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Research Article

STUDIES ON ETHANOLIC LEAF EXTRACT OF *PHYLLANTHUS NIRURI* AND ITS EFFECT ON AFLATOXIN INTOXICATED MALE ALBINO RATS

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ABSTRACT

Aflatoxin is an environmental toxicant which frequently contaminates foodstuffs in different parts of the world. The present investigation was an attempt to evaluate the possible ameliorative effects of *Phyllanthus niruri* on aflatoxins induced serological and biochemical changes in liver of rats. In the current study, toxicity was developed by oral administration of aflatoxin at a dose of (200 µg/kg body weight) for 40 days in male rats. *P. niruri* (300 mg/kg body weight) was given simultaneously for 40 days. Administration of *P. s. niruri* lowered the level of lipid peroxidation and enhanced the antioxidant status of animal. It can be concluded that the *P. niruri* acts as an effective drugs playing an important role in reduction of hepatotoxicity. In conclusion, *Phyllanthus niruri* was found to be safe and successful agent counteracting the aflatoxins toxicity and protected against the toxicity induced by aflatoxin. However, it suggests that a dose adjustment may be necessary to optimize the effects in clinical settings.

Key words: Aflatoxin, *Phyllanthus niruri*, hepatotoxicity, Enzyme activity, Biochemical studies.

INTRODUCTION

The word "aflatoxin" comes from a = *Aspergillus*, fla = flavus and toxin = venom. Aflatoxins (AF) are fungal secondary metabolites that form a group of toxic compounds that chemically correspond to furan coumarins. AF were discovered in Great Britain in 1960, after the death of one hundred thousand turkeys that were fed with AF contaminated peanuts from Brazil, the flour was contaminated with the mould *Aspergillus flavus*.

Humans are continuously exposed to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in environmental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. Great attention is focused on environmental health in the past two decades as a consequence of the increasing awareness over the quality of life due to major environment pollutants that affect it. Studies have shown that exposure to environmental chemical carcinogens have contributed significantly to cause human cancers, when exposures are related to life style factors such as diet (Ramamurthy and Maria Rajeswari, 2015).

Aflatoxin is an environmental toxicant which frequently contaminates foodstuffs in different parts of the world. Literatures have shown that complete eradication of aflatoxins from foodstuffs is difficult to attain because of a combination of factors such as climatic conditions that favour easy growth, proliferation and toxin production by fungi (Hendrickse, 1991).

Aflatoxins are well known for their hepatotoxic and hepatocarcinogenic effects (WHO, 1979). AFB1 is activated to AFB1-8,9-epoxide and forms adduct primarly at N-7 position of guanine and is responsible for its mutagenic and carcinogenic affects (Denissenko *et al.*, 1999). In goslings and chickens (Marvan *et al.*, 1983), experimentally studied the distriction of AFB1 where according to AFB1 concentration, the organs and tissues were catergorized in the order from high to low concentrations as follows: the gonades, the parencgymatous organs (Liver and kidney), the lymphopoietic organs

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(spleen, bursa and thymus), the endocrine glands, the muscles and the lungs, while the brain had the lowest concentration. Also, it has been reported that aflatoxins have a deleterious effect on the reproductive systems of a wide spectrum of domestic animals (Doerr and Ottinger, 1980). Naidu *et al.* (1991) observed multifocal hepatic necrosis, bile ductular proliferation, areas of altered hepatocytes, neoplastic nodulesand hepatocellular carcinoma constituted the total spectrum in both adult and newborn rats exposed to AFB1. Meanwhile, progressive hepatic degeneration, necrosis and bile duct hyperplasia were the constant pathological changes observed in rats and chickens (Salem *et al.,* 2001). In addition, aflatoxin administration induced degenerative changes in the hepatic and renal tissues of rats (Abdel-Wahhab *et al.,* 2002). Moreover, AFB induced mononuclear cell infiltration and/or focal lymphoid cell accumulation in the intertubular areas of the tests and epidedymis; degeneration and desquamation in the epithelium and decrease in the size and thickness of the germinative layer of the seminiferous tubules and lowered plasma testosterone levels in adult roosters (Ortatatli *et al*., 2002).

