Chlorpyrifos- Induced Oxidative Stress and Tissue Damage in the Liver of Swiss Albino Mice: the Protective Antioxidative Role of Root Extract of Withania Somnifera

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Abstract

Chlorpyrifos (o,o-diethyl-3,5,6-trichloro-pyridyl phosphothionate, CPF) is a pesticide which induces oxidative stress thought to be due to enhanced production of reactive oxygen species (ROS). The Toxicity of pesticides causes an exposure on activities of Glutathione reductase (GR), Superoxide dismutase (SOD), lipid Peroxidation (LOP), Glutathione peroxidase (GPx), Glutathione s-transferase (GST) and catalase (CAT) was studied in Swiss albino mice. Due to their antioxidant property, alkaloids and steroidal lactones in plant roots of Withania Somnifera extract may afford protection from CPF toxicity. One of the most important targets of reactive oxygen species (ROS) is the membrane lipids, which undergo Peroxidation that alters the lipid milieu and structural and functional integrity of cell membrane, it also affects the activity various membrane-bound enzyme, including AChE and different ATPases. The present study mainly focus on the protective antioxidative role of alkaloids and steroidal lactones of Withania Somnifera in the liver of SAM against toxicity caused by the organophosphorus pesticide; chlorpyrifos (CPF).

Keywords: Chlorpyrifos, oxidative stress, antioxidants, LOP, SOD, GST, GR, GPx, CAT ATPase & AChE.

I. INTRODUCTION

Withania somnifera, also known as Ashwagandha, Indian ginseng, Winter cherry, Ajagandha, Kanaje Hindi, Amukkuram in Malayalam and Samm Al Ferakh, is a plant in Solanaceae or nightshade family.

It grows as a stout shrub that reaches a height of 170 cm (5.6 ft). Like the tomato which belongs to the same family, it bears yellow flowers and red fruit, though its fruit is berry-like in size and shape. Ashwagandha grows prolifically in India, Nepal, Pakistan, Sri Lanka and Bangladesh. It is commercially cultivated in Madhya Pradesh (a state in India).

In Ayurveda ashwagandha is considered a rasayana herb. This herb is also considered an adaptogen which is an herb that works to normalize physiological function, working on the HPA axis and the neuroendocrine system. In Ayurveda, the fresh roots are sometimes boiled in milk, prior to drying, in order to leach out undesirable constituents. The berries are used as a substitute for rennet, to coagulate milk in cheese making.

The species name somnifera means "sleep-inducing" in Latin, indicating that to it are attributed sedating properties, but it has been also used for sexual vitality and as an adaptogen. Some herbalists refer to ashwagandha as Indian ginseng, since it is used in ayurvedic medicine in a way similar to that ginseng is used in traditional Chinese medicine.

The main constituents of ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, sommiferine, withananine, pseudo-withanine, tropine, pseudotropine, cuscohygrine, anferine and anhydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, having six membered lactone ring.
II. MATERIALS AND METHODS

Withania Somnifera is one of the major constituents used for reduce oxidative stress of an animal inducing humans. One of the major factors that induce the oxidative stress of an animal is by inducing its production of free radicals. Hence the present study was aimed to analysis the effect of Withania Somnifera on different parameter of the taken animals. W.S Root was taken for the experimental was collected from the “Vetdhapuri Agriculture Research Center” (Chithathoor). The root was washed with distilled water, dried for 15 days and used for the experiment. The dried roots were grinded nicely and used for the experiments.

III. EXPERIMENTAL DESIGN AND BIOCHEMICAL ANALYSIS

24 Swiss albino mice, 7-8 weeks old, approximately 25 to 30 grams weighed were bought from Animal House of Indo-American College, Cheyyar. The animals were maintained on standard laboratory condition; diet and water ad libitum. All animals are kept in a neat cage, bottomed with husk. All the animals received professional human care in compliance with the Guidelines of the ethical Committee of Indo-American College, (Regno: NO 1142/ab/07/CPCSEA)

After one week, animals were divided in to four groups (six mice each). Group I was orally administrated 100µl of corn oil. Group II received 100µl of corn oil containing ED50 Concentration of Somnifera root extract. Group III received 100µl of corn oil containing 20ml/kg/mice of o,o-diethyl-3,5,6-trichloro-pyridyl phosphothionate (CPF) and Group IV received ED50 Concentration of Somnifera root extract along with 100µl of corn oil followed by 100µl of corn oil containing 20ml/kg/mice of o,o-diethyl-3,5,6-trichloro-pyridyl phosphothionate (CPF) after 6 hours. This oral administration of the above mentioned were carried out for 28 days.

Mice were then sacrificed at the end of treatment. The liver, were quickily excised and plunged into sterile, ice-cold saline for removal of blood. The washed organ was blotted dry on sterile filter paper and immediately stored in deep freezer at -80°C. The blood samples were collected, Serum was separated an stored at -70°C. Serum AST, ALT were measured according to Reitman and Frankel . The Enzymic antioxidant Catalase CAT was assessed by the method of Luck,974 (CAT, EC.1.11.1.6) Lipid peroxidation (LPO), SOD, GSH, glutathione reductase (GSR) and glutathione-S-transferase (GST) were determined in the liver homogenate according to Esterbauer and Cheeseman, Niskikimi et al Eillman, Zanetti and Habig et al, respectively.

IV. STATISTICS ANALYSIS

The statistics difference between measurement of Test (CPF induced and Treated with root extract of Withania Somnifera) and Control where analyzed followed the alteration in the average standard deviation values. The difference various consider for the calculate percentage variation exhibited by root extract of Withania Somnifera treated oxidative stress induced mice against oxidative stress induced mice.

