

Full Length Research Paper

Effect of *Terminalia chebula* fruit extract on lipid peroxidation and antioxidative system of testis of albino rats

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The effect of aqueous extract of the *Terminalia chebula* was studied in male albino rats to explore its activities on testis. 1.0 ml of aqueous extract of *T. chebula* (500 mg/kg body weight) was given orally for 45 days. The activity of lipid peroxidation and superoxide dismutase were significantly increased but the concentration of antioxidant enzyme glutathione, and catalase were decreased in the aqueous extract administered rats rather than in control rats. Significant decrease in the activities of antioxidant enzymes, spermatogenesis and increase in the level of lipid peroxidation, indicate the severity of oxidative stress induced as a result of administration of extract of *T. chebula*. The aqueous extract also lead to changes in the parameters such as sperm count, motility and morphology, protein and cholesterol content of the rat testis. The long term (45 days) administration of the extract causes significant changes in the rat testis.

Key words: *Terminalia chebula*, antioxidant enzymes, extract, antifertility.

INTRODUCTION

Reactive Oxygen Species (ROS) such as superoxide anions (O_2^-) hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot) and nitric oxide (NO) are directly or indirectly involved in DNA damage leading to mutations. Some antioxidant defences are present in the plants and their products mainly edible vegetables and spices, have a key role in chemopreventers in human diet. For example, *Pleurotus florida*, possessed significant antioxidant enzyme activity (Nayana and Janardhanan, 2000), *Indigofera tinctoria* had strong antioxidant effect (Sreepriya et al., 2001) and *Coriandrum sativum* increased the antioxidant enzyme activity (Chithra and Leelamma, 1999). *T. chebula* is a home medicine; it is widely used for various ailments. But the available literature on *T. chebula* does

not reveal the effect of its antioxidant enzyme activities in testis. Since *T. chebula* is one of the commonly used nuts, we studied the role of *T. chebula* on lipid peroxidation and antioxidant defence mechanism in rat testis.

MATERIALS AND METHODS

Plant materials

T. chebula nuts were purchased from market and identified by the Department of Botany, Bharathidasan University, Thiruchirappalli, India.

Preparation of extract

100 g of powdered nuts of *T. chebula* were extracted in 200 ml of water by soxhlet apparatus. The extract of water was concentrated to dryness. The dried extract (20 g) was dissolved in 40 ml of water for further treatment.

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Table 1. Changes in the biochemical and antioxidant enzyme contents in *Terminalia chebula* extract treated rats.

Parameters	Control	Treatment
Protein ^a	5.72±0.082	4.821±0.022*
Cholesterol ^b	0.453±0.012**	0.662±0.156**
Lipid peroxidase ^c (LPO)	3.21±0.14	6.28±0.128*
Superoxide dismutase ^d (SOD)	4.86±0.211	12.32±0.210***
Glutathione ^e	82.22±0.22	41.0±1.02*
Catalase ^f	47.18±2.1	28.008±0.212*
Sperm count(million/ml)	54±9.16	43.6±1.24
Motility(%)	13.5±1.71	12.5±0.831

* P<0.001 ** P<0.02; *** P<0.5

^amg/100 mg tissue; ^bmg/100 mg tissue; ^cn moles of malondialdehyde/mg protein; ^dunit/minute/mg protein ; ^eµg/mg protein; ^fn moles of H₂O₂ decomposed/minute/mg protein.

Animals

Male Wistar rats were maintained in the laboratory in standard environmental conditions of temperature 32 ± 0.5°C. These animals were fed with commercial diet and water *ad libitum*. The institutional Animal Ethical Committee approved all experimental procedure (no. 687/02 /a/CPCSEA)

Experimental design

Group I animals served as control. These animals received water only, while groups II and III served as experimental groups, and received 1.0 ml of extract daily (500 mg/body weight) for 45 days. Body weight was monitored daily. At end of the experiment, the animals were sacrificed and testis was removed for further analysis. Statistical analysis was carried out using student's 't' test.

RESULTS AND DISCUSSION

Rats treated with the aqueous extract of *T. chebula* have decreased sperm count and motility. The lipid peroxidation increased and antioxidant enzymes showed significant changes. The present results indicate that the sperm morphology, count and motility are highly associated with the production and activity of free radicals and antioxidant enzymes (Table 1). The long term (45 days) administration of extract reduced the sperm count and induced the lipid peroxidation. It reveals that the extract or its component might be a toxic to the testis in the treated rats. This may be one of the factors responsible for the changes in sperm count, sperm motility, and broken head of the sperm. The extracts might have decreased the level of protective antioxidant enzymes. Sukcharoen et al. (1996) have previously reported the association of lipid peroxidation with mid-piece abnormality, decreased sperm count, motility. The increased lipid peroxidation activity is mainly due to the formation of highly reactive cytotoxic compounds like oxidative free radicals. The oxidative free radicals generated in living cells are superoxide anions (O₂⁻) and derivatives like hydrogen peroxide (H₂O₂), which induce peroxidation of cell membrane lipids (Bhattacharya et al., 1999). The improper balance between reactive

oxygen metabolites and antioxidant defence results in "oxidative stress" (Gibanananda and Hussain, 2002), and the severe oxidative stress gives the following negative effect (Irshad and Chaudhuri, 2002) in Figure 1.

