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Hepatoprotective and anti allergic study of various extracts of *Argyrea speciosa*, sweet root extracts in experimental animals

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ABSTRACT

The various sutras in ayurveda illustrates that a person undergoing rejuvenation therapy attains longevity, memory, intellect, freedom from diseases, youth, excellence of luster, true sense-organ and respect, brilliance and vak-siddhi. This rejuvenation therapy is known as Rasayana therapy. The materials used in the therapy are termed as Rasayana. These Rasayana may be inducers of enzymes and hormones, which the body needs for adaptations and survival during normal health, stress and during the diseases also. This concept of Rasayana is well known to ayurvedic physicians that the delicate cellular machinery of the body fluids from trauma (stress) resulting in wear and tear of different body structures and deterioration of the functional capacity such as liver. This procedure of revitalization and rejuvenation were adopted to increase the power of resistance to disease (increased immunity). The plant Argyrea speciosa, Sweet, is not explored scientifically for its medicinal values or for any of its use except few studies like antibacterial activity. There is a need for supplementation of exogenous antioxidants to protect the body from the onslaught of free radical induced damage, in such conditions. Hence our aim was to explore the hepatoproctective and antiallergic effect Argyrea speciosa, sweet root extracts in experimentally induced hepatotoxicity and allergy in animals. The roots were shade dried and powdered. The roots were defatted with petroleum ether (60-80º) and further extracted with distilled water (ASW), methanol (ASM) and under Soxhelet extraction and methanolic fraction of aqueous extract (ASMW) was prepared. The aqueous, methanolic and methanolic fraction of aqueous extract of Argyrea speciosa Sweet have exhibited significant protection against the deleterious effects like various diseases and conditions like liver damage and allergy. The results indicated potent hepatoprotective activity which may be attributed to the membrane and cellular stabilizing activity of extracts. The results also indicated potent anti allergic activity which may be attributed to prevention of sensitization or inhibiting the release of various allergic mediators.

Key words: Hepatoprotective, Argyrea speciosa, antiallergic

INTRODUCTION

The various sutras in ayurveda [1] illustrates that a person undergoing rejuvenation therapy attains longevity, memory, intellect, freedom from diseases, youth, excellence of luster, true sense-organ and respect, brilliance and vak-siddhi. This rejuvenation therapy is known as

Rasayana therapy. The materials used in the therapy are termed as Rasayana. These Rasayana may be inducers of enzymes and hormones, which the body needs for adaptations and survival during normal health, stress and during the diseases also. Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medicinal practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India [2]. Allergy is an immunological phenomenon. The disease results when an exposure to the allergen induced an immune response, referred to as sensitization rather than immunization. Once sensitization occurs, an individual will not be symptomatic until there is an exposure to the allergen. Then the reaction of allergen with specific antibody or sensitized effector T lymphocyte induces an inflammatory response, producing the signs and symptoms of allergic reaction [3]. The plant *Argyrea speciosa*, Sweet, is not explored scientifically for its medicinal values or for any of its use except few studies like antibacterial activity. There is a need for supplementation of exogenous antioxidants to protect the body from the onslaught of free radical induced damage, in such conditions. Hence our aim was to explore the hepatoprotective and anti allergic effect *Argyrea speciosa*, sweet root extracts.

MATERIALS AND METHODS

Collection of plant material: The roots of A. speciosa, sweet were collected from the botanical garden of Ayurveda Mahavidyalaya, Panchavati, Nashik and the roots of the plant were authenticated by Dr. D. R. Mahajan, Head, Dept. of Botany, K.T.H.M. Science College, Gangapur Road, Nashik.

Preparation of Argyrea speciosa, Sweet Extracts:
The roots were shade dried and powdered. The roots were defatted with petroleum ether (60-80º) and further extracted with distilled water (ASW), methanol (ASM) and under Soxhelet extraction and methanolic fraction of aqueous extract (ASMW) was prepared.

Adult albino rats and mice (Wistar strain) of either sex weighing 200-250gm and 20-30gm respectively were used in the entire study. Each pair of animal was housed in a spacious polypropylene cage containing wood shavings as nesting material, which was maintained at 23ºC ± 3°C in a well-ventilated animal house under natural photoperiod condition. The study protocol for the current study was prepared as per CPCSEA. All the study protocols were approved by Institutional Animal Ethical Committee of PDVVF's College of Pharmacy, Vilad Ghat, Ahmednagar. All the experiments were started only after the approval of IAEC and completion report was also submitted to the IAEC after the completion of experiments.

Statistical Analysis:
All observations were presented as Mean ± SEM. The data was analyzed by student's t-test for in-vitro studies and one-way ANOVA followed by Dunnett's test for in-vivo study. $p < 0.05$ is considered as significant and $p < 0.001$ as highly significant.

