Study of Ethanolic Leaf extract of *Tinospora cordifolia* and its Effect on Aflatoxin Intoxicated Female Albino Rats

V. Ramamurthy & H. Jacena Begum
P.G & Research Department of Biochemistry, Marudupandiyar College, Vallam Post, Thanjavur, 613 403, Tamil Nadu, India.

Received: September 18, 2018 Accepted: November 01, 2018

ABSTRACT: *Aflatoxins are unavoidable food contaminant and reducing their in vivo toxicity of major interest. The potential of Tinospora cordifolia was evaluated for reducing the aflatoxin induced toxicity in rats. The present investigation was an attempt to evaluate the possible ameliorative effects of Tinospora cordifolia on aflatoxins induced hormone and biochemical changes of rats. In the current study, toxicity was developed by oral administration of aflatoxin at a dose of (100 µg/kg body weight) for 45 days in female rats. T. cordifolia (500 mg/kg body weight) was given simultaneously for 45 days. Administration of manifestations includes severe hormonal changes in the ovaries and uterus associated with decrease in the levels of some reproductive hormones such as estrogen, progesterone, testosterone, LH and FSH. In conclusion, T. cordifolia 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, Tinospora cordifolia, hormone and 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.

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). AFB1 is activated to AFB1 -8, 9-epoxide and forms adduct primarily at N-7 position of guanine and is responsible for its mutagenic and carcinogenic affects (Denissenko *et al*., 1999). In goslings and chickens, experimentally studied the distraction of AFB1 where according to AFB1 concentration, the organs and tissues were categorized in the order from high to low concentrations as follows: the gonads, the parencgymatous organs (Liver and kidney), the lymphopoietic organs (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 nodules and 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).

Tinospora cordifolia (Willd.) Miers, (Guduchi) is one of the important dioecious plants belongs to the family Menispermaceae. In Hindi, the plant is commonly known as Giloe which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally

young. In Ayurveda, it is designated as Rasayana drug recommended to enhance general body resistance, promote longevity and as antistress and adaptogen. This significant plant is also mentioned in important pharmacopoeias. Phytochemistry of *T. cordifolia* belongs to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. *T. cordifolia* is widely used in folkloric veterinary medicine and traditional Ayurvedic medicine in India for its, anti-inflammatory, immuno-modulatory, anti-pyretic activity, antioxidant, anti-diabetic, anti-allergic and anti-arthritic activities and various other medicinal properties. Almost all the parts of the plant are documented to be useful in ethnobotanical surveys conducted by ethnobotanists. Details of various important aspects such vernacular names of *T. cordifolia* and its important ethnobotanical, Ayurvedic properties, pharmacological and phytochemistry have been published (Mathew George *et al*., 2016). Therefore, in the present study was aimed to investigate whether intoxication of aflatoxin induces oxidative stress and if so, *T. cordifolia* reduces the aflatoxin intoxicated oxidative stress in the female rats.

Materials and Methods

For the present study, the mature green leaves of *T. cordifolia* belongs to family Menispermaceae were collected from in and around area of Thanjavur District, Tamil Nadu, South India.

Preparation of plant extract

The *T. cordifolia* 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 ethanol extract of *T. cordifolia* was used for the entire study.

Experimental Animals

Adult Wistar albino rats weighing of 200 - 220 gm breed in the Central Animal House, Department of Pharmacology, College of Pharmaceutical Sciences, 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. The animal was in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD-404 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control group and other three groups were that of plant extract at different doses (1000, 2000 and 4000 mg/kg body weight). Single dose of the extract was administrated orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days.

Experimental design

The animals were randomly divided in to four groups, each containing six animals. Rats were dived in four groups (Group I, Group II, Group III and Group IV) and each group taken in six rats. Group – I: Served as normal, which received, feed and water only. Group – II: Animals of this group were orally administered 100 µg/kg of body weight of aflatoxin along with formulated feed for 45 days. Group – III: Animals of this group were orally administered 100 µg/kg of body weight of aflatoxin along with formulated feed. Then the animals were treated with the ethanolic extract of *T. cordifolia* daily for 45 days at concentration of 500mg/kg of body weight. Group – IV: Animals of this group were orally administered 100 µg/kg of body weight of aflatoxin dissolved in formulated feed. Then the animals were treated with Ethinyl estradiol for 45 days at concentration of 0.01mg of body weight. After 46th days of treated animals were fasted for 12hours after the last dose of drug treatment and were scarified cervical decapitation under mild chloroform anesthesia.