Statistical analysis was carried out using the SPSS for Windows Version 8.0 program. Data for each group of animals was subjected to analysis of variance (ANOVA). Values are given as mean ± standard deviation (SD). The statistical evaluation of the results was carried out using two-tailed, paired student's t-tests. Significance was set at P < 0.01. A student's t-test was used to compare the difference between treated and control groups. Statistical significances of differences were calculated with one-way analyses of variance followed by Student-Newman-Keul's multiple-range test.

V. RESULT

A. Protective effect of root extract of Withania Somnifera on CPF-induced hepatotoxicity

Our data revealed that the administration of CPF induced a significant increase (P<0.01) in ALT and AST activity in serum as compared to control. Treatment with root extract of Withania Somnifera showed a significant decrease in serum ALT and AST activity (P<0.01; Figure 1) induced by CPF.

Fig. 1: Effect of CPF after root extract of Withania Somnifera treatments on serum ALT and AST activity in S.A.M.
Data are represented as percentage of change ± percentage of standard error. Significance change is calculated as p<0.01(CPF) compared to untreated control and p<0.01 compared to CPF-W.S treated mice.

**B. Effect of W.S on CPF induced liver oxidative stress**

The CPF dose showed a significant increase (P<0.01) in hepatic lipid peroxidation level (LPO) compared to control groups (fig 2). S.A.M that received W.S showed a significant decrease (P<0.01) in CPF administrated S.A.M treated with W.S (fig 2). The effective of W.S showed a greatest improvement of LPO (fig 2).

Furthermore, the CPF dose caused a significant decrease (P<0.01) in hepatic glutathione level (GSH), Gluthione reductase (GSR), Catalase (CAT), Super oxide dismutase (SOD) & GST activity, compared to the control (fig 3).

**VI. DISCUSSION**

The protective role of Withania Somnifera on oxidative stress which is induced by CPF was proved in this project by 3 different parameter results. In addition to the reduction of oxidative stress, the Withania Somnifera also used as a medicine for various disorders with combination to some other herbal plants. The presence of CPF in excess will induces the oxidative stress to above normal by damaging tissue of Brain and Liver and also made some alteration in GSH, LOP, SOD, Bilirubin, AST, ALT, Urea and some other normal levels.

Brain and Liver tissues are the main target for alteration of GSH, LOP, SOD AChE, ATPases and Lipid Peroxidation in the respective tissues which was proved by some standard assay procedure of this respective enzyme.

Chlorpyrifos (o,o-diethyl-3,5,6-trichloro-pyridyl phosphothionate, CPF) is a major environmental toxic suggested to increase the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury. Since liver is the main site of CPF metabolism, the production of ROS in the liver may be responsible for its carcinogenic effects.

This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. Serum AST, ALT and ALP are biomarkers in the diagnosis of
hepatic damage because they are released into the circulation after cellular damage. W.S prevents liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes.

CPF is well known to generate free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress and carcinogenesis. Lipid peroxidation plays an important role in carcinogenesis and may lead to the formation of several toxic products, such as malondialdehyde (MDA) and 4-hydroxynonenal. These products can attack cellular targets including DNA, thereby inducing mutagenicity and carcinogenicity. In line with this finding there was a significant increase in the level of lipid peroxidation in the liver of rats treated with CPF. However, groups treated with W.S displayed a significant reduction in lipid peroxidation when compared to animals treated with CPF alone.

Free radical scavenging enzymes such as superoxide dismutase (SOD) protect the biological systems from oxidative stress. The current study showed a significant decrease in SOD activity in rats treated with CPF. On the other hand, there was a significant increase in SOD activities in groups treated with W.S. Oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance towards a lower oxidative stress status. The present data show that injection of CPF caused depletion of GSH, which may be responsible for the increased lipid peroxidation. Treatment with W.S increased the GSH content in the liver compared to animals treated with CPF alone.

A significant decrease in the activity of GSH dependent enzymes, GST and GSR was observed in CPF treated mice. This may be due to the decreased expression of these antioxidants during hepatocellular damage. Administration of W.S, to CPF induced rats significantly increased the activity of GSR. Our study shows that treatment with W.S improved the activities of GSR and GST. This improvement may have resulted from changing the tissue redox system by scavenging the free radicals and improving the antioxidant status in the liver during CPF hepatotoxicity.

The present study shows that serum NO level significantly increased in CPF treated animals. It has been reported that elevated levels of lipid peroxidation stimulates host cells, mainly monocytes/macrophages, to produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage. In the view of the current data, W.S has been found to decrease serum NO level in rats injected with CPF. The inhibitory effect of W.S on NO is mainly due to the inhibition of the induction of iNOS protein/enzyme.

VII. CONCLUSION

The present study suggests that the Alkaloids and Steroidal Lactones extract of Withania Somnifera can prevent or slow down the oxidative damage induced by CPF in SwissAlbinoMice. The effects of CPF on Lipid peroxidation(LPO), Acetyl choline esterase(AChE), ATPases activity and on Super oxide dismutase(SOD) activity by Reactive oxygen species which was induced by the taken CPF were reversed by treatment with Alkaloids and Steroidal Lactones root extract of Withania Somnifera. Further studies to identify the active compounds in the Alkaloids and Steroidal Lactones root extract of WithaniaSomnifera, and determine their structure and mechanism of action are in progress. In addition, further work is required to clarify how this Alkaloids and Steroidal Lactones root extract of Withania Somnifera increases antioxidant enzyme activity (directly by gene transcription or indirectly through superoxide production).

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