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Research Paper

Testicular toxicity of aqueous extract of *Phyllanthus niruri* in male Wistar rat

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Abstract

Most of the traditional medicine and their preparations have not been scientifically evaluated, thus limited information is available about their pharmacokinetic, efficacy and safety. The objective of the present study was to elucidate the level of testicular toxicity caused by aqueous extract of *Phyllanthus niruri* and its commercial formulation. Adult male Wistar rats (150 – 200 gm) were divided in three groups. Control group was given distilled water, *P. niruri* group was given *Phyllanthus niruri* whole plant extract (1000 mg/kg bw) orally for 20 days and Nirocil (market formulation of *P. niruri*) treated group received Nirocil tablet in distilled water (200 mg/kg bw) orally for 20 days. Antioxidant status of testes was assessed by determining the activities of antioxidant enzymes, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Gluthione S-transferase (GST), Glutathione reductase (GRD) as well as by determining the levels of reduced GSH and levels of lipid peroxides. *P. niruri* treated and Nirocil treated rats showed significant increase in lipid peroxidation level compared to control. The antioxidant status was also altered in both the treated groups. Thus our study demonstrated that the aqueous extract of *P. niruri* and its market formulation could cause oxidative stress in experimental animals leading to testicular toxicity.

Keywords: Antioxidant, GSH, *Phyllanthus niruri,* Lipid peroxidation, Nirocil, toxicity.

Introduction

Phyllanthus niruri is one of the species belonging to family *Euphorbiaceae* distributed throughout the tropical and subtropical regions of the world and is used in many Ayurvedic preparations which have been used for the treatment of various diseases treatment lasting for 4-5 months. In traditional medicine it is also used for treating jaundice, kidney and liver stones, malaria and diabetes. The most recent research on *P. niruri* has documented its antiviral, hepatoprotective, hypolipidemic, hypoglycaemic, antifertility ,antioxidative and antihyperuricemic properties[1]. In contrast to this, Manjrekar *et. al.* (2008)[2] and Valentine *et.al.* (2011)[3] have shown toxic effect of *P. niruri* extract on some organs including reproductive tissues and also reported eosinophilic protein casts in the testes and kidney tubules. In addition to this tubular disorganization and decreased amount of mature spermatozoa have also been reported which indicates testicular toxicity.

Antifertility actions of crude and alcoholic extract of *P. niruri* have also been reported in male and female mice respectively [4,5]. However, there are no reports on the effect of chronic exposure of *P. niruri* extract on testicular function. Since testicular toxicity was observed in short term exposure of *P. niruri* extract it may be possible that chronic exposure may lead to compromised testicular function leading to infertility.

The major aim of the present study was to examine the effect of *P. niruri* and its market formulation Nirocil on the testes of adult male Wistar rats. Oxidative stress was evaluated by measuring levels of GSH and extent of lipid peroxidation in testes and acitivity of antioxidant enzymes like Superoxide

dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Gluthione S-transferase (GST) and Glutathione reductase (GRD).

Materials and Methods

Plant Material Collection and Extraction

Phyllanthus niruri plant was obtained from Shikhar Shingnapur, Satara, Maharashtra and authenticated from Harbarium at Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and voucher specimen was deposited.

The whole plant was washed, shade dried for two weeks and final drying was done in oven at 40^0 C. It was ground into a fine powder using blender. 100 gms powder was mixed with distilled water in a beaker and kept in water bath at 80^0C for 4 hrs which was then filtered through muslin cloth and then by Whatman filter paper[6]. The filtrate was dried using rotary evaporator at 80^0C. Final drying was done in oven at 40^0 C to obtain the powder of extract which was then stored at 4^0C till further use. Total yield of the extract was 12.97 %.

Nirocil tablet, a market formulation of *P. niruri* whole plant was obtained from local Ayurvedic store (1tablet is equivalent to 1000 mg of *P. niruri*).

