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RESEARCH ARTICLE

DETERMINATION OF THE EFFECT OF AN AQUEOUS SEED EXTRACT OF *SESBANIA SESBAN* (L) MERR. ON THE LEVELS OF NON-ENZYMIC ANTIOXIDANT STATUS IN EXPERIMENTAL ANIMALS

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ABSTRACT

This study investigates the effect of aqueous extract of seeds of *Sesbania Sesban* (L) Merr. on the levels of non-enzymatic antioxidants such as reduced glutathione and vitamin-C in different organs of experimental animals subjected to inflammation with a standard drug. The experimental animals were induced inflammation with administration of a standard inflammatory drug, Carrageenan and two different concentrations of plant extract viz.150mg/kg body weight and 300mg/kg body weight of experimental animals were administered through oral mode one hour prior to Carrageenan induction. After induction and treatment, the organs such as spleen, thymus and hind paw were removed and was evaluated to test the effect of plant extract on non-enzymatic antioxidant levels in two experimental group of animals from the homogenate sample. The values obtained showed the protective effect of the plant extract in enhancing the levels of both the antioxidants in a dose-dependent manner to a significant level.

Keywords: Carrageenan, inflammation, aqueous extract, non-enzymatic antioxidants, experimental animals.

1. INTRODUCTION

Plants have been used since time immemorial for diverse purposes by humankind, particularly as food for nutrition and as medicine for treating diseases in both humans and animals. Plants are used in all cultures worldwide and have been relied upon for several millennia to support, promote, and restore human health (1). Plant contains enormous biochemical compounds called phytochemicals, which offer many benefits for human health (2,3). These phytochemicals or substrates encompass several biological compounds such as terpenes, polyphenols, and alkaloids (4).

Medicinal plants are used in alternative medicine because they have several therapeutic effects. Using such natural preparations is steadily increasing because it is cheaper than a commercial synthetic drug. These preparations are typically taken as a drink without a prescription. Furthermore, alternative medicine has limited some side effects than conventional treatment. Plants and some other organisms, such as fungi, are now considered important sources of potential medicines for several diseases, including cancer, heart disease, dementia, and malaria (1).

Sesbania sesban belongs to Fabaceae family has a long history of use in India, grows in a wide range of soils from loose sands to heavy clays. It is found widely in tropical Asia and Africa upto an altitude of 1200m^3. *Sesbania sesban* has synonyms such as Common sesban, Sevari, Shewari, Jayanti, Jait, Jaya, Jayantika etc (5,6). *Sesbania sesban* is a soft, slightly woody, 1-6 m tall perennial nitrogen fixing small tree. The leaves are compound 12-18 cm long and made up of 6-27 pairs of leaflets. The raceme has 2-20 flowers which are yellow with purple or brown streaks on the corolla. Pods are sub cylindrical, straight or slightly curved up to 30 cm long and 5mm wide containing 10-50 seeds (7).

Sesbania sesban is primarily used as a green manure and a source of cut and carry forage. It can be intercropped with corn, beans, cotton and many other field crops. Root and bark used as bitter tonic used in debility nervous disorders, CNS stimulant. Root of plant used as dysuria, retention of urine, hepatoprotective activity. Leaves are used as anthelmintic activity (8). Seeds are used in diarrhea, excessive menstrual flow, to reduce enlargement of spleen and in skin disease (9).

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This study has been designed to study the effect of aqueous seed extract of *Sesbania sesban (L) Merr.* on the levels of non-enzymic antioxidant status in experimental animals

2. MATERIALS AND METHODS

The plant was authenticated with Botanical Survey of India, Southern Regional Centre, Coimbatore. Seeds of *Sesbania sesban* were shade dried and healthy seeds were selected and grinded well to a coarse powder. Hot aqueous extract was prepared with 10g of the powdered sample in 300 ml of water. The extract that was obtained was condensed in an oven and was preserved in an air tight container and stored at 4°C for further use.

2.1. Selection of Animals

Female Sprague Dawley rats weighing approximately 180-200g obtained from Small Animal Breeding Station, Thrissur, Kerala, were used for the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (AVM Cattle Feeds, Coimbatore, and Tamil Nadu) and water *ad libitum.* Experimental animals were handled according to the university and institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.2. Experimental Design

The animals were divided into five groups of six animals each. Inflammation was induced by sub plantar injection of Carrageenan (CG) (1%) (10).

