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Research Article

COMPARATIVE STUDY OF IMMUNOMODULATORY AND ADAPTOGENIC ACTIVITY OF *HIBISCUS ROSA SINENSIS* EXTRACTS IN RATS

SANDHYA K. DESAI*, SMRUTI S. MULGAONKAR, CHANDAN H. PANDEY.

Department of Pharmacology & Toxicology, Prin. K. M. Kundnani College of Pharmacy, 23-Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai- 400 005. India. Email: sandhyakdesai@yahoo.com

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ABSTRACT

Objective: The present study was undertaken to study and compare the adaptogenic and immunomodulatory activity of *H. rosa sinensis* Linn by Forced Swim Test in rats.

Methods: Separate groups of rats were administered either one dose level of Ethyl Acetate Extract (EAE) of *H. rosa sinensis* petals (100 mg/kg) or two dose levels of Standardized Extract of *H. rosa sinensis* in terms of Cyanidin chloride (SEC4 and SEC8) i.e. 4mg/kg and 8mg/kg respectively.

Results: The treatment with EAE and SEC significantly increased mean swim time (MST) and restored back to near normal the levels of enzymes like Alanine aminotranferase (ALT) and other indicators of physical stress like Cholesterol and Triglycerides. Only SEC8 treated group showed significant restoration in the levels of Aspartate aminotransferase (AST) whereas both SEC4 and SEC8 groups showed significant restoration of Glucose levels as compared to toxicant group.

Conclusion: Thus it can be proved that these extracts can increase tolerance to stress and in turn have an immune potentiating action in such situations of severe physical stress.

Keywords: Immunomodulatory, Ethyl Acetate Extract, Forced Swim test, SEC4 and SEC8.

INTRODUCTION

Herbal medicines constitute about 75-80% of therapeutic agents in developing countries. Herbs with antioxidant activity peculiarly free radical scavenging activity are increasingly proving their benefits in treatment of diseases caused by oxidative stress [1]. Plants are a potential source of natural antioxidants and produce various anti-oxidative compounds to counteract ROS in order to survive. A large number of plants, their extracts, decoctions and pastes have been used by tribals and folklore traditions for treatment of cuts, wounds and burns [2]. Several plant polyphenolic compounds are reported to be potential antioxidant and are less toxic and less carcinogenic then the synthetic antioxidants currently in use in many foods, beverages and medicines [3]. Oxidative stress is often caused by imbalance in production and scavenging of free radicals. Oxidative stress can cause cancer, cardiovascular diseases, neurological diseases like Alzheimer's and Parkinson, rheumatoid arthritis, pulmonary diseases like COPD and skin diseases [4]. The forced swim stress test developed by Porsolt et al (1977) has been a widely used model for induction of physical stress [5]. Rodents when allowed to swim in a restricted area become immobile after some time which may signify a state of mental depression [6]. During stressful situations the energy requirement of organism increases resulting in enhanced generation of free radicals [7]. Phytoconstituents are an important source of antioxidants and are capable of terminating free radical chain reactions [1]. *H. rosa sinensis* Linn (Malvaceae) commonly known as 'Hibiscus' or 'Jaswanda' has shown to possess abundant phenolic and flavonoids contents and exhibits excellent antioxidant activities compared to synthetic antioxidants [8]. These flowers are known to be rich in cyanidin, quercetin, flavonoids, hentriacontane, thiamine, riboflavin, niacin and ascorbic acid. Many of these compounds have proven their excellent 'natural antioxidants' status in various oxidative stress models. *H. rosa sinensis* has shown pharmacological activities like anti-fertility, abortifacient, analgesic, anti-inflammatory, anti-ovulatory, anti-estrogenic, hypoglycaemic, hypotensive, CNS-depressant, antifungal, antiviral and as a hair growth stimulant [9]. Other clinically relevant activities attributed to *H. rosa sinensis* include *in-vitro* anti-oxidant activity of its leaves [8], anti-bacterial activity of flower extracts [10], treatment of a motor disorder like tardive dyskinesia by root extract [11], anti-hyperlipidaemic activity of ethanolic flower extract [12].