Animal Model

Fifteen male albino Wistar rats (weighing between 150 – 200 gms) were procured from Bharat Serum Pvt. Ltd, Thane, Navi Mumbai, India and acclimatized before starting the experiment. They were caged in an air conditioned animal house under standard conditions of humidity, temperature (25 ± 2^0C) and light (12hr light/dark). They were fed standard rat pellete diet obtained from Lipolin India and water *ad libitum.* Animals were handled according to the Institutional legislation, regulated by the Committee (CPCSEA) after institutional ethical clearance.

Experimental Design

Animals were randomly divided in three groups consisting of 5 animals each. One group served as Control and received sterile distilled water. Other group received *Phyllanthus niruri* whole plant extract (1000 mg/kg bw). Third group received Nirocil tablet in distilled water (200 mg/kg bw) orally for 20 days.Animals were sacrificed at the end of experiment and Testes were excised, washed with ice cold saline and a portion was homogenized in 0.01 M Tris-HCL buffer (pH – 7.4), centrifuged and homogenate was used for following analysis.

Determination of Lipid Peroxidation

Lipid peroxidation in testes was determined by measuring the malondialdehyde (MDA), formed as an end product of lipid peroxidation by the method of Okhawa *et al.*(1979) [7]. MDA reacts with thiobarbituric acid to generate a colored product which can be measured optically as 532 nm. Tetramethoxy propane was used as standard.

Estimation of antioxidant enzymes

Superoxide Dismutase (SOD) activity was estimated by monitoring the oxidation of epinephrine by the method of Misra and Fridovich*et. al.*(1972) [8].The unit of enzyme activity was expressed as the enzyme required for 50 % inhibition of epinephrine auto-oxidation (unit/mg protein).

Catalase (CAT) activity was determined by the method of Clairborne (1985)[9] in which hydrogen peroxide decomposition is measured at 240 nm. The enzyme activity was expressed as micromoles H_2O_2decomposed/min/mg of protein.

Glutathione Peroxidase (GPX) was measured by the method of Paglia and Valentine(1967)[10] in which oxidation of NADPH using hydrogen peroxide was recorded spectrophotometrically at 340 nm and the enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein.

Gluthione S-Transferase (GST) was assayed using the method of Habig *et al.*(1974)[11]. The method based on enzyme catalyzed condensation of glutathione with the 1-chloro, 2,4-dinitrobenzene to give 2,4-dinitrophenyl-glutathione which can measured at 340 nm. The enzyme activity was expressed as micromoles CDNB conjugates formed/min/mg of protein.

Glutathione reductase (GRD) was determined by the method of Carlberg and Mannervik (1975) [12]. The method is based on transfer of hydrogen from NADPH to GSSG (reduction of GSSG to GSH) and decrease in absorbance was monitored spectrophotometrically at 340 nm. The enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein.

Estimation of non-enzymatic antioxidant

Total GSH content was estimated by the method of Moron *et al.* (1979)[13] using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) as coloring agent and absorbance was read at 412 nm against a blank containing TCA. The amount of GSH was expressed as nmoles of GSH/mg protein.

Estimation of Protein content was doneby method of Lowry *et.al*. (1951)**[14]**

Statistical Analysis

The values were expressed as mean ± SEM. The results were analysed by Student's t-test and given respective symbols in the tables.

Results

There were no distinctive clinical signs, mortality or morbidity observed in any of the experimental groups during the experiment. Both *P. niruri* and Nirocil treated groups showed no noticeable change in body weight and tissue weight.

Effect of extracts on lipid peroxidation level

MDA content, a product of lipid peroxidation in testes tissue homogenates of all the groups are shown in Figure 1. MDA levels in both the treated groups animals increased significantly (***p<0.001) as compared to control group. The lipid peroxide level was very high Nirocil treated group than the *P.niruri* group suggesting greater tissue damage due to free radicals.

