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EFFECT OF ASHWAGANDHA ON LIPID PROFILE AGAINST ENDOSULFAN INDUCED TOXICITY IN MICE

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ABSTRACT

India is an agrarian country with crops cultivated at a huge scale. Pesticides in recent times have caused serious health hazards in the population which are widely used by the farmers for the better yield of crops. Endosulfan is an organochlorine pesticide, which is widely used by the farmers. But, in the present times, it has caused serious health hazards in the exposed population causing various diseases, including cancer. Hence, the present study on animal aims to observe the protective effect of *Withania Somnifera* (Ashwagandha) against endosulfan induced toxicity in Swiss albino mice. Endosulfan at the dose of 3.0mg/Kg body weight per day was administered orally to Swiss albino mice for 4 weeks. Then after, *W. somnifera* at the dose of 1000 mg/Kg b.w. was orally administered for 4 weeks. Mice were sacrificed after the completion of the entire treatment. After dissection, the blood samples were collected for biochemical assay, especially for lipid profile analysis. The lipid profile study showed inclination on in the Total cholesterol level (117±6.686 mg/dl), Cholesterol (LDL) (78.83±4.151mg/dl), level and Triglycerides level (60.83±2.613mg/dl), while declination in Cholesterol (HDL) (13.50±1.33mg/dl), level after Endosulfan exposure. But, upon *Withania somnifera* treatment to the endosulfan treated group showed significant ($p<0.001$) normalisation in the lipid profile levels. Therefore, it was concluded that *Withania somnifera* played a vital role to control the endosulfan induced toxicity.

KEYWORDS: Endosulfan; Lipid profile; Swiss albino mice; *Withania somnifera.*

INTRODUCTION

Indiscriminate use of agrochemicals under conventional agriculture not only causes severe health hazards for human beings but also has numerous other side effects on the environment including destruction of the biodiversity. Endosulfan (hexachloro-hexahydro-methano-benzodi oxathiepinoxide) is an organochlorine (OC) insecticide belonging to class cyclodiene. It is a cyclic sulphurous acid ester with a molecular formula C9H6O3Cl6S and molecular weight 407. Despite its life-threatening toxic effects, endosulfan continues to be one of the most widely used agricultural pesticides, largely in the developing countries, due to its high efficacy, low cost and environmental stability. It is easily absorbed in the gastrointestinal tract, lungs and skin and exposure through various routes and is very hazardous.

Commercially produced endosulfan consists of two iso-mers α endosulfan and β endosulfan. Both these forms have been proved to be genotoxic to human gonads (ATSDR, 2000 and Helle *et al.*, 2002). It has been classified by the World Health Organization (WHO) as Class II – moderately hazardous to human health. However, the United States' Environmental Protection Agency (EPA) rates endosulfan as Category Ib – highly hazardous compound (WHO, 2005; USEPA 2010). Evidence of the threats to human health posed by endosulfan are abundant, and the chemical has been banned outright or severely restricted in a number of countries as a result. Independent of LD50 results, these threats warrant the immediate upgrading of endosulfan to WHO Class I b (Pradhan *et al.*, 1997; EPA 2002; Yavuz *et al.*, 2007). Ashwagandha (*Withania somnifera*, WS), belongs to the family Solanaceae, and is known to be an Ayurvedic herb worldwide for its numerous beneficial health activities since ancient times. It is widely used for the treatment of various diseases such as epilepsy, depression, arthritis, diabetes, and palliative effects such as analgesic, rejuvenating, regenerating, and growth-promoting effects. It has a multifarious effect on vital organs of the body (Mirjalili *et al.*, 2009; Devi *et al.*, 1992; Satyavati *et al.*, 1976). Hence, the present work was aimed to study the protective role of *W. somnifera* against endosulfan induced toxicity in Swiss albino mice.

MATERIALS AND METHODS

Animals

Thirty Swiss albino mice (Mus musculus) were reared in the animal house. 12 weeks old mice weighed 30±2 grams were selected for experiments. The mice were kept in the polypropylene cages with paddy husk at room temperature 28±2ºC and humidity 50±5% in a controlled light (12 hrs light and 12 hrs dark).

Test Chemicals

Pesticide endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment. The pesticide was prepared to 3.0 mg/Kg b.w, which was administered orally to mice for 4 weeks. Commercially available kit for chemical analyses like Serum total Cholesterol, HDL, LDL and Triglyceride were used of crest coral clinical system.