Blood sample collection

Blood (2ml) was collected from each animal via the retro-orbital sinus with 70µl heparinized capillary tube (Ezzai, 1995) and put into plain sample bottle for estrogen analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyze the level of estrogen.

Organ collection

After parturition, the animals were killed by cervical dislocation. The ovaries, uterus heart, kidney and liver were removed and cleared of adherent tissues before they were weighed immediately with an electronic weighing balance with a capacity of 0.1 to 1000g.

Hormonal Assay

Blood samples were collected from the orbital sinus by eye puncture in sterile centrifuge tubes just before scarification, and centrifuged at 3000 rpm for 15 min. for serum collection and stored at -20C°for further hormonal analysis.

Result

Effect of *T. cordifolia* on body weight of female albino rats

There was no significant difference in the mean body weight of all the groups, before and throughout the treatment period when compared with the control group. However, there was a weight gain of 7.4%, 8.5%, 9.6%, 9.7%, 8.9%, 8.9%, 8.3% and 7.2% in the control, aflatoxin, *T. cordifolia* and standard treated groups and their respective recovery, when the weight after treatment (45 days) was compared with the weight before treatment.

Effect of *T. cordifolia* on organ weight of female albino rats

There was significant decrease ($P< 0.05$) in the mean weight of ovary of rats treated with aflatoxin, *T. cordifolia* and standard treated organ weight of *T. cordifolia* when compared with the control group but there was insignificant decrease in the mean weight of ovary in their respective recovery groups when compared with the control as shown in table 2. There was significant decrease ($P < 0.05$) in weight of uterus in different treated group. There was insignificant increase in the mean weight of the uterus in the ethanolic extract of *T. cordifolia* treated group body weight of aflatoxin when compared with the control group.

Hormonal Assay

Treatment had no significant effect on estrogen concentrations ($P>0.05$). Estrogen concentrations were significantly low in animals of treatment aflatoxin compared with animals of the control group. A slight increase of estrogen concentrations was observed in animals of both treatment ethanolic extract of *T. cordifolia* and standard compared to control (Table 3). Progesterone concentrations were significantly influenced by the treatment ($P < 0.05$), whereas a significant decrease in progesterone concentrations compared with the control group. Significant increase of progesterone concentrations was observed in animals of both treatment ethanolic extract of *T. cordifolia* and standard compared to control. Testosterone concentrations are also significantly influenced by the aflatoxins treatment ($P<0.05$). Animals of control group have significantly low testosterone concentrations compared to those of the control group but animals of treatments with ethanolic extract of *T. cordifolia* and standard have insignificantly low testosterone concentrations compared to control one. Table 3 shows that there were significant higher levels of LH and FSH significant lower levels of progesterone in infertile females group compared to controls.

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. Female infertility accounts for approximately 40% of all infertility cases, ovulatory disorders are a predominant cause for women not being able to conceive and accounts for 25% of female infertility (Paul and Lauren, 2004).AFB was reported to exert deleterious effects on the reproductive capacity of lab and domestic female animals (Abdelhamid *et al*., 2007).

The delay in estrous circle is probably due to the fact that aflatoxin treatment inhibits the hormonal function probably while compromising cellular integrity and function. Aflatoxicosis may impair reproductive efficiency including abnormal estrous cycle (too short and too long) (Cassel *et al*., 1988). Compounds that have been reported to destroy or impair the growth of ovarian follicles can have marked effects on cyclicity.

Aflatoxins are very potent toxins affecting the growth of all animals, delay in genital system growth (Hafez *et al*., 1982), high disturbances in estrous cycle, reduced pregnancy rate and number of live new born, failure in nidation and intrauterine death of the foetus (Shapour and Saeedeh, 2013).

In this study aflatoxin administration led to significant decrease in the levels of some reproductive hormones such as estrogen, progesterone, testosterone, LH and FSH. After prolonged AFB administration and also during the oestrus cycle in goats, decreased progesterone concentrations during the luteal phase and decreased oestradiol-17β concentrations during oestrous synchronization were also confirmed by Kourousekos *et al*. (2008) who also proposed that a direct effect of AFB either on ovarian secreting cells or on the hypothalamus–hypophysis–ovarian axis.

Most authors hypothesise that aflatoxin may affect the reproductive system by its toxic effect on the liver, where the cellular hepatic damage could inhibit enzyme synthesis and/or enzyme activity or inhibition of lipid metabolism or fatty acid synthesis, causing decreased synthesis of precursor molecules for gonadal as well as gonadotropic hormones, e.g. FSH, luteinizing hormone (LH) oestrogen, testosterone and progesterone (Handan and Güleray 2005).