The present study was designed to explore the adaptogenic and immunomodulatory activity of Ethyl Acetate Extract (EAE) and Standardized Extract of *H. rosa sinensis* in terms of Cyanidin chloride (SEC) of *H. rosa sinensis* using Forced Swim Model.

MATERIALS & METHODS

Experimental animals

Albino Wistar rats of either sex, weighing 120-150 gm were used for the experiment. They were housed under standard conditions (temperature 24°- 28°C, relative humidity 45-60% and 12 hr dark-light cycle) in clean polypropylene cages and were fed with standard rodent diet (Amrut laboratory animal feed, Pune, India) and water *ad libtium*. Experimental protocol no. 111201 was reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 25/1999/CPCSEA).

Drugs, Chemicals and Kits

All the reagents and solvents used in the study were of analytical grade and commercially available viz: Cyanidin chloride (Extrasynthese Pvt Ltd. France), Alanine aminotransferase (ALT) kit (Span Diagnostics Ltd.), Aspartate aminotransferase (AST) kit (Span Diagnostics Ltd), Cholesterol kit (Span Diagnostics Ltd.), Triglycerides kit (Bio Lab Ltd.) and Glucose kit (Bio Lab Ltd.).

Plant Material and Preparation of Extract

Fresh flowers of *H. rosa sinensis* were obtained from local market, authenticated at St. Xavier's Institute, Mumbai and processed further for obtaining the following extracts.

Ethyl Acetate Extract (EAE)

Fresh petals of *H. rosa sinensis* were shade dried for 2 days after which they were subjected to Soxhlet extraction using ethyl acetate as solvent at 55^{0}C. The cycles were run till the yellow colour of the extract persisted. The extract was further dried on water bath in an evaporating dish at 55^{0}C till a yellowish brown residue was obtained.

Standardized Extract of *H. rosa-sinensis* in terms of Cyanidin chloride (SEC)

40 g of freshly harvested flowers of *H. rosa-sinensis* were macerated in 400 ml methanol: 2M HCl (85:15 v/v) solution for 72 hrs by the

slightly modified method of Ukwueze et al [13]. The extract was concentrated to about 100 ml by heating in a water bath and then filtered. 20 ml of concentrated Hydrochloric acid was added and the mixture was heated in a round bottom flask under reflux for 2 hrs. The mixture was introduced into a flask, stoppered and placed in a refrigerator until a reddish brown powder precipitated out. This powder was filtered under suction, dissolved in methanol, filtered and dried on water bath at 40°C. The content of Cyanidin chloride was determined by UV analysis based on the standard curve of pure Cyanidin chloride.

Adaptogenic activity (Forced Swim Test) [6,14]

The rats were divided into six groups of six animals each viz: Normal control (untreated), Stress control, Standard treatment i.e. Dried alcoholic root powder extract of Ashwagandha (100 mg/kg, p.o.), EAE treatment group (100 mg/kg p.o.) and SEC4 and SEC8 treatment groups (two dose levels 4 mg/kg and 8 mg/kg p.o. respectively). All animals except normal control group and stress control group were given standard or test drug treatment for seven consecutive days. On the 8th day, all the rats except normal control group were subjected to forced swim test by keeping them in propylene buckets filled with water at temperature 20^{0}C to a height of 17 cm. Rats were allowed to swim till complete exhaustion and the end point was taken when the animals began to drown. The mean swim time for each group was calculated. The blood (2-4ml) was collected from retro orbital puncture for estimation of biochemical parameters like Glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Triglycerides and Cholesterol.