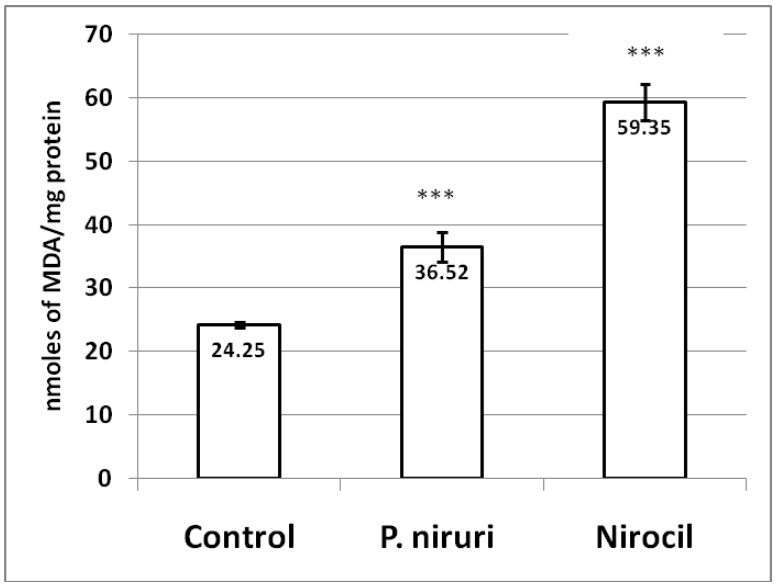

Figure 1: Effect of aqueous extract of *P. niruri* and Nirocil tablet on lipid peroxidation in rat testes

Values are expressed as Mean ± SEM (n=6). Comparison of control with treated groups,***p<0.001.

Effect of extracts on GSH

P. niruri treated group showed significant depletion in GSH (p<0.05) and significant loss of GSH was also observed in Nirocil treated group (p<0.01) compared with control group. (Figure 2)

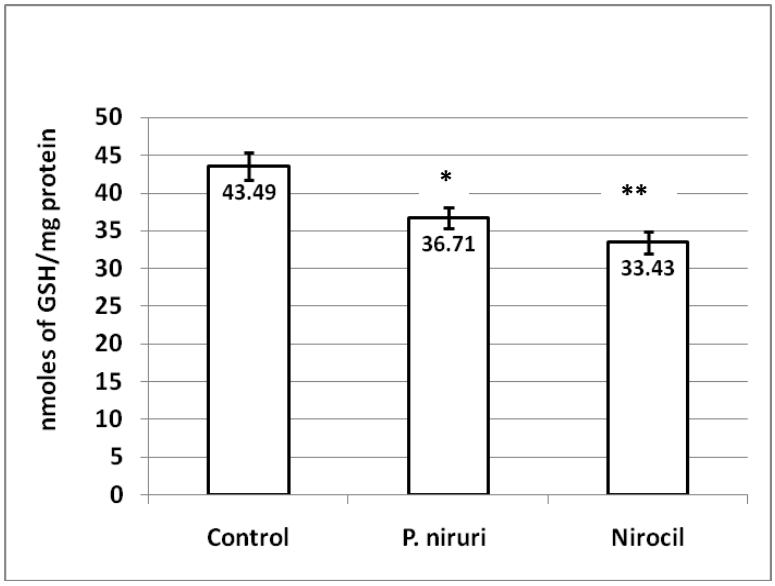

Figure 2: Effect of aqueous extract of *P. niruri* and Nirocil tablet on the levels of GSH in testes of rats

Values are expressed as Mean ± SEM (n=6). Comparison of control with treated groups *p<0.05, **p<0.01

Effect of extracts on antioxidant enzymes

Table 1 represents the altered activity of antioxidant enzymes SOD, CAT, GPx, GST and GRD in testes tissue of all the groups.

Table 1: Effect of aqueous extract of *P. niruri* and Nirocil tablet on the activities of antioxidant enzymes in testes of rats

Antioxidant enzyme	Control	*P. niruri* treated	Nirocil treated
SOD	0.397 ± 0.034	0.760 ± 0.007 ***	0.836 ± 0.007 ***
CATALASE	13.09 ± 0.234	59.41 ± 0.776 ***	22.63 ± 0.610 ***
GPx	0.600 ± 0.024	0.843 ± 0.020 ***	1.660 ± 0.097 ***
GST	0.225 ± 0.027	0.309 ± 0.024 *	0.373 ± 0.052 *
GRD	0.0686 ± 0.003	0.0430 ± 0.002 ***	0.0424 ± 0.005 **

