markers: Large decreases in all markers after cinnamon treatment bring in first-hand information of antioxidant activity through free radical scavenging by polyphenolics, metal chelating, and breakage of oxidative chain reactions [28]. Etoposide data: Antioxidant enzyme data indicate that etoposide impairs the cellular defenses with a decrease in the activities of SOD, CAT, GPx and a decrease in GSH levels, which is similar to the results of Bayram *et al*., [29]. The meaningful restoration of cinnamon extract suggests that cinnamyl compounds confer direct antioxidant effects and improve the endogenous capacity by transcriptional upregulation [30]. The phytochemical characterization of Mutlu *et al*., [31], and the high polyphenol composition were revealed by Arachchige *et al*., agree with a variety of protective mechanisms that work in a synergistic way [32].

The pathways of the protective action of cinnamon are not limited to direct antioxidant action. The information of Adarthaiya and Arivarasan, concerning the metabolic effects [33], and the molecular evidence presented by Akter *et al*., of cellular targets would provide the context of testicular protection [34]. Anti-inflammatory effects could also play a role in decreasing the production of cytokines and regulating immune infiltration [35], and findings by Anderson *et al*., indicate that mitochondrial function is also preserved and mitochondrial-mediated apoptosis is prevented [36]. The implication of chemotherapy interfering with the various pathways as indicated by Kilarkaje and Al-Bader, implies that total protection can only be achieved by targeting many pathways, which can be done through a variety of cinnamon bioactive compounds [37]. A comparison with the other studies demonstrates the similarity in the protection patterns, where El-Wafaey *et al*., [38], and Bayram *et al*., [29], reported the benefit in the form of antioxidant mechanisms. The works by Famurewa *et al*., [39], prove the therapeutic potential of plant-derived antioxidants. The benefit of cinnamon is its safety history, accessibility and full characterization [7].

There are important clinical implications of male cancer patients with fertility issues during chemotherapy treatment [40]. Cinnamon extract is a viable candidate of clinical translation that has a history of safety profile and protective effects [40]. Nevertheless, issues such as the review of possible interactions with chemotherapeutic agents to guarantee the absence of anticancer effects, the formation of optimal dosing regimens using dose-response and pharmacokinetic analyses, and the identification of particular bioactive compounds that provide protection with the explanation of molecular mechanisms should be taken into account [1]. Limitations of the study are relatively short periods of four weeks may not be a fully representative of a long-term clinical regimen, the use of a single dose of the drug not requiring systematic dose response studies, and a requirement of molecular studies of gene expression, steroidogenic enzyme protein, and apoptotic markers. Even when limited, extensive evaluation results are excellent indications of the protective role of cinnamon extract against chemotherapy-induced reproductive toxicity and future studies into clinical practice are justified.

CONCLUSION

This paper demonstrates that alcoholic extract of cinnamon (*Cinnamomum zeylanicum*) offers great protection against the etoposide induced testicular damage in the male Wistar rats through intensive antioxidant action. The treatment using cinnamon maintained the level of testosterone, inhibited the occurrence of gonadotropins, preserved the structure of seminiferous tubules and the spermatogenesis, significantly lowered the evidence of oxidative stress (lipid peroxidation, protein oxidation, nitrosative stress), and recovered the activity of antioxidant enzymes (SOD, CAT, GPx) and glutathione content. Correlation studies have confirmed that a better antioxidant status correlates positively with a betterreproductive status which is mainly mediatedby bioactive polyphenols present in cinnamon such as cinnamaldehyde and eugenol through free radical scavenging and regulation of cellular pathways. These results propose cinnamon extract as a promising, safe adjuvant treatment in preserving male fertility in cases of chemotherapy. But additional research is needed to determine, and understand possible interactions with anti-cancer agents, optimal dosage, molecular pathways, and test in patients to confirm safety and effectiveness and may represent a useful adjunct to fertility protection in men with cancer.

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