Phyllanthus niruri are originated in India, usually occurring as a winter weed throughout the hotter parts. The Phyllanthus genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of hot hemispheres. Unfortunately, there remains a great deal of confusion among scientists regarding plant identification and many cases, plant misidentification make evaluation of published information difficult. *P. amarus* and *P. sellowianus* are often considered a variety of *P. niruri*, or no distinction is made among these three species in published clinical research. Often time's one name is indicated tube synonymous with another and, sometimes, both names are used interchangeably as if referring to one plant. It became so confusing that, in the 1990s, a major reorganization of the Phyllanthus genus was conducted (which classified *P. amarus* as a type of *P. niruri*). *Phyllanthus niruri* is an herb of Euphorbiaceae family that grows up to 60 cm. Phyllanthus means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf. *Phyllanthus nirui* is a common kharif (rainy season) weed found in both cultivated fields and wastelands.

Phyllanthus niruri is an annual herb belonging to the family Euphorbiaceae grows 50 to 70 centimeters tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. It produces phyllanthid branches with the presence of flowers and fruits at the base of each leaf, one of the identification characteristics of this plant (Ramamurthy and Abarna, 2014). *Phyllanthus niruri* is used in the treatment of various ailments like jaundice, diabetes, kidney stones and liver disorders and for treatment of Hepatitis B viral infection. Therefore, in the present study was aimed to investigate whether intoxication of aflatoxin induces oxidative stress and if so, *Phyllanthus niruri* reduces the aflatoxin intoxicated oxidative stress in the liver of rats.

MATERIALS AND METHODS

For the present study, the mature green leaves of *Phyllanthus niruri* belongs to family Euphorbiaceae were collected from in and around area of Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora (Gample, 1967 and Matthew, 1983) with standard references (Kirtikar and Basu, 1993).

Preparation of plant extract

The *Phyllanthus niruri* was collected, washed, cut into small pieces and dried at room temperature (28±1ºC) for two weeks and made into powder for further analysis. The aerial parts were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether and then extracted with ethanol by using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The percentage yield of ethanol extract was 36%. The ethanol extract of *Phyllanthus niruri* was used for the entire study.

Experimental Animals

Adult Wistar albino rats weighing of 200 - 250 gm breed in the Central Animal House, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, were used in this study. They were housed in Tarson`s polypropylene cages with metal grill tops and provided with food and water *ad libitum.* They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. In the laboratory, rats were fed with standard rat pellet diet.

Experimental design

The animals were randomly divided in to four groups, each containing three animals. Four groups (Group I, Group II, Group III and Group IV) of rats, six rats in each group were taken. Group – I: Served as normal, which received, feed and water only. Group – II: Animals of this group were orally administered 200 µg/kg of body weight of aflatoxin along with formulated feed for 40 days. Group – III: Animals of this group were orally administered 200 µg/kg of body weight of aflatoxin along with formulated feed. Then the animals were treated with the alcoholic extract of *Phyllanthus niruri* daily for 40 days at concentration of 300mg/kg of body weight. Group – IV: Animals of this group were orally administered 200 µg/kg of body weight of aflatoxin dissolved in formulated feed. Then the animals were treated with Silymarin for 40 days at concentration of 25 mg/kg of body weight. After 41st days of treated animals were fasted for 12 hours after the last dose of drug treatment and were scarified cervical decapitation under mild chloroform anesthesia. The blood was collected for

serum separation. The organs were excised and they were washed in ice-cold saline until homogenized. Liver-10%, homogenate was prepared in 0.1 ml Tris HCl buffer pH 7.4. The serum separated by the centrifugation process and was used for following estimation.

At the end of the study, all animals were fasted for 12 h and then under mild ether anesthesia, animals were sacrificed and blood samples were collected. Blood was collected immediately into tubes containing EDTA for analysis of hematological parameters viz. hemoglobin, total red blood cells (RBC), packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, basophiles, total platelet count (Theml *et al.,* 2004) using hematology analyzer Sysmex XS800i (Sysmex corporation, USA).

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) by the methods of Reitman and Frankel (1957), alkaline phosphatase (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and protein (Lowry *et al.,* 1951) were analyzed.

RESULTS

The treatment with the extract did not decrease waterand food consumption rats. The body weight of the rats treated with alcoholic extract once a day during sub-acute treatment did not show any significant change when compared with the control group, although had a tendency to decrease body weight. This decrease can be associated with the decrease of liver weight at the dose of 100 mg/kg in comparison with the control group without any concomitant alteration in the activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Estimation of the serum activity of total bilirubin, protein, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury (Table 1).

Effect of aflatoxin and *Phyllanthus niruri* leaf extract either alone or in different combination on hematological variables in albino rats are illustrated in table 2. Supplementation of aflatoxin led to significant fall ($P<0.01$) in Hb, PCV, RBC, lymphocyte count and remarkable rise ($P<0.01$) in WBC, platelet and neutrophil count as compared to respective values of normal rats. Co-administration of different treatment of *P. niruri* leaf extract along with aflatoxin led significant improvement in Hb, RBC content and bought them back near to normal. Whereas, PCV and lymphocyte count were also significantly elevated ($P<0.01$) in these groups as compared to group aflatoxin treated rats. Moreover *P. niruri* leaf extract led to significant fall ($P<0.01$) in WBC, platelet and neutrophil count as compared to respective values of aflatoxin alone receiving group of rats.