Estimation of Biochemical Parameters

Biochemical Parameters were determined using diagnostic kits by the following methods; Glucose by GOD-PAP End point Method, Triglycerides by GPO-PAP End Point Method, Alanine aminotranferase (ALT) and Aspartate aminotransferase (AST) by 2, 4-DNPH Reitmann and Frankel Method [15] and Cholesterol by Wybenga and Pileggi Method [16].

Statistical Analysis

The results were expressed as mean ± S.E.M (n=6). The statistical significance of differences between groups was determined by one way analysis of variance (ANOVA), followed by Tukey-Kramer test for multiple comparisons among groups by using GraphPad Instat version 5.0 of Graphpad software. Values of p <0.05 were considered statistically significant.

RESULTS

The results of the above study revealed that the extracts significantly increased (p<0.001) mean swim time in EAE and SEC8 groups and (p<0.01) in SEC4 group as compared to stress control group (Fig 1).

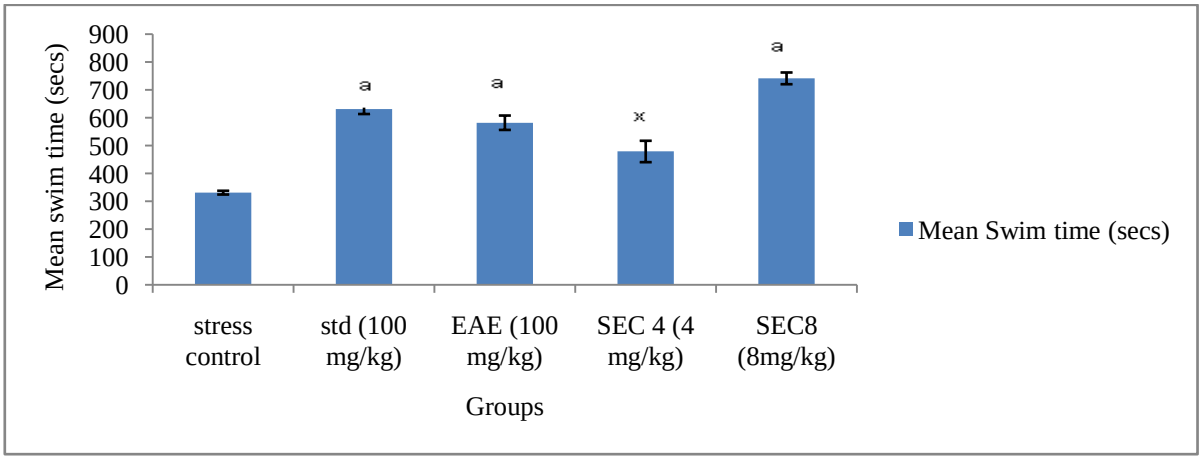

Fig. 1: Effect of EAE, SEC4 and SEC8 on Mean Swim Time in Forced Swim Test

All values expressed as mean ± SEM, n = 6; One-way ANOVA followed by Tukey-Kramer test is applied for statistical analysis. P value: ^{a}< 0.001 treated group compared to stress control group, ^{x}< 0.01 treated group compared to stress control group

AST levels were restored back significantly in SEC8 treated group as compared to stress control group. The levels of ALT, cholesterol and triglycerides were significantly restored back to near normal in all the treated groups when compared with stress control group. The glucose levels were restored back significantly in both SEC8 group and in SEC4 group when compared with stress control group (Table 2).

Table 2: Effect of EAE, SEC4 and SEC8 on Biochemical parameters:

Groups	AST (SGOT) (IU/L)	ALT (SGPT) (IU/L)	GLUCOSE (mMOL/L)	TRIGLYCERIDES (MG/DL)	CHOLESTEROL (MG/DL)
Normal	93.61 ±4.49	66.79 ±1.44	103.93 ±3.56	111.54 ±2.79	95.02 ±3.04
Stress Control	116.1 ±3.746 ^{y}	92.83 ±4.18^{x}	134.92 ±2.55^{y}	140.93 ±1.69^{x}	221.55 ±12.41^{x}
STD (100mg/kg)	84.49 ±9.052^{b}	64.14 ±1.84^{a}	82.87 ±1.94^{a}	112.185 ±1.88^{a}	92.34 ±7.46^{a}
EAE (100mg/kg)	101.6 ±1.93^{NS}	73.81 ±1.88^{a}	106.97 ±3.57^{NS}	122.97 ±2.12^{a}	97.00 ±6.17^{a}
SEC4 (4mg/kg)	104.5 ±4.56^{NS}	74.79 ±3.31^{a}	91.43 ±15.63^{b}	126.93 ±4.79^{a}	97.57 ±4.00^{a}
SEC8 (8mg/kg)	84.32 ±3.21^{b}	56.97 ±1.52^{a}	73.9 ±2.90^{a}	107.36 ±2.28^{a}	73.17 ±2.59^{a}

All values expressed as mean ± SEM, n = 6; One-way ANOVA followed by Tukey-Kramer test is applied for statistical analysis. P values: ^{x}<0.001 when stress control group compared with normal group, ^{y}< 0.05 when stress control group compared with normal group, ^{a} <0.001 when treated groups compared with stress control group, ^{b} <0.01 when treated group compared to stress control group

DISCUSSION

The rats of Stress control group showed a significant decrease in MST as compared to normal group, whereas the EAE, SEC4 and SEC8 treated groups showed a significant elevation in the MST. This could be because when rodents are made to swim in a restricted area become immobile after the initial period and this immobility signifies mental depression. Increase in Mean Swim Time indicates better tolerance to stressful situations [6].

A state of significant hyperglycaemia had been induced in rats of the stress control group after exposure to acute stress. This can be attributed to the fact that Forced Swim test induces physical exercise as well as psychological stress [17]. This stress leads to depletion in liver glycogen content accompanied by increased blood glucose levels which indicates glycogenolysis, but this cannot be sustained for too long and hence another source of glucose is required. This leads to activation of gluconeogenesis which is accompanied by increase in glucose and Glucose-6-Phosphatase (G6Pase) activity. To support this further, increased need of substrates for gluconeogenesis is taken care of by glucogenic amino acids that are acted upon by AST (SGOT) and ALT (SGPT) to form pyruvate and oxaloacetate. This explains the further rise in levels of liver enzymes i.e. ALT and AST in stress control group of animals. Thus enhanced gluconeogensis and glycogenolysis leads to hyperglycaemia in stress control animals [18]. This increase in levels of glucose, ALT and AST has been significantly restored back in treated groups which thus indicate adaptogenic activity for *H. rosa sinensis*.

The rats of stress control group also showed significant rise in cholesterol and triglyceride levels as compared to normal group. Stress induced rise in serum cholesterol levels is likely to be attributed to stimulation of Hypothalamo-Hypophyseal Axis (HPA) and sympathetic nervous system which inhibits the immune functions of liver and kidney. The resultant rise in levels of catecholamines and corticosteroids leads to rise in serum cholesterol levels [17], [19].

These increased levels of catecholamines mobilize lipids from adipose tissue which lead to increase in serum triglyceride levels [17], [19]. The treated groups showed significant restoration in levels of cholesterol and triglycerides.

Stress alters the levels of various hormones which have significant impact on the immune system in general. Stress and depression affect the immune system functioning with respect to both immune suppression and immune activation. Thus the activity could be due to presence of flavonoids like quercetin in EAE, anthocyanins like Cyanidin in SEC4 and SEC8 and some other polyphenolics which could probably act as smooth pro-stressors that reduce the reactivity of host defence system by decreasing the damaging effects of various stressors due to increased basal levels of mediators involved in stress response. Thus a drug induced state of resistance to such stress situations is termed as adaptogenic activity and the plant is named as 'Adaptogen' [6].

Hence the above results could be indicative that the EAE, SEC4 and SEC8 extracts of *H. rosa sinensis* possess adaptogenic and immunomodulatory activity.

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