The sperm DNA is vulnerable to oxidative stress impart, because semen has a weak antioxidant system (Zini et al., 1993; Aitken et al., 1996). It also supports the present results that due to the presence of weak antioxidant systems it is not able to tolerate the stress induced by the plant extract and its compounds. Oxidative stress at the testicular level has also been implicated in the disruption of spermatogenesis during cryptorchidism and exposure to genobiotics (Peltola et al., 1994; Peltola et al., 1995). Similarly, the oxidative damage is a possible cause of idiopathic male infertility involving disruption of spermatogenesis (Lenzi et al., 1993) and high level of free radicals were reported in the seminal fluid of those that are infertile (87%) and fertile (55%) (Mazzilli et al., 1994). Reduction in the sperm count and motility may be associated with the increasing formation of free radicals. The spontaneous formation of free radicals has been associated with decreased sperm-egg interaction (Aitken et al., 1987).

The reduced level of catalase and glutathione peroxide might be due to the excess production of anions in response to the extract of *T. chebula*. The most abundant oxidative free radicals generated in living cells are superoxide anions and derivatives, particularly the highly reactive and damaging hydroxyl radical which induces peroxidation of cell membrane lipids (Arunabh Bhattacharya et al., 1999). Superoxide anions (O₂⁻) itself directly affects the activity of catalase and peroxidase by affecting intracellular enzymes (Ghosh and Myers, 1998), creatine phosphokinase (Lee et al., 1998). Superoxide dismutase (SOD) was found to be increased in the treated animal's testis. The high level of SOD in the animal might be due to the oxidative stress caused by the extract. Similarly SOD is considered to be a stress protein which is synthesized in response to oxidative stress (McCord, 1990). Elevation of intracellular SOD increased the cell damage, allowing more H₂O₂ to be generated (Simon et al., 1981). Increase in the level of SOD activity leads to various diseases.

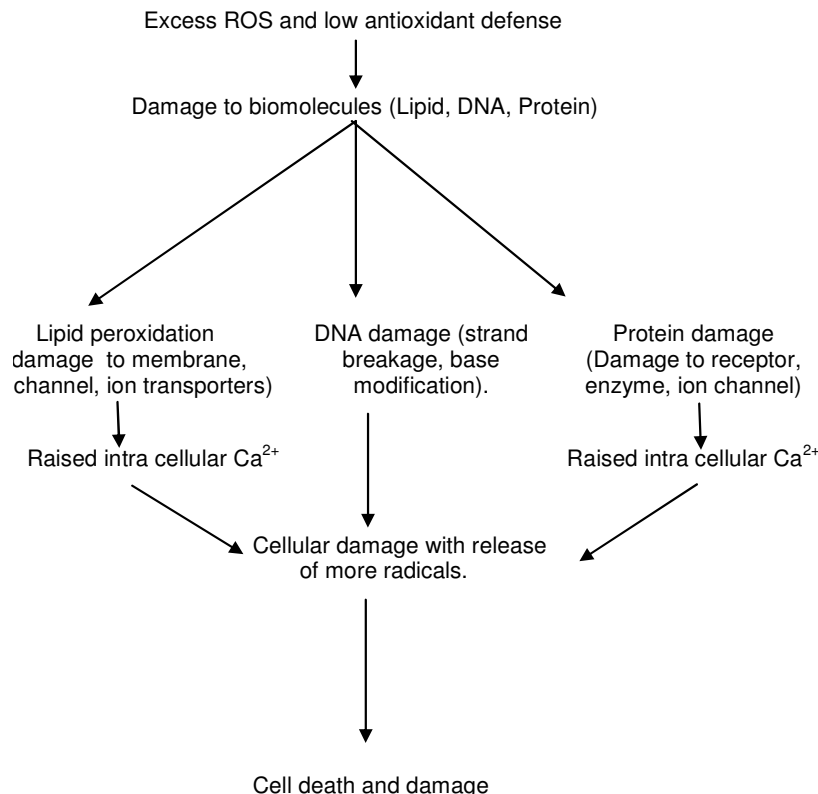


Figure 1. Negative effects arising from severe oxidative stress (Irshad and Chaudhuri, 2002).

Significantly high SOD was observed in breast cancer (Kokaglu et al., 1989) and in Hodgkin's disease (Abdel – Aziz et al., 1997).

Glutathione (GSH) concentration was also reduced in the animals treated with the extract. It is suggested that the utilization of glutathione by glutathione peroxidase. The GPX catalyses the oxidation of GSH to GSSG (Gibanananda and Hussain, 2002). This oxidation reaction occurs at the expense of (H₂O₂). The decreased level of protein concentration in the testis could be the change in the androgen production (Nocenti et al., 1968). The significant reduction in the levels of protein is due to the interference like atrophy of accessory organs in androgen production. The increasing testicular cholesterol might regulate the process of spermatogenesis (Dixit, 1977), and it acts as precursor for androgen synthesis (Verma et al., 1980). It is evident that the protein concentration decreases and increases the concentration of cholesterol in treated testis. It is concluded that the long term effect of the extract of *T. Chebula* in rats may be toxic to the testis and it affects the spermatogenesis process by altering the antioxidant enzymes activities.

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