Group I **:** Control

Group II **:** Inflammation was induced by subcutaneous injection of freshly prepared 0.1ml Carrageenan in saline into the right hind paw

Group III **:** Treated with 150mg/kg body weight of aqueous extract of *S. sesban* (SSAE) orally, 1 hour prior to Carrageenan induction as group II rats.

Group IV **:** Treated with 300 mg/kg body weight of aqueous extract of *S. sesban* (SSAE) orally, 1 hour prior to Carrageenan induction as group II rats.

Group V **:** Treated with 10mg/kg body weight of Diclofenac sodium orally, 1 hour prior to Carrageenan induction as group II rats.

The odema was measured after 1, 2, 3 and 4 h. After 4 h, the rats were killed by cervical dislocation, and then the whole spleen, thymus and hind paw were removed and washed with ice-cold saline. The tissues were homogenized using 0.1 M Tris-HCl buffer (pH 7.4) to give 10% homogenate.

2.3. Collection of Samples

At the end of experimental regimen, the animals were sacrificed, blood was collected and the spleen, thymus and hind paw were excised and washed in saline. 10% homogenate of the organs were prepared with 0.1 M Tri-HCl buffer, pH 7.4. The homogenates were centrifuged at 3000 rpm for 15 min at 4ºC for cytosolic separation. Non-enzymatic antioxidants such as reduced glutathione and vitamin C were assayed in the cytosolic fraction of spleen, thymus and in hind paw tissue.

2.4. Analysis of non-antioxidant enzymes

2.4.1. Estimation of Reduced Glutathione

Reduced Glutathione estimation was done by following the procedure described by Moron *et al.*, (11). A known weight of tissue was homogenized in phosphate buffer. From this 0.5ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of supernatant was taken after centrifugation and added 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm.

Pipetted out 0.2 to 1.0 ml standard solution corresponding to a µg of 40-200. The volume in all the tubes were made up to 1.0 ml with distilled water. Then added 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer to all the tubes. The absorbance was read at 412 nm within 2 min against the reagent blank. The amount of glutathione was expressed as µg/mg protein.

2.4.2. Estimation of Vitamin-C

Estimation of vitamin-C was done by following the procedure of Omaye *et al*., (12). 1.0 ml of 10% homogenate was precipitated with 5% ice-cold TCA and centrifuged for 20 min at 3,500 g. 1.0 ml of the supernatant was mixed with 0.2 ml of DTCS reagent and incubated for 3 hours at 37°C.

Then 1.5 ml of ice-cold 65% sulphuric acid was added. Mixed well and the solutions were allowed to stand at room temperature for an additional 30 min. Absorbance was determined at 520 nm. The results were expressed as µg/mg protein.

2.5. Statistical analysis

Statistical comparison was done at significance level, P<0.05 using SPPS package version 16.0. One way ANOVA followed by post hoc analysis of LSD was performed.

3. RESULTS AND DISCUSSION

The results pertaining to this study are given as follows and discussed. The drug administered and plant extract & standard drug treated organ samples were excised and estimated for the levels of non-enzymatic antioxidants such as reduced glutathione and vitamin-C and the results are tabulated in table given below. As per the experimental design, a total of five groups each group consisting of 6 animals were taken for the study and sample obtained were analyzed using the standardized procedures. In the spleen sample, the control group values are 13.60 ± 0.65, drug induced group are 6.48 ± 0.21, plant extract administered group were 7.37 ± 0.51 and 9.63 ± 0.39, and standard drug treated group was 12.05 ± 0.83. From these values, it is observed that in the carrageenan induced group, the levels of antioxidant – reduced glutathione was declined. Upon treatment with plant extract, the levels of the antioxidant was found to restore to different levels responding to two different extract concentrations with reference to the standard drug treated animals. Similarly, levels of vitamin-C when declined in the carrageenan treated group, was found to increase in the animals treated with plant extracts with reference to standard drug treated animals. Similarly, in the thymus and hind paw samples, the levels of reduced glutathione and vitamin-C were increasing gradually upon treatment with different concentration of plant extracts. The levels of enzymes restored upon treatment varies from one organ to another, but on the whole, the plant extract was found to be effective in treating the damage caused due to the drug effect by restoring the levels of antioxidant enzymes to possible levels indicative of the fact that the plant extract is of anti-inflammatory in action.

