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Research article

EFFECT OF SHAKTI DROPS® ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN VARIOUS ORGANS OF SWISS ALBINO MICE *MUS MUSCULUS*

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ABSTRACT

There are hundreds of Ayurvedic and herbal products in the market sold under the banner of various spiritual leaders. Common public believe on the attractive advertisements and get impressed to use such products. Even though the products are prepared and marketed by the experts of reputed spiritual Gurus, the research and development work is limited. In the present investigations Shakti Drops® a formulation of Sri Sri Ayurveda was tested for its beneficial effects in liver, kidney, pancreas, spleen and cerebral cortex of Swiss albino mice *Mus musculus.* The animals were supplemented with Shakti drops® twice a day for three months at a dosage of 6µl/ kg body weight. There was a decrease in the lipid peroxidation in Shakti drops® treated group as compared to sham control. The difference was significant in the cerebral cortex and nonsignificant in other organs. There was a significant increase in the activity of antioxidant enzyme: superoxide dismutase in the spleen and pancreas of Shakti drops® treated group as compared to sham control. The catalase activity was significantly increased only in the pancreas of Shakti drops® treated group.

Key Words:- Shakti Drops, Lipid peroxidation, Superoxide dismutase, Catalase.

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INTRODUCTION

Some of the spiritual leaders from India have launched various products for consumers by advertising through different media like television, radios, mass gathering, etc. In 2003, Sri Sri Ayurveda (SSA) and in 2006, Patanjali have initiated marketing of their Ayurvedic products. Sri Sri Ayurveda claims that their products are based on the ancient philosophy and practice of Ayurveda. They offer effective treatments by providing herbal remedies, personal care products and nutritional products. Their some herbal medicinal products are Samahan (claims that it is antipyretic, analgesic, anti-inflammatory and expectorant), AmrutadiVati (claimed to be used for Skin disorders, viral fever, rhinitis, bacterial infection), Chyawanprash (Memory Sharpener, Luster of the skin), Karelajamun juice (Anti-diabetic). SSA products are primarily sold through 600 franchise stores names as 'Divine Shops' at Sri Sri Ravi Shankar's gatherings. They are also available online at bigbasket.com, amazon.com.

'Shakti drops'® is one of the products sold by SSA on a large scale. On the leaflet, it is mentioned that Shakti drops contain pure water extract of Ashwagandha, Amla, Amruth, Brahmi, Brihngraj, Shatavari, Shakhpushpi. SSA claims that the product is helpful in building all round immunity, it acts as a rejuvenator and builds strength. The herbs used in Shakti drops have many benefits as per Ayurveda.

The ingredients of Shakti drops individually have their own beneficial effects. The colour, appearance and the test of the drops are just like water. Therefore, to understand the efficacy of Shakti drops, the present investigations were carried out in Swiss albino mice *Mus musculus*.

MATERIALS AND METHODS

Sri sri Ayurveda Shakti drops were used for the present study which was purchased from a local Ayurvedic shop.

Animal model: All the experimental procedures and protocol performed in this study were reviewed by the Institutional Animal Ethical Committee (IAEC) and were in accordance with the guidelines of CPCSEA (RegistrationNo.1825/PO/EReBi/S/15/CPCSEA). Adult male mice *Mus musculus* were maintained in the animal house of the Department of Zoology, Shivaji University, Kolhapur, Maharashtra, India. The animals were kept in polypropylene cages at an ambient temperature 25±2ºC, 55-65% humidity and 12:12 hours light:dark cycle. The Animals were fed *ad libitum* with commercially available standard rat chow (Nutrivate life sciences, Pune) and drinking water.

Experimental design: In these experiments 6 healthy male mice of age 12 month were used. These animals were randomly separated into two groups.
Group I : Untreated Sham Control
GroupII : Treated with Sri Sri Ayurveda Shakti drops

Administration and dose:-

Sri Sri Ayurveda recommends 5-6 drops twice a day (i.e.250-300 µl) in a cup of water. This accounts for 5-6µl/ kg body weight for an average man of weight 50 Kg. Accordingly the dosage was calculated. Three hours prior to dose administration the animals from sham control group and experimental group, were made thirsty by removing the water bottle from the cage. Then, animals from the experimental group were supplied with water bottle containing Shakti drops at the concentration of 0.15µl/ml for 30 min. Similarly animals from sham control group were supplied with water without Shakti drops.

Prior trials in our lab have shown that each animal drinks 2 ml of water after three hours of water derivation. The dose was given twice a day for a period of three months. Animals were sacrificed by diethyl ether anesthesia and used for the biochemical studies.

Biochemical parameters:-

After completion of the three months treatment mice were scarified by cervical dislocation. Liver, Kidney, Spleen, Pancreas and Cerebral cortex were dissected out and used for various biochemical studies.

