Efficacy of *Withania somnifera* on lipid profile of endosulfan induced toxicity in Swiss albino 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* against endosulfan induced toxicity in Swiss albino mice. Endosulfan at the dose of 3mg/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 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 *W. somnifera* treatment to the endosulfan treated group showed significant (p<0.001) normalisation in the lipid profile levels. Therefore, it was concluded that *W. somnifera* played a vital role to control the endosulfan induced toxicity.

**Keywords:** Endosulfan, Efficacy, Hyperlipidaemia, Swiss albino mice, *Withania somnifera*

**INTRODUCTION**
India is an agriculture-based country with a production of the crops at very large scale. But, unfortunately, due to the pests, there is significant damage in crop production. The pest burden is increasing every year due to the appearance of new pests and diseases (Rajendran, 2003; Pimentel 1995). Among all types of pesticides present, organochlorines have proven more mortality of pests or are the best to be used in the pest management (Jayaraj et al., 2016; Akhtar 2009; Gupta 2004).

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 C2H4O2Cl6S 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 (Coutselinis et al., 1976; Yadla et al., 2013). 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 isomers 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 LD95 results, these threats warrant the immediate upgrading of endosulfan to WHO Class I b (Pradhan et al., 1997; EPA 2002; Yavuz et al., 2007; Wilson et al., 2014; Lee et al., 2015).
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 (Pratte et al., 2014; Mirjalili et al., 2009; Rai et al., 2016; Devi et al., 1992; Kumar et al., 2015; Satyawati 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**

**Ethical approval:** Ethical approval was taken from the Post Graduate Research Council (PGRC) of Patna University, Patna with no. PGRC No. Acad - / 464, serial No. 7, dated 12/02/2007.

**Animals:** Twenty-four Swiss albino mice (28g to 32 g) were obtained from the animal laboratory of Dr. A. Nath, Department of Zoology, Patna University, Patna, India. The research work was approved by the Post Graduate Research Council of the Patna University. Food and water to mice were provided *ad libitum* (prepared mixed formulated feed by the laboratory itself). Animals were maintained in colony rooms with 12 hrs light/dark cycle at 22 ± 2°C.

**Chemicals:** The commonly used pesticide- endosulfan was obtained (Excel India Pvt. Ltd. Mumbai with EC 35%). The pesticide was prepared to 3 mg/Kg b.w., which was administered orally to mice for 4 weeks. Commercially available kit for chemical analyses like Serum Cholesterol, HDL, LDL Cholesterol and Triglyceride were used of crest coral clinical system, Goa, India.

**Plant material:** The fresh, dried rhizome of *W. somnifera* (WS) (Ashwagandha) was purchased from the herbal store in Patna, and aqueous extract was made by dissolving it in distilled water using by mortar and pistol. The dose was finally made to 1000 mg/kg body weight for oral administration.

**Experimental Design:** In the present study 24 mice (18 endosulfan treated and 6 as control mice) were taken and divided into groups - control (untreated group), endosulfan treated and *W. somnifera* treated. The endosulfan at the rate of 3mg /kg body weight daily were administered orally for 4 weeks. To this endosulfan treated group aqueous extract of WS at the rate of 1000mg / kg body weight was administered for 4 weeks. After the completion of the experiment blood samples were collected by orbital sinus puncture method and then serum was extracted for lipid profile 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). The difference among means has been analyzed by applying Dunnet’s ‘t’ test at 99.9% (p< 0.001) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

**RESULTS AND DISCUSSION**

The lipid profile study showed inclination 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 to mice. But, after WS treatment total cholesterol levels (90±5.23mg/dl), LDL cholesterol levels (54.67±3.75mg/dl) and triglycerides levels show decreased (41±2.066mg/dl) in the levels while there was significant (P<0.001) increase in the HDL cholesterol levels (20.83±1.77mg/dl) denoting the protective effects (Fig.1,2,3 and 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 biochemical 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; Jenkins et al., 2003; Rajamanickam and Muthuswamy 2008; Yekeen and Fawole, 2011) and on bacteria (Saravanan et al., 2011).