Aflatoxin may also affect the reproductive system by causing lysis of germ cells, as it was known that aflatoxin at graded doses induced severe oxidative damage in the testis and accessories that promoting their apoptosis (Kawkab *et al*., 2012).In vitro evidence from animal model and clinical studies suggests that reactive oxygen species (ROS) plays a role in the aetiology of adverse reproductive events (Walsh *et al*., 2000; Acevedo *et al*., 2001; Sikka, 2001) causing the generation of radical species exceeds scavenging by antioxidants as a result of excessive production of ROS and/or inadequate intakes or increased utilization of antioxidants. Increased ROS is associated with decreased steroidogenesis and their cyclic productions contribute to a decline ovarian function (Kodaman and Behrman 2001).

In the current work, the severity of the pathological alterations observed in the Ovaries and uteri of aflatoxins treated female rats were dose dependent and both treatment ethanolic extract of *T. cordifolia* and standard compared to control, since the ovaries showed severe atrophy in most of examined cases, decreased number of functional ovarian follicles compared to the presence of multiple corpra lutae, these lesions agreed with that mentioned by Shapour and Saeedeh (2013).

Table 1: Effect of *T. cordifolia* extract on body weight of aflatoxin intoxicated female rats

Treatment group	Before weight (g)	Weight of 15th day (g)	Weight of 30th day (g)	Weight of 45th day (g)
Control	213 ± 2.43	228 ± 3.25	236 ± 3.11	248 ± 2.17
Aflatoxin treated group	216 ± 3.18	218 ± 3.37	224 ± 2.09	228 ± 3.12
Aflatoxin + *T. cordifolia*	214 ± 3.09	219 ± 3.14	228 ± 3.25	235 ± 2.98
Aflatoxin + Standard	215 ± 3.18	221 ± 2.97	230 ± 3.18	241 ± 3.25

Table 2: Effect of *T. cordifolia* extract on organ weight of aflatoxin intoxicated female rats

Treatment group	Ovary (g)	Uterus (g)	Heart (g)	Kidney (g)	Liver (g)
Control	0.125 ± 0.05	0.53 ± 0.02	0.63 ± 0.06	0.71 ± 0.12	6.12 ± 0.15
Aflatoxin treated group	0.062 ± 0.01	0.41 ± 0.03	0.54 ± 0.11	0.67 ± 0.17	4.89 ± 0.19
Aflatoxin + *T. cordifolia*	0.089 ± 0.04	0.46 ± 0.02	0.56 ± 0.12	0.68 ± 0.11	5.10 ± 0.18
Aflatoxin + Standard	0.112 ± 0.03	0.49 ± 0.06	0.60 ± 0.02	0.69 ± 0.10	5.65 ± 0.22

Table 3: Effect of *T. cordifolia* extract on hormonal assay in the aflatoxin intoxicated female rats

Treatment group	Estrogen (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/dl)	LH (IU/L)	FSH (IU/L)
Control	39.5 ± 2.58	22.8 ± 1.27	0.72 ± 0.17	7.81 ± 0.15	4.92 ± 0.18
Aflatoxin treated group	21.9 ± 2.12	15.8 ± 1.31	0.44 ± 0.12	20.15 ± 0.72	9.12 ± 0.22
Aflatoxin + *T. cordifolia*	32.4 ± 1.98	19.5 ± 1.42	0.65 ± 0.28	10.56 ± 0.81	6.12 ± 0.15
Aflatoxin + Standard	35.8 ± 2.19	21.1 ± 1.67	0.69 ± 0.12	9.14 ± 0.17	5.52 ± 0.12

Conclusion

The investigations led to the conclusion that aflatoxins are severely toxic to female reproductive system. The manifestations include severe hormonal changes in the ovaries and uterus associated with decrease in the levels of some reproductive hormones such as estrogen, progesterone, testosterone, LH and FSH. Thus, chronic exposure of female rats to aflatoxins can bring about deterioration of female reproductive health. The *T. cordifolia* could become helpful for patients with damaged liver and ovaries possibly by reducing hormone parameters. These results finding shows that *T. cordifolia* extract have the ability to rectify anti-ovary cyst or toxicity. Hence it is advised that if one happens to take any ovarian toxic drugs in overdose they can consume *T. cordifolia* extract as a anti-ovarian agent. Thus always have in mind that "Prevention is better than cure".

References

1. Abdelhamid, A.M., M.F.I. Salem, A.I., Mehrem, M.A.M. El-Shaarawy. 2007. Nutritious attempts to detoxify aflatoxic diets of tilapia fish: fish performance, feed and nutrient utilization, organs indices, residues and blood parameters. Egypt J Nutr Feeds **10**(1):205–223.