Table 1. Effect of *Phyllanthus niruri* extracts on some biochemical and serum marker enzyme parameters in aflatoxin intoxicated rats.

Treatment group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)	Protein (g/dl)
Normal Control	121 ± 2.11	38.2 ± 1.2	115 ± 2.2	0.85 ± 2.15	7.5 ± 1.22
Aflatoxin control (200µg/kg of aflatoxin)	198 ± 2.13	99.5 ± 1.9	258 ± 2.9	2.89 ± 2.35	4.5 ± 1.18
200µg/kg of aflatoxin+300 mg/kg extract of *P. niruri*	131 ± 2.25	46.1 ± 1.5	145 ± 2.5	1.22 ± 2.14	6.5 ± 1.32
200µg/kg of aflatoxin+25 mg/kg of silymarin	135 ± 2.09	43.5 ± 1.5	137 ± 2.3	0.98 ± 2.18	6.9 ± 1.41

Table 2. Effect of *Phyllanthus niruri* extract on haematological variables in aflatoxin intoxicated rats.

Parameters	Normal Control	Aflatoxin treated groups		
		200µg/kg of aflatoxin	300 mg/kg *P. niruri*	25 mg/kg Silymarin
RBC (10^6/µl)	7.48 ± 0.18	4.54 ± 0.12	6.62 ± 0.17	7.02 ± 0.15
Hb (g/dl)	13.8 ± 0.32	9.2 ± 0.26	11.9 ± 0.12	12.2 ± 0.21
PCV (%)	45.5 ± 0.21	21.8 ± 0.37	39.2 ± 0.15	41.1 ± 0.18
MCV (fL)	53.1 ± 0.25	44.2 ± 0.32	51.1 ± 0.18	51.8 ± 0.42
MCH (pg)	17.5 ± 0.31	14.6 ± 0.25	16.9 ± 0.33	17.1 ± 0.22
MCHC (g/dl)	35.4 ± 0.18	27.5 ± 0.16	33.4 ± 0.21	34.2 ± 0.18
Lymphocyte(%)	86.5 ± 0.15	70.2 ± 0.17	82.5 ± 0.15	82.8 ± 0.25
Platelet (10^3/µl)	894 ± 1.05	1315 ± 1.08	1195± 1.32	1225 ± 1.15
WBC (10^3/µl)	7.25 ± 0.12	15.5 ± 0.31	9.01 ± 0.43	8.87 ± 0.18
Neutrophils (%)	18.5 ± 0.25	23.2 ± 0.24	19.8 ± 0.08	22.5 ± 0.33

RBC - red blood cell; Hb - hemoglobin; PCV - packed cell volume; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular haemoglobin concentration; WBC - white blood cell.

DISCUSSION

A lot of medicinal plants, traditionally used for thousands of years, by the Indian traditional health care system (ayurvedic) named 'Rasayana' for their antioxidative properties. *Phyllanthus niruri* was a very good antioxidant and hepatoprotective agent (Ramamurthy and Abarna, 2014; Ramamurthy and Gowri, 2015). *Phyllanthus niruri* (300mg/kg) increased cell viability of rat hepatocytes being treated with aflatoxin intoxicated rats. The present study was carried out to evaluate the hepatoprotective activity of *P. niruri* against aflatoxin induced hepatocellular degenerative in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. *P. niruri* possessed very high levels of alkaloids and flavonoids and are employed in medicinal uses. The plants studied here can be seen as a potential source of useful drugs. The results of biochemical parameters revealed the elevation of enzyme level in aflatoxin treated group, indicating that aflatoxin induces damage to the liver. Liver tissue rich in both transaminases increased in acute hepatic diseases SGPT, which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease (Murugaian *et al.,* 2008 and Sukumaran *et al.,* 2008). A significant reduction ($P < 0.005$) was observed in SGPT, SGOT, ALP, total bilirubin and protein levels in the groups treated with silymarin and extract of *Phyllanthus niruri*. The results confirmed that the enzyme levels were almost restored to the normal levels (Ayyadurai and Ramamurthy, 2009; Ramamurthy and Sagaya Giri, 2013).