Calculation of LD50 and Maximum Permissible Dose (MPD) of Withania Somnifera (Ashwagandha) aqueous root extract

For calculating the LD50 value of Withania somnifera for mice by standard method was reported by Balachandran and Govindrajan (2005), as 2500 mg/kg b.w as LD50. At 600 mg/Kg & 1100 mg/Kg b.w. although there were no death reports but the no side effects were seen at 1100 mg/kg b.w. So, 1000 mg/Kg b.w. was selected as Maximum Permissible Dose (MPD) for the experiment.

Experimental design

In the present study 30 mice (20 Endosulfan treated and 10 as control mice) were taken and divided into groups - control, Endosulfan treated and ashwagandha treated. The endosulfan at the rate of 3.0mg /kg body weight daily was administered orally for 4 weeks. To this endosulfan treated group ashwagandha at the rate of 1000mg / kg body weight per day was administered for 4 weeks. After the completion of the experiment blood samples were collected by orbital sinus puncture method and Blood samples of each mice group were incubated for 30 min. and then serum samples were separated by the process of centrifugation at 3000 rpm for 15 minutes. These serum samples were kept for further biochemical and hormonal analysis.

Statistical analysis

Results are presented as mean ± S.D and total variation present in a set of data was analyzed through one-way analysis of variance (ANOVA). Difference among means has been analyzed by applying Dunnet's 't' test at 99.9% (p< 0.001) confidence level.

RESULTS AND DISCUSSION

The lipid profile study showed inclination in the total cholesterol level (112±5.73 mg/dl), cholesterol (LDL) (82± 4.51mg/dl), level, and triglycerides level (62±2.82mg/dl), while declination in cholesterol (HDL) (14±1.43mg/dl) level after Endosulfan exposure to mice. But, after ashwagandha treatment total cholesterol levels (82±4.32mg/dl), LDL cholesterol levels (52±3.75mg/dl) and triglycerides levels show decreased (43±2.23mg/dl) in the levels while there was significant (P<0.001) increase in the HDL cholesterol levels (19.83±1.65mg/dl) denoting the protective effects (Fig.1-4).

Lipids are an important component of the living system as it holds in the central position in the metabolism of various functions of the body such as precursor of steroid hormones and is an important constituent of the membrane which maintains the fluidity and fragility (Shell, 1961; Lehninger, 1975; Suhail *et al.*, 1988). They are mainly synthesized in the liver, while bio-chemical parameters such as lipid profile are the important indicator of the status of internal metabolic function of the body. Most of the pesticides usually cause a deleterious effect on the membrane causing serious damage to them (Agrahari and Gopal, 2009). The plethora of studies have been carried out which shows the impact of pesticides on the biochemical parameters of different fishes (Pant and Singh, 1983; Bhushan *et al.*, 2002; Mohamed and Gad, 2008; Jen-kins *et al.*, 2003).

In the present study, endosulfan caused significant (P<0.001) hyperlipidemia in the exposed mice as it had caused hepatotoxicity in them, leading to malfunctions in cholesterol synthesis and storage in the liver. Although the liver is the vital organ of the body which detoxifies the toxicity of the pesticides but to regulate the function, it requires lots of energy for the detoxification. Lipid metabolism is activated by due to which biosynthesis of different classes of lipids takes place. Endosulfan, unfortunately, enhances the levels of lipid profile parameters such as cholesterol and triglyceride levels.

Hence, it can be speculated that increased lipid profile is one of the compensatory mechanisms of the animal to detoxify the pesticide toxicity impact. The inclination in serum lipid content (total cholesterol level, cholesterol (LDL) level; triglycerides level, while Cholesterol (HDL) levels resulting in hyperlipidaemia is certainly due to stress-induced by pesticide poisoning for longer periods. The test animals in the present study were observed to be restless throughout the exposure period. They were in constant fast movements aided by muscular action. Lots of extra energy was required to minimize the stress induced by endosulfan. The observed high lipo-protein level during the present investigation may be also due to impairment in the membrane organization and damage to the liver. Various studies have observed the impact of pesticide on increased cholesterol levels. Medicinal plants have a potent protective effect in controlling the hyperlipidemic effect (Deokar, 1998; Cuvelier *et al.*, 1992).