2. Acevedo, C.G., G. Carrasco, M. Burotto, S. Rojas, I. Bravo. 2001. Ethanol inhibits L-arginine uptake and enhances NO formation in human placenta. Life Sci. **68:**2893–2903.
3. Cassel, E.K., S.M. Barao, D.K. Carmal, 1988. Aflatoxicosis and ruminants. Texas Vet. Med. Diagnostic lab, Tesas collage. The national dairy database (1992) NDB, Health, Test, Hf100200. TxT.
4. Denissenko, M.F., Cahill, J., Kondriakova, J.B., Berger, N., Pfeifer, G.P. 1999. Quantitative and mapping of aflatoxin B1-8,9-exposed and microsomal activation systems. Mutation Res., **425:** 205-211.
5. Doerr, J.A, Ottinger, M.A. 1980. Delayed reproductive development resulting from aflatoxicosis in Juveile Japanese quail. Poultry Science, **59**: 1995-2001.
6. Ezzai S. El-Daly. 1995. Effect of intrathecal morphine on blood glucose, glucagon and tissue glycogen in rat, comparison with the effect of Xanthan gum on blood glucose. J. Isl. Aca. of Sci. **8 (1):** 3-8.
7. Hafez, A.H., S.E. Megalla, H.M. Abdel-Fattah, Y.Y. Kamel, 1982. Aflatoxin and aflatoxicosis. II. Effects of aflatoxin on ovaries and testicles in mature domestic fowl. Mycopathlogica **77**(3):137–139.
8. Handan, U. and A.Güleray, 2005. Selenium protective activity against aflatoxin B1 adverse effects on Drosophila melanogaster. Braz. Arch. Biol. Technol. **48:**2.
9. Hendrickse RG. 1991. Clinical implications of food contaminated by aflatoxins. Ann.Acad. Med., **20P:** 84-90.
10. Hossam El-Din, M.O. 2013. Mycotoxins-Induced Oxidative Stress and Disease, Mycotoxin and Food Safety in Developing Countries, Dr. Hussaini Makun (Ed.), ISBN: 978-953-51-1096-5, InTech, DOI: 10.5772/51806.
11. Kawkab A.A., M.M. El Mahady, S.A. Badawy, Y.F. Ahmed, M.A. Aly, 2012. Pathological Consequences of Aflatoxins in Male Rabbit: Cellular, Genetic and Oxidative Damage. Global Veterinaria, **8** (7): 721-731.
12. Kodaman, P. H. and H. R. Behrman, 2001. Endocrine-regulated and protein kinase C-dependent generation of superoxide by rat preovulatory follicles. Endocrinology, **142:** 687–693.
13. Kourousekos, G.D., E. Theodosiadou, H. Ververidis, S. Belibasaki, C. Boscos, P. Saratsis, A.G. Lymberopoulos, 2008. Effect of aflatoxin B1 administration on serum steroid hormones concentrations of goats. Reproduction in Domestic Animals, **43**, 102.
14. Mathew George, Lincy Joseph, Minu Mathew. 2016. Tinospora cordifolia; A Pharmacological Update. The Pharma Innovation Journal, **5(7)**: 108-111.
15. Naidu, NRG., Sehgal, S., Bhaskar, KVS., Aikat BK. 1991. Cystic disease of the liver following prenatal and perinatal exposure to aflatoxin B1 in rats. Journal of Gastroenterology and Hepatology, **6** (4): 359-362.
16. OECD, 2008. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints. OECD Series on Testing and Assessment, No.19. Organisation for Economic Cooperation and Development. Paris, 39 pp.
17. Paul, D.S and A.R. Lauren, 2004. Management of ovulatory dysfunction in the infertile couple. Gunderson Lutheran Med. J. **3:**21–25.
18. Salem MH, Kamel KI, Yousef MI, Hassan GA, El-Nouty, FD. 2001. Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B1. Toxicology, **162** (3): 209-218.
19. Shapour, H and A.Saeedeh, 2013. Aflatoxin B1 effects on ovarian follicular growth and atresia in the rat. Comp. Clin. Pathol. **22:** 563–572.
20. Sikka, S.C. 2001. Relative impact of oxidative stress on male reproductive function. Curr. Med. Chem. **8:** 851–862.
21. Walsh, S.W., J.E. Vaughan, Y. Wang, L.J. Roberts. 2000. Placental isoprostane is significantly increased in preeclampsia. FASEB J., **14**(10): 1289 – 1296.