WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 1, 1477-1485. Research Article ISSN 2277– 7105

ANTI-HYPERLIPIDAEMIC EFFECT OF *OCIMUM SANCTUM* ON ENDOSULFAN INDUCED TOXICITY

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Article Received on 21 Nov. 2018,
Revised on 11 Dec. 2018,
Accepted on 01 January 2019
DOI: 10.20959/wjpr20191-14009

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ABSTRACT

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 is an organochlorine insecticide effective against a wide range of pests of cereals, coffee, cotton, fruits, oilseeds, potato, tea and vegetables. Moreover, it is easily absorbed by the stomach, lungs and skin and exposure through any route can be hazardous. In the present study, endosulfan was administered orally at the dose of 3mg/Kg body weight per day to Swiss albino mice for 4 weeks. Upon 4 Weeks endosulfan pretreated mice group *Ocimum sanctum* (Tulsi) leaf extracts at the dose of 1000 mg/Kg body weight per day was orally administered for 4 weeks to these groups to observe the ameliorative effect. Mice were sacrificed at each interval and their blood and tissue samples were collected for biochemical assay (for lipid profile). After endosulfan exposure, the lipid profile level shows inclination in the total cholesterol level, cholesterol (LDL) level and triglycerides level while declination in cholesterol (HDL) level. But upon *Ocimum sanctum* leaf extract treatment to the endosulfan treated group showed significant declination in the total cholesterol level, cholesterol (LDL) level and triglycerides level while inclination in cholesterol (HDL) level. Thus, from the above study it can be concluded that although the entrance of these hazardous pesticide (endosulfan) into our body cannot be stopped but by the use of these medicinal plant extracts as potent antidote can solve the problem at much extent, normalizing the physiology of the body and maintaining the cellular integrity and normal functioning of the system.

KEYWORDS: Endosulfan, *Ocimum sanctum*, hyperlipdiaemic effect.

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 is an organochlorine insecticide effective against a wide range of pests of cereals, coffee, cotton, fruits, oilseeds, potato, tea and vegetables. Moreover, it is easily absorbed by the stomach, lungs and skin and exposure through any route can be 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).

Pesticide safety is classified by the World Health Organisation (WHO) according to the results of LD_{50} tests, which document the amount of a chemical required to kill 50% of a population of laboratory rats. Under this system, endosulfan is currently classified as Class II – *moderately hazardous to human health*. However, the United States' Environmental Protection Agency (EPA) rates endosulfan as Category Ib – *highly hazardous*. LD_{50} data for endosulfan are equivocal, with some published results indicating that the chemical should be in the WHO's Class Ib, according to the organisation's own criteria (WHO, 2005). 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 LD_{50} results, these threats warrant the immediate upgrading of endosulfan to WHO Class Ib (EPA 2002).

The World Health Organisation (WHO) estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care needs Medicinal plants being natural, non-narcotic, having no side effect, cost effective, preventive and curative therapies which could be useful in achieving the goal of "Health for all" in a cost effective manner. Demand for medicinal plants is increasing in both developing and developed countries but 90% harvested from wild sources without applying scientific management hence many species are under threat to become extinct.

Ocimum sanctum, Holy Basil (also *tulsi*), is an aromatic plant in the family Lamiaceae which is native throughout the Old World tropics and widespread as a cultivated plant and an escaped weed. It is an erect, much branched subshrub, 30–60 cm tall with hairy stems and simple, opposite, green leaves that are strongly scented. Recent pharmacological studies have established the anabolic, hypoglycemic, smooth muscle relaxant, cardiac depressant, antifertility, adaptogenic and immunomodulatory properties of *Ocimum sanctum* (Chaturvedi et al, 2007).

MATERIALS AND METHODS

Ethical Approval: Ethical approval was taken from 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 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 $22 \pm 2^{o}C$.

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 Cholesterols and Triglyceride were used of crest coral clinical system, Goa, India.

Plant material: The fresh leaves of *Ocimum sanctum* (Local name- Tulsi) were procured from the local garden of (Patna). The leaves were washed with distilled water and crushed and aqueous extract was made by dissolving it in distilled water using by mortal and pistal. The dose was finally made to 1000 mg/kg body weight for oral administration.