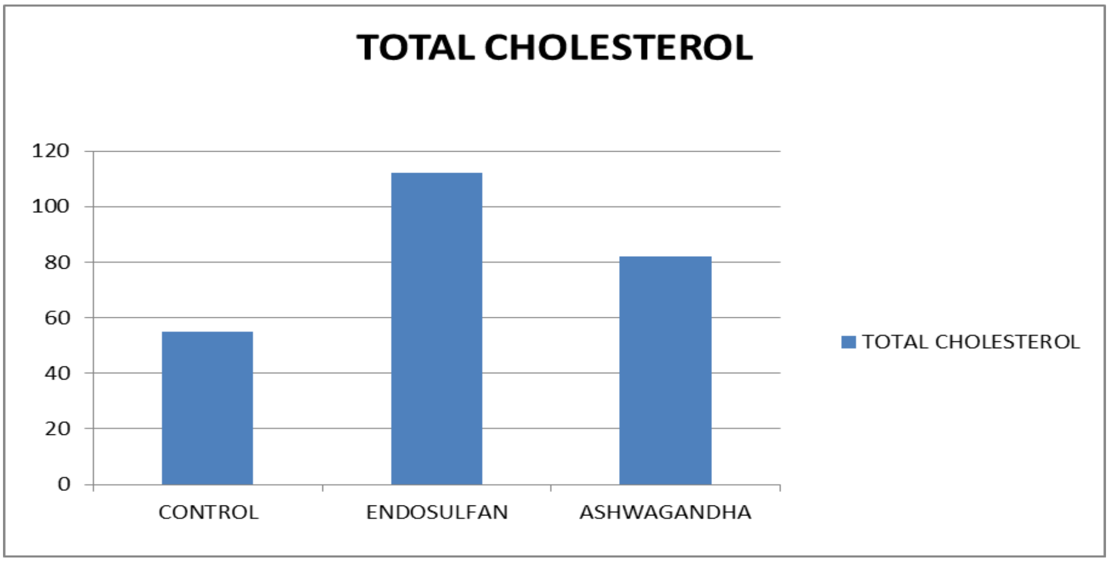

Fig.1. Effect of WS on Endosulfan treated group showing total cholesterol levels (n=6, values are mean ± S.D).

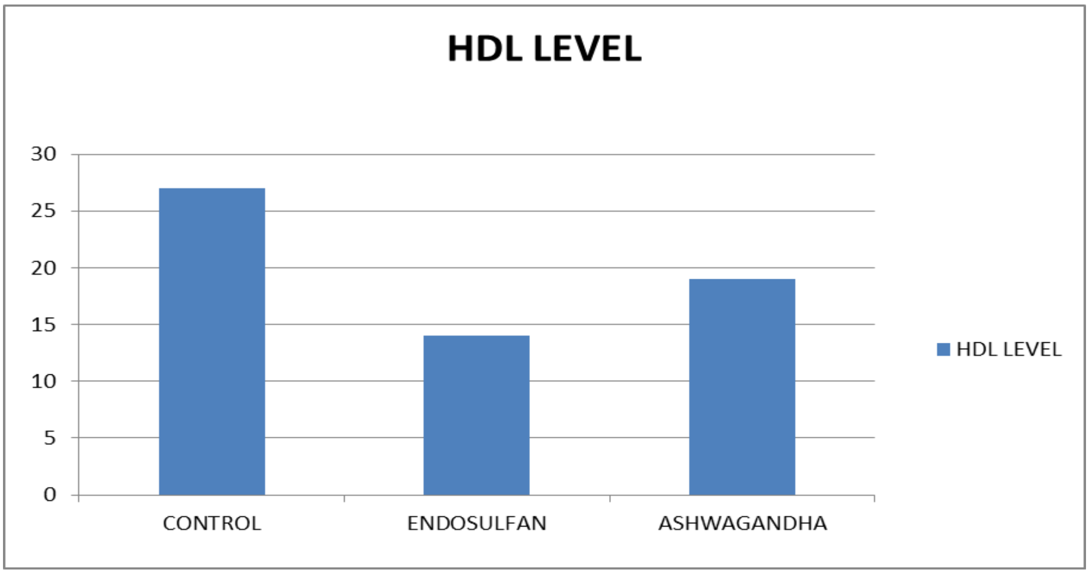

Fig.2. Effect of WS on Endosulfan treated group showing cholesterol HDL levels (n=6, values are mean ± S.D).