Assay of Superoxide dismutase (SOD), Catalase, Lipid peroxidation and Protein estimation in Liver, Kidney, Spleen, Pancreas and cerebral cortex were carried out.

Estimation of protein (Lowry et al 1951): 0.5 ml homogenate was added in 3.0 ml distilled water in a test tube and 5.5ml of 0.5 % alkaline CuSO4 was added into it. After 10 minutes 0.5 ml Folin-Ciocalteau reagent was added and kept for 30 min and absorbance was read at 660 nm against blank by setting O.D. to zero density. Each test was carried out in triplicate.

Estimation of lipid peroxidation (Wills 1966):-

For this purpose, the tissue was frozen for one hour and homogenized in a reaction mixture containing 75 mM phosphate buffer pH 7.04, 1mM ascorbic acid, 1mM ferric chloride at the concentration of 5mg/ml. 0.2 ml homogenate was added in a test tube containing 1.8 ml distilled water. Then 1 ml 20 % TCA and 2 ml 0.67 % TBA were added into it. All the test tubes were placed in a boiling water bath for 10 minutes and cooled. For the blank, 0.2 ml distilled water was added instead of homogenate. Absorbance was read at 532 nm against blank by setting the O.D. to zero. Lipid peroxidation is calculated by the formula-

Lipid peroxidation/mg tissue = O.D. of sample/0.156 x amount of tissue in mg

Where, 0.156 is the absorbance for 1 nM solution of malondialdehyde in 1 cm thick cell at 532 nm.

Estimation of Superoxide Dismutase (SOD) (Beauchamp and Fridovich 1971): Homogenate was prepared at the concentration of 20 mg/ml in chilled homogenization medium containing 0.25M sucrose and 1mM EDTA using chilled pestle and mortar.

Enzyme assay:

3.4 ml of 0.1M phosphate buffer pH 7.8, 0.3 ml 10mM EDTA, 1.2ml of 130mM methionine, 0.6ml of 750 µM NBT were added in each test tube. 0.4 ml of 60 µM riboflavin was added in each test tube to initiate the reaction and exposed to fluorescent lamp of 28W and at a distance of 12 inches for 15 minutes. The tube of the negative control i.e. without any enzyme was also exposed to fluorescent lamp for 15 min. The tube containing blank was kept in complete dark. 0.1ml of the homogenate was added in blank just before setting the OD to zero. Absorbance was read at 560 nm. One unit of SOD activity is the amount of enzyme required to reduce the O.D. to half of the control.

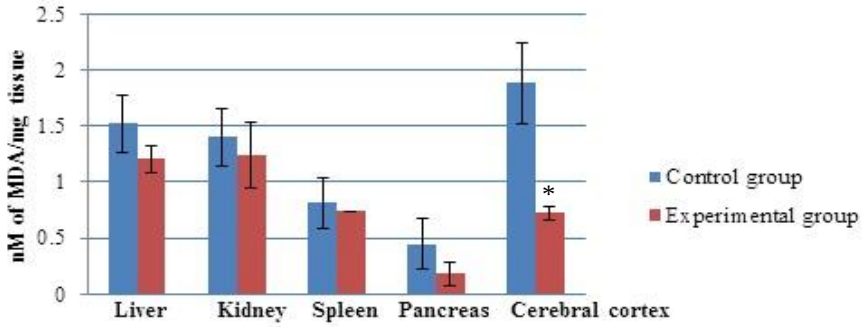
Estimation of Catalase:- (Beers and Sizer 1952) Tissue homogenate was prepared in chilled 75mM phosphate buffer (pH 7) at a concentration of 20 mg/ml using chilled pestle and mortar. Increments of Hydrogen

peroxide in the range of 50 to 100 microliters were added in 100 ml of 75mM phosphate buffer and the OD was set to 0.45 at 240nm. This was used as a substrate buffer for the estimation of catalase activity. 2ml of the substrate buffer was transferred into the cuvette and 0.1ml homogenate was added. The time required to decrease the OD from 0.450 to 0.400 was recorded as Δt. Enzyme activity was calculated using the formula,