Aflatoxin has a harmful and stressful influence in the serum, hepatic and renal tissue. In the present study, aflatoxin treatment was found to cause an increase in ALT, AST and alkaline phosphatase levels. These results may indicate degenerative changes and hypofunction of liver (Abdel-Wahhab and Aly, 2003). The activity of ALT and AST are sensitive indicators of acute hepatic necrosis. The reduced level of total protein is indicative of the toxic effect of Aflatoxin in serum and blood. Aflatoxin is known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibits RNA synthesis, DNA-dependent RNA polymerase activity and causes degranulation of endoplasmic reticulum. Reduction in protein content could also be due to increased necrosis in the liver. Thus reduction in protein biosynthesis as well as increased necrosis could be responsible for a decrease in protein content. Many other investigators have also reported a decrease in protein content in skeletal muscle, heart, liver and kidney of aflatoxin fed animals (Sharma *et al.,* 2011).

In the present investigation, *Phyllanthus niruri* significantly decreased serum alkaline phosphatase (ALP) of rat after 30 days of experiment. Decreased level of ALP with *P.niruri* in this experiment is similar to previously reported by Pal *et al.* (2005). ALP is a sensitive marker of liver damage. In hepatitis, hepatocytes start to die due to liver damage and there by ALP concentration in the bile ducts increases. Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that is why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. The abnormal high level of serum ALT, AST, ALP and protein observed. In our study (Table 1) are the consequence of aflatoxin induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with *P. niruri* reduced the enhanced level of serum ALT, AST, ALP and protein, which seem to offer the protection and maintain the functional integrity of hepatic cells. This result is support by stimulations of protein synthesis have been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the protection of liver cell (Ramamurthy *et al.,* 2014).

Effect of aflatoxin and *Phyllanthus niruri* leaf extract either alone or in different combination on hematological variables in albino rats are illustrated in table 2. Supplementation of aflatoxin led to significant fall ($P<0.01$) in Hb, PCV, RBC, lymphocyte count and remarkable rise ($P<0.01$) in WBC, platelet and neutrophil count as compared to respective values of normal rats. Co-administration of different treatment of *P. niruri* leaf extract along with aflatoxin led significant improvement in Hb, RBC content and bought them back near to normal. Whereas, PCV and lymphocyte count were also significantly elevated ($P<0.01$) in these groups as compared to group aflatoxin treated rats. Moreover *P. niruri* leaf extract led to significant fall ($P<0.01$) in WBC, platelet and neutrophil count as compared to respective values of aflatoxin alone receiving group of rats. Administration of *P. niruri* leaf extract was effective in reducing the adverse effect of aflatoxin on hemopoitic system supporting the hypothesis that plant extract exhibits effective antioxidant property. The plant extract showed improvement in biochemical variables with an increase in TEC, Hb and PCV in current study indicated that component present in *Phyllanthus niruri* leaf extract prevent oxidative damage, such as lipid peroxidation associated with many diseases, including cancer and immune deficiency.

Aflatoxin has also harmful and stressful effect on blood variables, serum variables and hepatic tissues. In present study reduced level of total erythrocyte count (TEC) was observed in aflatoxin treated rats. The mechanism of action by which aflatoxin aggravated pathogenesis of anemia could involved down-regulation of erythropoietin activity (Reddy *et al.,* 1987). Decreased level of TEC has been contributed to reduction of erythropoisis in bone marrow and showed rapid rate of destruction of peripheral RBC in spleen. Decreased level of Hb can be related with reduced size of RBC, impaired biosynthesis of haem in bone marrow or due to reduction in rate of formation of TEC (Sharma *et al.,* 2011). There was a significant increase in WBC count, which mainly consisted of neutrophils. The increase level of WBC and percentage of neutrophils suggest that aflatoxin elicited an inflammatory response and cause alteration in bone marrow and function of immune system (Abdel-Wahhab and Aly, 2003). Animals treated with aflatoxin also showed lower

level of PCV due to development of anemia in aflatoxicosis bearing mice (Veena Sharma *et al.,* 2011).

In rats with damaged liver the treatment with *Phyllanthus niruri* herb shows decrease in liver enzymes and biochemical level with extremely significant, while there is an improvement in haemoglobin level. The *P. niruri* could become helpful for patients with damaged liver possibly by reducing liver enzymes and biochemical parameters. It increases haemoglobin level and possibly improves in life style of such patients. These results finding shows that *P. niruri* extract have the ability to rectify hepatic damage or toxicity. Hence it is advised that if one happens to take any liver toxic drugs in overdose they can consume *P. niruri* extract as a hepatoprotective agent. Thus always have in mind that "Prevention is better than cure".

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