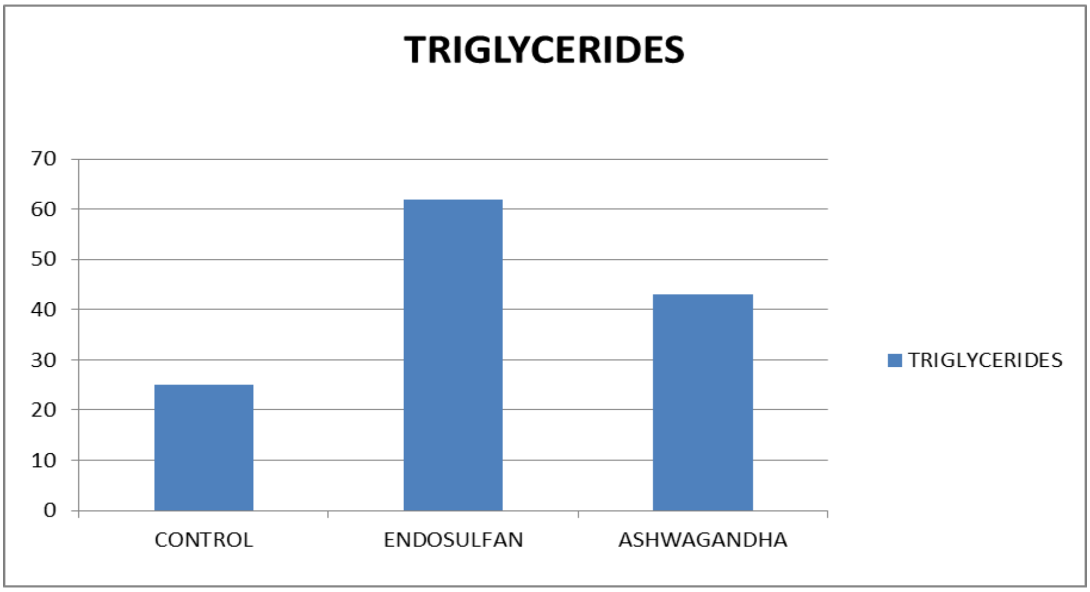

Fig.3. Effect of WS on Endosulfan treated group showing triglycerides levels (n=6, values are mean ± S.D.)

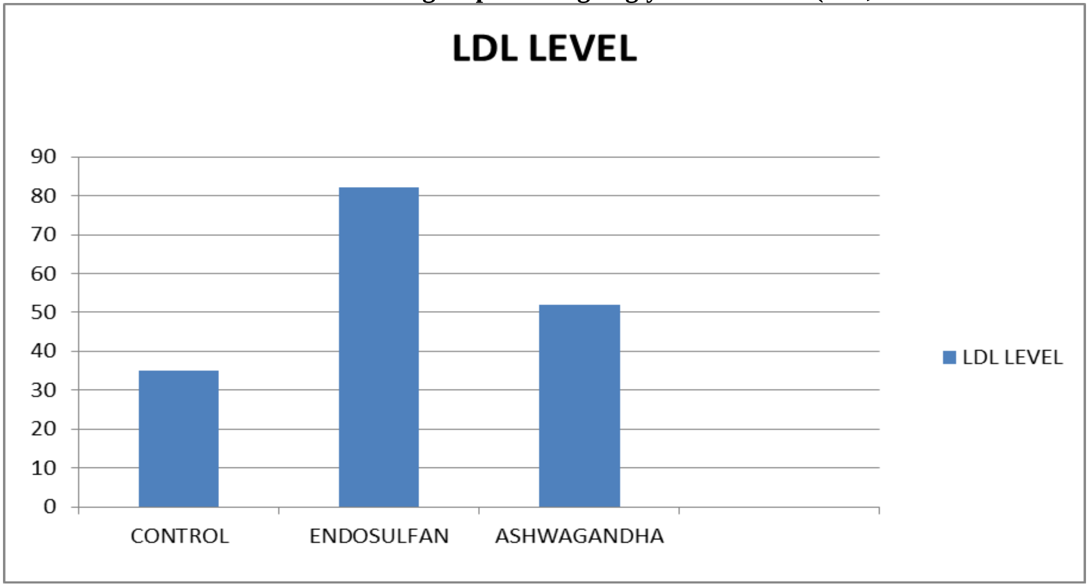

Fig. 4: Effect of WS on Endosulfan treated group showing cholesterol LDL levels (n=6, values are mean ± S.D).

In the present study, there was a significant restoration in the lipid profile levels Hence, from the entire study, it can be concluded that *Withania somnifera* plays a vital role to combat the deleterious effect of endosulfan as *withania somnifera* protects cardiovascular functions.

CONCLUSION

Endosulfan caused a deleterious effect on the lipid profile of the Swiss albino mice at the dose of 3.0mg/Kg b.w but after the treatment of root extract of WS at the dose of 1000mg/Kg b.w, there was a significant restoration in the lipid profile levels such as in the total cholesterol, LDL cholesterol, HDL cholesterol and tri-glycerides levels. Thus, *Withania somnifera* possessed anti-hyperlipidaemic, anti-cholesteric activity controlling the cardiotoxicity caused by the pesticide-induced toxicity

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