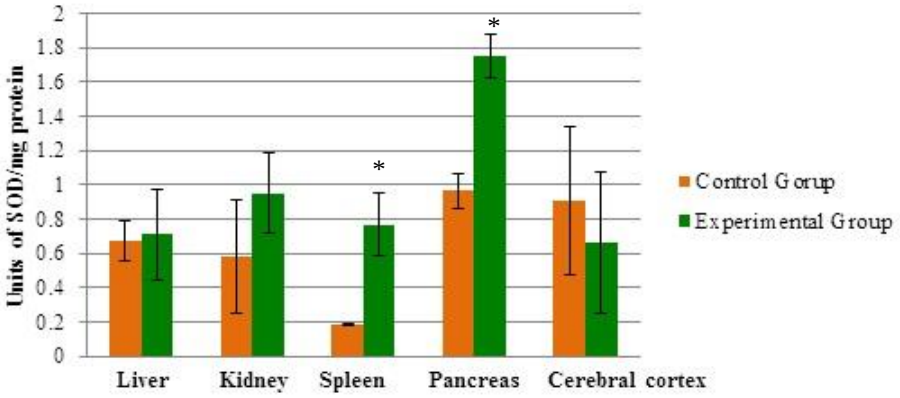
Unit of catalase activity = 17/ Δt

Where, 17 is extinction coefficient of H_2O_2 at 25°C. Δt is time in seconds required for the decrease in OD from 0.450 to 0.400

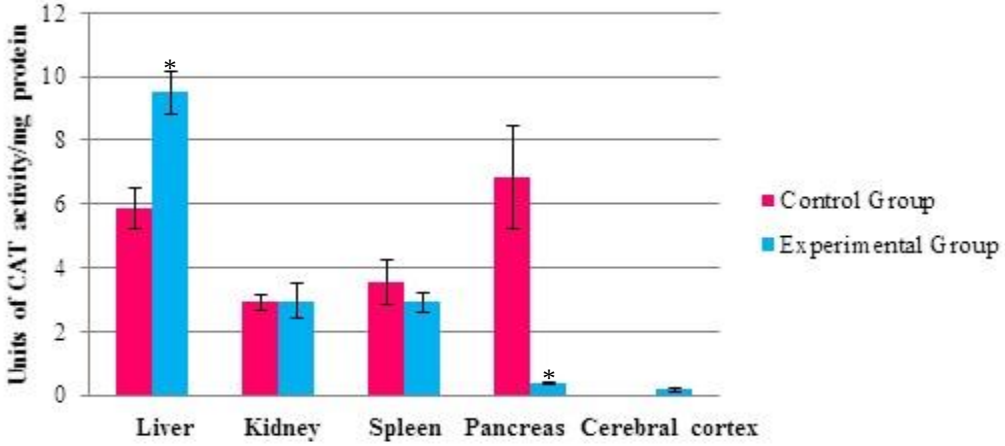
Graph 1. Effect of Shakti drops on lipid peroxidation in the form of nM of MDA/mg tissue in various organs of Swiss albino mice.



Graph 2. Effect of Shakti drops on Superoxide dismutase activity in the form of units /mg protein in various organs of Swiss albino mice.



Graph 3. Effect of Shakti drops on catalse activity in the form of units /mg protein in various organs of Swiss albino mice.



* indicates $p<0.05$ i.e. the difference is statistically significant.

Table 1. Effect of Shakti drops on lipid peroxidation in the form of nM of MDA/mg tissue in various organs of Swiss albino mice

Group	Liver	Kidney	Spleen	Pancreas	Cerebral cortex
Control	1.52 ± 0.25	1.40 ± 0.26	0.81 ± 0.22	0.45 ± 0.23	1.88 ± 0.36
Experimental	1.20 ± 0.12	1.23 ± 0.29	0.74 ± 0.001	0.18 ± 0.11	0.72 ± 0.060
p Value	0.148117	0.581612	0.604126	0.1794	0.027139
Significance	NS	NS	NS	NS	Significant (p<0.05)

Table 2. Effect of Shakti drops on Superoxide dismutase activity in the form of units /mg protein in various organs of Swiss albino mice

Group	Liver	Kidney	Spleen	Pancreas	Cerebral cortex
Control	0.67 ± 0.12	0.58 ± 0.33	0.18 ± 0.003	0.95 ± 0.10	0.90 ± 0.43
Experimental	0.71 ± 0.27	0.95± 0.23	0.76 ± 0.18	1.75 ± 0.12	0.66 ± 0.41
p Value	0.861082	0.115133	0.04729	0.02274	0.627883
Significance	NS	NS	Significant (p<0.05)	Significant (p<0.05)	NS

Table 3. Effect of Shakti drops on Catalase activity in the form of units /mg protein in various organs of Swiss albino mice.