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 (117±6.686 mg/dl), cholesterol (LDL) (78.83±4.151mg/dl) level; triglycerides level (60.83±2.613mg/dl), while Cholesterol (HDL) levels as (13.50±1.33mg/dl) 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 hyperlipidaemia during the present investigation may be also due to impairment in the membrane organization and damage to the liver (Alva et al., 2012). Various studies have observed the impact of pesticide on increased cholesterol levels. A study has also reported the increased level of serum cholesterol and triglycerides, (Gad and Saad 2008; Vinson et al., 2001). Endosulfan also causes oxidative damage to the membrane due to release of ROS leading to lipid peroxidation (Hincal et al., 1995; El-Shenawy 2010; Oliveira et al., 2017). This altogether causes the onset of pathogenesis, causing disease in the animal (Di Rosa et al. 1971; Kobljakov et al., 2001; Sood et al. 2009).

Endosulfan is a known xenoestrogen which causes severe damage to the membrane due to lipid peroxidation. The depletion of the lipids from the membrane makes the blood more viscous, causing the risk of cardiovascular disease. Hence, increased lipid profile parameters can lead to the cause of atherosclerosis and cardiovascular disease. In the present study also, there were significant changes in lipid profile parameters such as an increase in the total cholesterol, LDL cholesterol and Triglycerides while the decrease in the HDL cholesterol hence correlates the above-mentioned pathway.

Thus, there are multiple possible pathways that might lead to selective alteration in rates of synthesis or metabolism of some classes of serum lipids. Lipid profile abnormalities play a significant role in atherosclerosis and cardiovascular disease. The findings of the present investigation indicate that exposure to endosulfan alters the metabolism of cholesterol and thus increases the risk of cardiovascular disease and atherosclerosis in mice (Vandenberg 2020; Xu et al., 2017).

Medicinal plants have a potent protective effect in controlling the hyperlipidemic effect (Deokar, 1998; Cuvelier et al., 1992; Dongmo et al., 2019; Wu et al., 2019).
2019). In the present study, there was a significant restoration in the lipid profile levels such as total cholesterol levels (90±5.23mg/dl), LDL cholesterol levels (54.67±3.75mg/dl), triglycerides levels (41±2.066mg/dl) and HDL cholesterol levels (20.83±1.77mg/dl) due to WS rhizome extract treatment. The active ingredient Withaferin A in WS plays a vital role as the cholesterol-lowering effect as it increases the excretion of cholesterol and bile acids through faecal sterol excretion. The enhanced faecal neutral sterol excretion could be due to the higher fiber content in WS root. Various studies on dietary phytoconstituents of WS rhizome revealed that the dietary fibers are effective in depressing the absorption of exogenous cholesterol from micelles through increased resistance for diffusion in the aqueous luminal medium (Vahouny et al., 1980; Arjmandi et al., 1992; Moundras et al., 1997; Duangjai and Saokaew 2018; Visavadiya et al., 2007 and Duangjai, et al., 2019; Teymouri et al., 2019). Due to greater hydrophobicity, phytosterols have a greater affinity for micelles rather than for the cholesterol. The displaces the intestinal cholesterol from micelles to reduce cholesterol absorption (Miettinen and Gylling, 1999; Ikeda and Sugano, 1998). The consequent reduction in hepatic cholesterol level leads to a compensatory increase in endogenous cholesterol synthesis (Miettinen and Gylling, 1999). The decrease in the triglyceride levels due to WS may be attributed to the fibre contents of the WS which decreases the intestinal transit time for cholesterol and carbohydrate absorption (Ebihara and Schneeaman, 1989) causing decreased hepatic lipogenesis and reduction in hepatic and plasma triglyceride concentrations (Mamo et al., 1991). Hence, from the entire study, it can be concluded that WS plays a vital role to combat the deleterious effect of endosulfan as WS protects cardiovascular functions.

Conclusion
Endosulfan caused a deleterious effect on the lipid profile of the Swiss albino mice at the dose of 3mg/Kg b.w but after the treatment of rhizome 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 triglycerides levels. Thus, W. somnifera possessed anti-hyperlipidaemic activity controlling the cardiovascular disorders caused by the pesticide-induced toxicity.

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Conflict of interest
The authors declare that they have no conflict of interests.

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