Table 1. Effect of aqueous extract of *Sesbania sesban* on the activities of non-enzymatic antioxidants in the spleen, thymus and hind paw of experimental animals

Groups	Control	Carrageenan (1mg/ml)	SSAE (150mg/ kg b wt) + CG	SSAE (300 mg/ kg b wt) + CG	Diclofenac sodium (10 mg/kg) + CG
Spleen					
GSH	13.60 ± 0.65 [b]	6.48 ± 0.21 [a]	7.37 ± 0.51 [b]	9.63 ± 0.39 [b]	12.05 ± 0.83 [b]
Vitamin C	19.59 ± 0.88 [b]	15.77 ± 0.72 [a]	17.05 ± 0.93 [a b]	17.39 ± 0.68 [b]	19.52 ± 0.49 [b]
Thymus					
GSH	12.93 ± 0.72 [b]	3.75 ± 0.19 [a]	6.02 ± 0.31 [b]	9.17 ± 0.06 [b]	11.38 ± 0.07 [b]
Vitamin C	22.53 ± 1.52 [b]	16.28 ± 0.95 [a]	18.68 ± 0.79 [a b]	19.19 ± 0.62 [b]	21.79 ± 1.27 [b]
Hind paw					
GSH	26.90 ± 1.01 [b]	8.98 ± 0.51 [a]	15.03 ± 0.69 [b]	20.09 ± 0.97 [b]	22.10 ± 1.13 [b]
Vitamin C	23.18 ± 0.99 [b]	14.79 ± 0.77 [a]	18.91 ± 1.44 [b]	20.15 ± 1.02 [b]	21.47 ± 1.35 [b]

Values are expressed as mean ± SD (n=6)
Units: GSH, Vitamin C - µg/ml or mg protein;
Group comparison and statistical significance at p<0.05: [a]: Group I vs. II, III, IV, V; [b:] Group II vs. I, III, IV, V

Seed and bark of *Sesbania sesban* are used as astringent, emmenagogue, in menorrhagia, spleen enlargement and diarrhoea. Leaves are used as anthelmintic and also useful in diabetes, colic and skin diseases. Seeds are stimulant, emmenagogue, astringent and also useful in diarrhea. Reports suggest that, previous phytochemical investigations of the plant led to the isolation of oleanolic acid, stigmasta-5,24(28)-diene-3-ol-3-0-β-D-galactopyranoside, fatty acids and amino acids. Various types of lignins composed of guaiacyl, syringyl and P- hydroxyphenylpropane building units (13), and also antitumor principal kaempferol disaccharide (14,15).

Reduced Glutathione (GSH) is a major non-protein thiol in living organism, which plays a central role in coordinating the body's antioxidant defense processes. Decreased GSH concentration contributes to the pathogenesis of complications associated with the diabetic state (16). Reduced glutathione, synthesized mainly in the liver, is an important non-enzymatic antioxidant in the antioxidative defense system (17).

GSH, an important protein thiol in living organisms plays a central role in coordinating the body's antioxidant defense process (18). Reduced GSH constitutes the first line of defense against free radicals (19). The levels of GSH was analyzed to check the antioxidant status in experimental animals. Upon induction with carrageenan, the levels decreased significantly at $p<0.05$. The treatment group showed an increase in levels of GSH to 9.63 ± 0.39 µg/mg protein similar to the standard drug treated group.

Vitamin C is an important water soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. It readily oxidizes to dehydroascorbic acid. Human beings have no ability to synthesis vitamin C due to mutation in the gene coded for L-gulonolactone oxidase, an enzyme required for biosynthesis of vitamin via the glucuronic acid pathway. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins (17, 20).

The levels of vitamin C in spleen, thymus and hind paw showed the effect of the aqueous extracts of *Sesbania sesban* in treating inflammation. Levels of vitamin C in thymus showed high value compared to spleen and hind paw tissues.

4. CONCLUSION

Antioxidants play a central role in maintaining the health of a biological system. The levels of antioxidants is vital for the optimum functioning of the cells which is accomplished through different mechanisms of action. This study revealed the protective nature of the *Sesbania sesban* seed extract in curbing inflammation and restoring the antioxidant status in the experimental animals. The vital organs of action during inflammation were analyzed for their activity at different states of health, which showed the plant extract has the potential to influence the antioxidant levels in *in-vivo* system. Further studies on molecular mechanisms of healing and lead compound isolation will make the traditional folklore plant to a modern therapeutic drug source which will benefit the society in a large.

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Conflict of interest

The author declares no conflict of interest**.**

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