Grouping and treatment: Hepatoprotective study.
The study protocol was designed for the treatment of thirty days. The initial treatments and design of groups was as given in table no 1. Biochemical investigations were carried out for

estimation of SGOT, SGPT, Alkaline phosphatase, Acid phosphatase and Total bilirubin was carried out at the end of study.

Table No. 1 Hepatoprotective Study

The study protocol was designed for the treatment of thirty days. The initial treatments and design of groups was as follows:

Group 1 (Control)	Animals were received single dose of saline at the dose of 1ml/kg body weight, i.p. for five days and received water *ad libitum* along with normal diet.
Group 2 (Galactosamine hydrochloride)	Animals were received two doses of D-Galactosamine hydrochloride 400mg/kg, i.p., 4-6 hrs apart and third dose was given 24 hrs later.
Group 3 (ASW)	Animals received aqueous extract ASW (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.
Group 4 (ASW + GAL)	Animals were pretreated with extract ASW (1ml/kg body weight, i.p.) for five days. On third day of treatment animals were received 02 doses of D-Galactosamine hydrochloride (400mg/kg body weight, i.p.) 4-6 hrs apart and third dose was given 24 hrs later along with normal diet and water *ad libitum*.
Group 5 (ASM)	Animals received methanolic extract ASM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.
Group 6 (ASM+GAL)	Animals were pretreated with extract ASM (1ml/kg body weight, i.p.) for five days. On third day of treatment animals were received 02 doses of D-Galactosamine hydrochloride (400mg/kg body weight, i.p.) 4-6 hrs apart and third dose was given 24 hrs later along with normal diet and water *ad libitum*.
Group 7 (ASWM)	Animals received methanolic fraction of aqueous extract ASWM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.
Group 8 (ASWM+STZ)	Animals were pretreated with extract ASWM (1ml/kg body weight, i.p.) for five days. On third day of treatment animals were received 02 doses of D-Galactosamine hydrochloride (400mg/kg body weight, i.p.) 4-6 hrs apart and third dose was given 24 hrs later along with normal diet and water *ad libitum*.

Grouping and treatment: Anti allergic study.

The mice were divided into various groups. Every group was consisting of five animals study design was as given in table no 2. Parameters such as mast cell degranulation, milk induced leukocytosis were investigated after the end of study.

Table no. 2 Anti-Allergic Study

The mice were divided into various groups. Every group was consisting five animals and given various treatments as shown under:

Group 1 (Control)	Animals were received single dose of normal saline at the dose of 1ml/kg body weight, i.p. and water *ad libitum* along with normal diet.
Group 2 (STD)	Animals were received standard drugs as follows: **Model-I:** Disodium chromoglycate (DSCG) at the dose of 50mg/kg body weight, i.p. and water *ad libitum* along with normal diet for mast cell study. **Model-II:** Diazepam at the dose of 1mg/kg, ip, body weight, i.p. and water *ad libitum* along with normal diet for leukocytocsis study.
Group 3 (ASW)	Animals received aqueous extract ASW (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.
Group 5 (ASM)	Animals received methanolic extract ASM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.
Group 7 (ASWM)	Animals received methanolic fraction of aqueous extract ASWM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.

RESULTS AND DISCUSSION

Table No.3 Effect of administration of several extracts of *Argyrea speciosa*, sweet extracts on the D-Gain induced hepatotoxicity in rats

Sr. No.	Serum Biochemical parameters	Groups							
		Control	GAL	ASW	ASW+GAL	ASM	ASM+GAL	ASWM	ASWM+GAL
1	SGOT (U/dl)	87.2±5.4	418.8[a]±31.1	88.1±5.3	128[a]±10.9	87.8±6.2	108.3[a]±11.2	86.9±8.2	89.1[a]±4.8
2	SGPT (U/dl)	112.08±9.8	376.3[a]±3.9	113.3±8.4	121[a]±13.5	112.9±8.9	121[a]±13.6	112.8±9.2	118[a]±11.2
3	$ACID_P$ (KA units/dl)	12.8±1.1	37.6[a]±1.6	21.3±0.3	12.6[a]±1.3	20.5±1.1	12.7[a]±1.4	11.9±0.9	11.9[a]±1.6
4	ALK_P (KA units/dl)	108±9.8	257.8[a]±13.2	107±8.4	132[a]±13.2	108.3±6.8	125[a]±11.5	107.9±5.3	107.6[a]±6.1
5	TBil (mg/dl)	0.4±0.01	1.48[a]±0.14	0.39±0.03	0.520[a]±0.01	0.402±0.04	0.484[a]±0.01	0.448±0.03	0.467[a]±0.03

Values are expressed as mean ±SEM for six animals in each group.

GAL is d-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of *Argyrea speciosa*, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.).