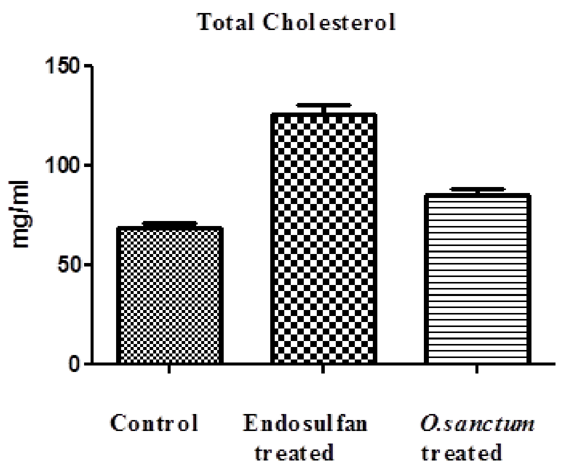
Experimental Design: In the present study 24 mice (18 Endosulfan treated and 6 as control mice) were taken and divided into groups - control, Endosulfan treated and *Ocimum sanctum* treated. The Endosulfan at the rate of 3mg /kg body weight daily were administered orally for 4 weeks. To this Endosulfan treated group *O.sanctum* 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.

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

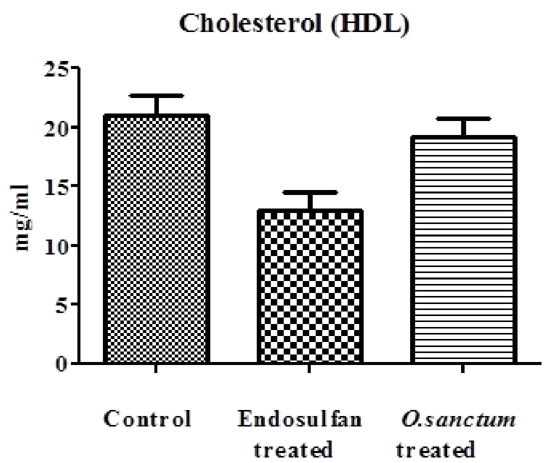
Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

RESULTS

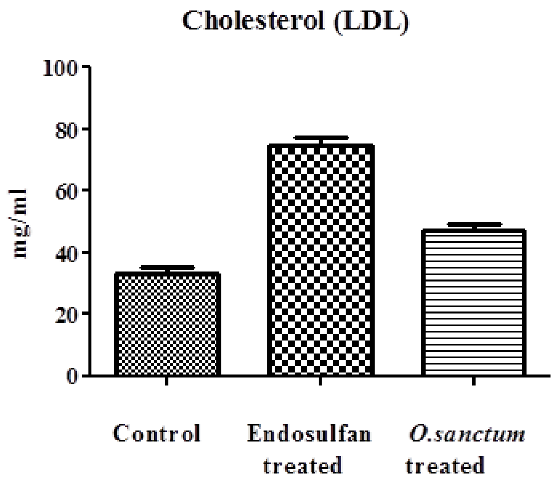
The lipid profile tests total Cholesterol levels, LDL cholesterol levels and triglycerides levels shows inclination in the levels while HDL Cholesterol decreased levels in the Endosulfan treated group in comparison to control group was observed. But, after *O.sanctum* treatment total Cholesterol levels, LDL cholesterol levels and triglycerides levels shows decreased in the levels while there was significant increase in the HDL Cholesterol levels denotes the anti-hyperlipidaemic effects (Graph fig.1,2,3 & 4).



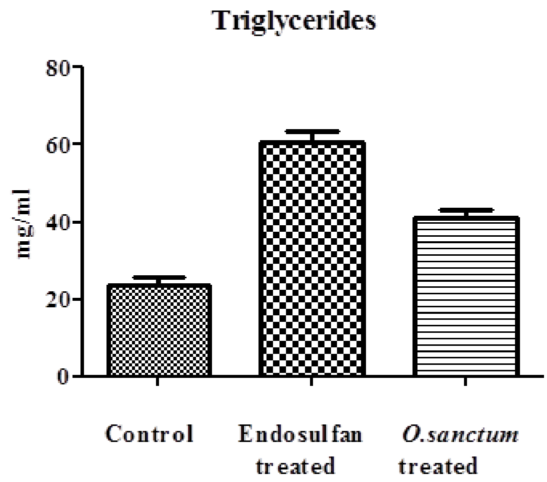
Graph Fig.1. Effect of *O. sanctum* on Endosulfan treated group showing total cholesterol levels (n=6, values are mean ± S.D).



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



Graph Fig.3. Effect of *O. sanctum* on Endosulfan treated group showing cholesterol LDL levels (n=6, values are mean ± S.D).



Graph Fig.4. Effect of *O. sanctum* on Endosulfan treated group showing triglycerides levels (n=6, values are mean ± S.D).