Group	Liver	Kidney	Spleen	Pancreas	Cerebral cortex
Control	5.88 ± 0.64	2.90 ± 0.23	3.56 ± 0.703	6.86 ± 0.62	--
Experimental	9.50 ± 0.66	2.95± 0.52	2.90 ± 0.32	0.39 ± 0.028	0.15 ± 0.051
p Value	0.030864	0.483012	0.455217	0.0491733	0.197219
Significance	Significant (p<0.05)	NS	NS	Significant (p<0.05)	NS

RESULTS AND DISCUSSION

i. Lipid peroxidation:

The results are displayed in table No.1 and Graph No.1. There was a decrease in the lipid peroxidation in Shakti drops treated animals. In the cerebral cortex, there was significant difference at p<0.05 in the sham control group and experimental group. However, in the liver, kidney, spleen and pancreas the difference was non-significant.

ii. Superoxide dismutase activity:

The results are displayed in table No.2 and Graph No.2. In the spleen and pancreas, there was significant increase in the SOD activity as compared ti sham control (p<0.05). In liver and kidney there was an apparent increase in the SOD activity in Shakti drops treated group, however, this increase was nonsignificant. In the cerebral cortex, there was nonsignificant decrease in the SOD activity in the experimental group than the control.

iii. Catalase activity:

The results are displayed in table No.3 and Graph No.3. There was nonsignificant difference in the catalase activity of kidney and spleen. In pancreas of experimental group there was a significant decrease in the catalase activity as compared to control group at p<0.05. In the cerebral cortex, control group exhibited no catalase activity, even at the end of 30 min of the assay, whereas, in Shakti drops treated group it was 0.15 ± 0.051U/mg protein.

In the liver of Shakti drops treated group there was a nonsignificant decrease in the lipid peroxidation, coupled with a nonsignificant rise in the SOD activity and significant increase in the catalase activity. This indicates that three months treatment of Shakti drops has just initiated antioxidant protection. There is a need to extend the treatment to verify if at all Shakti drops can exert a hepatoprotective effect.

In the kidneys of Shakti drops treated group, there was no any significant difference in the lipid peroxidation and in the activities of SOD and catalase indicating that Shakti drops are ineffective on kidneys with three months of treatment.

In the spleen of Shakti drops treated group, there was an apparent decrease in the lipid peroxidation which was statistically nonsignificant. The SOD activity was significantly increased; however, there was no concomitant increase in the catalase activity.

In the pancreas of Shakti drops treated group there was a nonsignificant decrease in the lipid peroxidation. SOD activity was significantly increased. Kozo (1977) demonstrated in post-partum developing liver that the increase in SOD activity causes concomitant decrease in lipid peroxidation. In the present study there was a significant decrease in the catalase activity. Bhattacharya et al. (2000) described that increase in the superoxide dismutase activity and concomitant decrease

in the catalase activity is a cellular stress. The paradox observed in SOD and catalase activity in the pancreas of Shakti drops treated group may be an alarming situation. However, there was no increase in lipid peroxidation. The Product of SOD activity is hydrogen peroxide which has to be degraded into H_2O and O_2 by catalase.An increase in the SOD activity indicates increase in production of H_2O_2, however, there was no concomitant increase in the Catalase activity.

In the spleen and pancreas there was a significant increase in the SOD activity. Chowdhuri *et al.* (2002), demonstrated that bacosides of *Bacopa monniera* modulate the expression of SOD in the rat brain. In the liver, there was an increase in Catalase activity, whereas in other organs there was no any significant effect.

In the cerebral cortex, there was statistically significant decrease in lipid peroxidation in Shakti drops treated animals. There was an insignificant decrease in the SOD activity, suggesting that the ingredients in the Shakti drops might have given the antioxidant protection. Brain is very vulnerable to oxidative injury because it is rich in polyunsaturated fatty acids, which are prone to oxidative damage. Moreover, it is metabolically more active tissue, possesses high levels of pro-oxidant iron (Arivazhagan et al. 2002). Present findings suggest that in the cerebral cortex, Shakti drops might have triggered the expression of catalase activity, because in the cerebral cortex of the control group there was no any catalase activity. Although the blood brain barrier prevents many exogenous anti-oxidants to reach in the brain tissue (Gilgun-Sherki *et al*. 2001), the results of the present investigation seem that Shakti drops exert beneficial effects on the cerebral cortex. It is claimed by Sri Sri Ayurveda that Shakti drops contain aqueous extract of Amla, Ashwagandha, Bringaraj, Brahmi, Amruth, Shankapushpi, Satavari, Yashtimadhu. There are numerous research articles documenting the potential antioxidant role of these plants (Tripathi et al. 1996, Prince et al. 2004,Gajare et al. 2005, 2007, Bihagi et al. 2011, Aguiar and Borowski 2013, Gani and Devi 2015).

The observed effects on lipid peroxidation suggest that even though the treatment of Shakti drops did not show significant decrease in lipid peroxidation in all the organs studied, but it could be the initiation of the protective role of Shakti drops.

CONCLUSION

Thus, with the dosage used in the present study and the duration of the treatment, i.e. of three months, Shakti drops have a varying role from organ to organ and there is need to increase the duration of the treatment to make any conclusion about the efficacy of the Shakti drops. Prima facie the results indicate that Shakti drops may have beneficial effects.

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