Table No.4 Effect of administration of several extracts of *Argyrea speciosa*, sweet extracts on the clonidine induced mast cell degranulation in mice.

Group No	Groups	Mast Cell Protection
1	Control	39 ± 3
2	DSCG	80 ± 5[b]
3	ASW	63 ± 4[b]
4	ASM	59 ± 4[b]
5	ASWM	48 ± 4[c]

Values are expressed as mean ±SEM for six animals in each group.

DSCG is d-disodium chromoglycolate administered at the dose of (50mg/kg, body weight, i.p.), ASW is aqueous extract of *Argyrea speciosa*, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b:<0.01, c:<0.001 When group 2 is compared with group 1; Group 4, 5 and 6 are compared with group 2.

The metabolites of β-D-galactosamine (GaIN), uridiophosphogalactosamine may deplete several uracil nucleotides such as UDP-galactose, UDP-glucose and UTP, causing reduction of mRNA and glycoprotein synthesis (i.e. reduction of ATP and glycogen synthesis), which leads to cellular membranes alteration **[**4]. The Ca^{++} homeostasis perturbation, inhibition of oxidative of NADPH and FADH substrate at the dehydrogenase co enzyme level [5] are also considered to be responsible for pathogenesis of GaIN induced hepatitis. There was significant increase in SGOT, SGPT, ACIDp, ALKp and Total bilirubin levels in animals intoxicated with d-galactosamine indicating the hepatic damage in animals. Administration of aqueous, methanolic and ethylacetate soluble fraction of methanolic extract of *Argyrea speciosa*, sweet significantly brought the levels of these enzymes to near normal in animals upon completion of thirty days of treatment indicating the hepatoprotective effect of these extracts as in table no.3.

Table No. 5 Effect of various treatments with Diazepam and extracts of *Argyrea speciosa* on Milk Induced Leukocytosis in mice

Group No	Groups	Number of leukocyte count (after 24 hrs. of injection of milk)
1	Control	3800
2	Diazepam	800[c]
3	ASW	1200 [c]
4	ASM	900 [c]
5	ASWM	810 [c]

Values are expressed as mean ±SEM for five animals in each group.

Group II, III, IV & V are compared with group I.

P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

ASW is aqueous extract of *Argyrea speciosa*, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of *Argyrea speciosa*, sweet (1ml/kg body weight, i.p.).

The probable mechanism of hepatoprotective activity may be attributed to prevention of cellular membranes alteration of hepatocytes and also may be attributed for prevention of Ca^{++} homeostasis perturbation. Histamine is an endogenous mediator of many (patho)-physiological processes, such as the regulation of gastric acid secretion and cardiac output, while functioning as a neurotransmitter in the brain. During allergic reactions, histamine is released in large quantities from intracellular stores from mast cells and basophils after cross-linking of cell surface IgE by allergens. Histamine possessing immunomodulatory activity through H_2 receptor on dendritic cells (DCs) [6]. During allergy, there is sensitization of the mast cells which cause the relase of mediators like histamine, cytokines, leukotrienes etc. as well as desposition of the proinflammatory cells like eosiniphils and leucocytes [7]. IgE initiates the activation of signal transduction pathway which leads to the release of histamine. Compound 48/80 increases the permeability of lipid bilayer membrance by causing the perturbation in the membrane. Clonidine causes mast cell degranulation in similar way as that of compound 40/80 by dynamic expulsion of granules without damaging cell wall. [8]. Subcutanceous injection of milk was known to produce an infection like condition by acting as an antigen and increasing the leukocyte count. The leucocytes are recirculated during stress and allergic responses and release the inflammatory mediators like cytokines, histamine, and major basic proteins promoting the ongoing inflammation. An adaptogen may have normalization that revealed itself irrrepective of the direction of previous pathologic shift [9, 10]. The antiallergic studies were one of the part of this study, it has be noted that there was significant protection of mast cells with co-administration of aqueous, methanolic and methanolic fraction of aqueous extract of *Argyrea speciosa*, sweet was seen indicating the antiallergic property of these extracts. Milk when administered subcutaneously causes leukocytosis. There was significant increase in milk induced leukocytosis in animals on subcutaneous administration of milk. Simillarly, the antiallergic compounds protects such type of leukocytosis. It is been found this present study that, the all the extracts have shown significant protection to the leukocytosis induced by milk in experimental animal's table no.4 and 5.

CONCLUSION

Thus the present study have shown that the oxidative stress in animals is through the generation of free radicals is an important consequence of intoxication with d-galactosamine. It is further shown that the administration of aqueous, methanolic and methanolic fraction of aqueous extract of *Argyrea speciosa*, sweet exhibited the hepatoprotective and antiallergic action by virtue of their organoprotective and antioxidant property.

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