Values are expressed as Mean ± SEM for five animals in each group. Comparison were made between control and treated groups *p<0.05, **p<0.01, ***p<0.001

When compared to control, *P. niruri* treated and Nirocil treated rats showed increased activities of SOD (p<0.001), CAT (p<0.001), GPx (p<0.001), GST (p<0.05) whereas significant decrease in the GRD activity in *P. niruri* treated (p<0.001) and Nirocil treated (p<0.01) was found as compare to control group.

Discussion

Toxic effect of Phyllanthus extract on testes in relation to testicular spermatogenesis, sperm morphology, motility and viability have been reported[4]. However, limited information is available

regarding the oxidative stress caused in the testes due to the *P. niruri* extract. Therefore, in the present study, toxicity of the aqueous extract of *P. niruri* and its tablet form Nirocil for a period of 20 days was studied. Although there was a decreasing trend seen in the body weight and relative organ weight of the treated groups of rats, oral feeding of the aqueous extract of the whole plant *P. niruri* and Nirocil to the adult male showed no significant change in the body weight and relative organ weight compared to control group for a period of 20 days indicating that the general body growth was normal as reported by Rao *et. al.*(1997)[4].

Lipid peroxidation is regarded as one of the primary causes of cellular damage and the increase in lipid peroxides results in oxidative stress[15]. Our finding reveals that *P. niruri* and Nirocil administration caused noticeable oxidative stress as evidenced by the significant increase in lipid peroxides. Increased free radicals initiate the peroxidation of membrane polysaturated fatty acid causing damage to the phospholipid membrane of the cell which lead to alteration in membrane permeability and cell function [16,17]. Increased lipid peroxidation causes depletion of Glutathione (GSH) along with loss of endogenous antioxidant enzyme activity[18].Depletion of GSH in tissue is prime factor which can impair the cell's defence against the ROS toxicity and may lead to peroxidative cell injury[19].The significant decrease in the level of GSH as observed in the current investigation probably results in oxidative stress in testis. GSH depletion increases the sensitivity of cells to various aggressions and has several metabolic effects which can lead to tissue disorder and injury [20].

Under physiological conditions, the damaging effects of ROS are also prevented by endogenous antioxidant enzymes viz , SOD, CAT, GPx, GST and GRD which are considered as cellular antioxidant defense system. SOD, CAT, GPx and GST are class of antioxidant enzymes responsible for scavenging H_2O_2 and organic hydroperoxides. In the present work changes in anti-peroxidative enzymes were also noted. The treated groups showed significant increase in SOD levels compared to the control group. Superoxide Dismutase (SOD) has been reported as one of the most important enzymes in the endogenous antioxidant defence system which scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical[21].

Increased rate of lipid peroxidation causes inducible synthesis of CAT, GST and GPx as an adaptive mechanism to detoxify the reactive species in order to minimize tissue damage [22], while GRD is an enzyme crucial for regeneration of GSH that ensures it availability to GSH-dependent enzymes for their proper functioning [23]. The significant elevation of CAT and GPx in treated groups implies that the testis is trying to combat the attack of ROS, which is also indicated by the depletion of GSH. The significant reduction in the activity of GRD in both the treated groups as compared to normal also could be factor responsible for reduced level of GSH. Since GRD can be inactivated by ROS, the decrease in the activity of GRD on treatment with aqueous extract and Nirocil could be due to the observed increase in the level of peroxides. The reduced GRD activity may be justified by the fact that under high oxidative stress GSSG can be effluxed out of the cell [24].

Thus, the study reveals that the aqueous extract of *P. niruri* is capable of altering the antioxidant milieu of the test is resulting in oxidative stress in testes which could probably lead to compromised testicular function. Further research are underway to reveal the underlying mechanism of testicular toxicity on treatment with *P. niruri*. In view of the potential use of this herbal extract for medicinal purpose caution should be exercised given its capacity to induce oxidative stress in tissues.

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