DISCUSSION

Pesticides cause cellular damage to all cells, cardiomyocytes damage can cause irreversible changes in the cells which cannot be rectified. Furthermore, elevated levels of cholesterol & triglycerides can also add damage to cardiomyocytes. But, medicinal plants have potent ameliorative effect in controlling the hyperlipidaemic effect. Mohanty et al (2006) have well studied on effect of *Ocimum sanctum* on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injury. The progressive loss of cardiomyocytes in a heart that is already compromised leads to further deterioration of cardiac function, conduction disturbances due to degeneration of SA, AV and inter-nodal pathway, cardiac remodeling and cardiomyopathy (MacLellan, et al 1997). The fact that apoptosis plays a role in the tissue damage seen after myocardial infarction has pathological and therapeutic implications. If indeed cardiomyocyte apoptosis plays an important role in initiation and progression of cardiac diseases, drugs that effectively and specifically inhibit apoptosis might be useful therapeutic agents for attenuating myocardial injury due to I-R] (Cook et al 1999).In reperfused ischemic hearts increase in oxidative stress, and decrease in antioxidant defense has been reported to lead to cardiac dysfunction partly due to apoptosis (Palojoki et al, 2001).

In the present study, there has been significant increase the lipid profile levels especially the cholesterol and triglycerides levels after endosulfan exposure. The causative reason is that the endosulfan acts as a xenoestrogen binding with the lipid membranes and causing severe damage to them due to lipid peroxidation activities. The lipid peroxidation process in turn causes depletion of lipid from the membranes making the blood more viscous and enhancing the cardiovascular function damage leading to cardiac failure. But, the *Ocimum sanctum* in the study plays a significant role in combating the damage made by endosulfan. The active ingredient eugenol possesses the restorative activity of the lipid membranes and making their functions normal. The cholesterol and triglyceride levels have been significantly controlled by the *Ocimum sanctum*. Various other studies have been carried out to by researchers who have observed the efficacy of *Ocimum sanctum*. Other effects of Tulsi have been well documented by many authors Leaf extract of *Ocimum sanctum*, 10 mg/kg (i.p.) for 5 days, protected mice to radioactivity (Ganasoundari 1998). 0.5 g/Kg for 15 days in mice decreased serum T4, hepatic LPO and G-6-P activity, the activities of endogenous antioxidant enzymes, SOD and CAT were increased by the drug. Unaffected were T3 level, T3/T4 ratio and cholesterol (Panda 1998). Inflammation induced by PGE2, leukotriene or arachidonic acid is inhibited by the fixed oil. Activity is proportional to the linolenic acid content (Singh 1998). Radiation effects are inhibited by an aqueous extract of *Ocimum sanctum* leaves 10 mg/kg ip (Ganasoundari 1997). Diet of 1% level Tulsi (*Ocimum sanctum*) leaf powder for a month reduced fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids in rats (Rai 1997). Antiinflammatory activity of *Ocimum sanctum* fixed oil is believed due to linolenic acid inhibition of cyclooxygenase and lipoxygenase pathways of arachidonate metabolism (Singh 1997).

Leaf extract orally at 400 & 800 mg/kg for 15 days to mice increased the activities of cytochrome p-450, cytochrome b5, ary+l hydrocarbon hydroxylase & GST, which help detoxify carcinogens (Banerjee 1996). DMBA induced skin papillomagenesis in mice was decreased by topical treatment with *O. sanctum* leaf extract. The protective GST was increased (Prashar 1994). Diet of 1-2% O. sanctum fresh leaves lowers total cholesterol, triglyceride, phospholipid and LDL-cholesterol levels and increased HDL-cholesterol and total faecal sterol contents in rabbits (Sarkar 1994). Glucose lowering by *O. sanctum* leaves alcoholic extract, orally, was 91% & 70% of tolbutamide in normal and streptozotocin induced diabetic rats (Chattopadhyay, 1993). Carcinogen induced stomach cancer was decreased in rodents by cumin or poppy seed or basil (*O. sanctum*) leaves but not by asafoetida, kandathipili, turmeric, drumstick leaves, solanum leaves and alternanthera (Aruna, 1992). Inflammation induced by carrageenan or croton oil was inhibited by *O. sanctum* aqueous suspension (Godhwani 1987). DMBA-DNA adducts were decreased by pretreatment of hepatocytes with *O. sanctum* extract for 24 h; 93% by 500 microg (Prashar 1998). Preventive and protective effects of *Ocimum sanctum* in ethanol-induced liver toxicity in rats (Chaturvedi et al, 2007). Effect of *Ocimum sanctum* (OS) leaf extract on hepatotoxicity induced by antitubercular drugs in rats were observed (Ubaid et al, 2003). *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice (Sharma et al, 2002).

CONCLUSIONS

Endosulfan causes deleterious effect on the cardiovascular functions in the Swiss albino mice, but after the treatment of *Ocimum sanctum* there was significant control in the lipid profile levels. Thus, *Ocimum sanctum* possesses anti- hyperlipidaemic activity normalizing the physiology of the body and maintaining the cellular integrity and normal functioning of the system.

ACKNOWLEDGEMENT

The authors are thankful to the department and the university for the infrastructural support.

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

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