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Phytochemical Analysis and Anti-Stress Adaptogenic Potential of Hydro-Alcoholic Extract of *Ocimum sanctum*

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Abstract: The aim of the present study was to investigate phytochemical analysis of major bioactive compounds present in hydro-alcoholic extract of *Ocimum sanctum*. Soxhlet apparatus was used for the extraction. Further, this research examined the adaptogenic potential of hydro-alcoholic extract of *Ocimum sanctum* in experimental Swiss albino mice. Three doses, 100, 150, and 200 mg/kg of the hydro-alcoholic extract prepared as a suspension in 2 ml of 2 % gum acacia were used. Swim endurance and anoxic stress tolerance tests were performed in Swiss albino rats. Potential effects of *Ocimum sanctum* were observed for reducing the stress level in a dose dependent manner. These findings indicated that *Ocimum sanctum* has significant adaptogenic activity with a therapeutic potential in the treatment of autoimmune disorders.

Key words: *Ocimum sanctum,* adaptogenic activity, Phytochemical analysis, Hydro-alcoholic extract.

Introduction

The concept of "Rasayana" is an ancient remedy in Ayurveda, indicating that adversary effect on body cells may cause stress or trauma which may result in the severe complications on the functional ability of human beings. These therapies of revitalization and rejuvenation were adopted to increase the power of resistance against diseases [1].

The basic concept of disease diagnosis and drug development in Ayurveda is based on "Tridosa" principle, i.e., *Vayu* (Air inside the body), *Pitta* (Acid content of the body) and *Kapha* (lymph content of the body) [2]. Many Indian medicinal plants such as *Aloe vera*, *Andrographis paniculata*, *Asparagus racemosus*, *Azadirachta indica*, and *Phyllanthus emblica* have been screened by various investigators in the search of novel immunomodulatory compounds and have also reported natural products that are having a reputation in ethnomedical practices giving an advance picture in the field of disease cure [3,4].

Ocimum sanctum as a medicinal herb is found various parts of India with a significant importance in the traditional Indian Ayurvedic Medicinal System, where it is known to be the universal remedy for many diseases. James *et al*., reported that *Ocimum sanctum* pretreatment exhibited significant increase in the lipid peroxidation and the level of scavenging enzymes in animal model exposed to acute, sub-acute and chronic noise stress [5]. Chemical constituents of *Ocimum sanctum* extracts such as water-soluble flavonoids have been isolated from the leaf and were found to possess potent antioxidant properties [6]. Free radical scavenging properties appear to be the mechanism of protection against oxidative stress by these flavonoids.

Extract derived from *Ocimum sanctum* leaves has been found to induce changes in the neutro-

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phil functions *in vivo* [7]. Tanzanian populations have used this plant as a mosquito repellent as well as flavor material in foods. In addtion, other *Ocimum* species have also been found to exhibit various pharmacological properties such as antimicrobial [8,9] and insecticidal activities against croppest insects [10].

Adaptation confers to the development of a various physiological states which may enable organisms to compete in the stressful environment [11]. These involve behavioral, neuroendocrine and neurochemical changes. Temporal prolongations of this adaptation to the stresses result in sustained over-activation of several stress-related psychopathologies, which cut across the boundaries of diseases including central and peripheral systems. Initial studies on plants originated from folk medicine along with an exponential increase in knowledge regarding the interactions among components of the stress system have encouraged various investigators to evaluate the potential of plant adaptogens for usages in modern day medicine. Further enrichment of the study on plant derived adaptogens was enabled by the substantial work carried out on plants such as *Ocimum sanctum* [12], *Emblica officinalis*, *Piper longum* and *Terminalia chebula* [13] and several other plants whose anti-stress activity was partially evaluated.

There are few scientific reports available in the literature on the anti-stress activities of *Ocimum sanctum*. However, the present study was undertaken to assess the *in vivo* adaptogen activities of the hydro-alcoholic extract derived from the *Ocimum sanctum* in relation with its folklore medicinal properties along with its phytochemical analysis.

Materials and methods

Plant material

Ocimum sanctum as a whole plant was collected, shade-dried, and ground. Powdered sample was room stored and a clean closed bottle for further studies

Preparation of extract

A 350 g powdered sample was repeatedly extracted with 70 % hydro-alcoholic solvent (1500 ml) using a round bottomed flask (2 liter). A 40 cycled solvent reflexing system was used for extraction purposes and the extract was filtered, evaporated and processed at room temperature.

Experimental animals

Protocol for animal studies was approved by the Dr. Hari Singh Gour University, Sagar, MP, India following an International Standard on animal experimentation. Animals (Swiss albino rats) of either sex (100 and 125 g) were used for the *in vivo* experiments. Animals were housed under standard conditions of temperature (25°C), 12 h/ 12 h light/dark cycles and fed with standard pellet diet and tap water.

Preliminary phytochemical screening

To identify the essential constituents of the hydro-alcoholic extract of *Ocimum sanctum* such as alkaloids, terpenes and steroids, saponins, flavonoids, polysaccharides and tannins, phytochemical screening was performed following various method as described previoulsy [14].

Effect of *Ocimum sanctum* extract on swim endurance

The procedure described by Bhargava and Singh [12] was used for swim endurance test. Animals were divided in nine groups of six mice each. The group I animals served as control (Saline 10 ml/ kg BW PO), group II animals received standard drug, group III animals were treated with *Ocimum sanctum* extract (100, 150, 200 mg/kg BW p.o. for 14 days). On the 14th day, mice of all the groups were allowed to swim till exhausted in separate cylindrical containers filled with water maintained at 25°C. The end point, i.e. "swimming time" for each animal was taken when the animals drowned. The mean swimming time in min for each group was calculated. The data obtained were subjected to statistical analysis.

Effect of *Ocimum sanctum* extract on anoxic stress tolerance test

The procedure described by Krupavaram *et al*. [15] was used for evaluating anoxic stress tolerance test. Animals were divided in nine groups of six mice each. The group I animals served as control (Saline 10 ml/kg BW PO), group II animals re-

ceived standard drug, group III animals were treated with *Ocimum sanctum* extract (100, 150, 200 mg/kg BW p.o. for 14 days). A 250 ml capacity air-tight conical flaks were employed for this experiment. Each animal was kept in the air-tight vessel and the time was noted using a stop-watch. The moment animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of "anoxic stress tolerance". The appearance of convulsion is a sharp end-point, as a delay of even 1 min in removal, kills the animal. The "anoxic stress tolerance time" was determined for each animal individually, after one week and two weeks of drug treatments. The data obtained were subjected to statistical analysis and observations were tabulated.

Results and discussion

Preliminary phytochemical screening

In the present study, *Ocimum sanctum* extract illustrated the presence of tannins, flavonoids, glycosides, cardiac glycosides, carbohydrates, steroids, terpenoids and proteins, whereas alkaloids, saponins, antharaquinone, gums and mucilage were absent (Table 1). These phyto-constituents although, could be extracted through successive extraction with different solvent systems, such as petroleum ether, chloroform, acetone, methanol and ethanol; recent studies have shown that the majority of active phytochemicals having medicinal importance are readily extracted when subjected to hydro-alcoholic (70 % ethanol) extraction [16].

Effect of *Ocimum sanctum* extract on swim endurance

In the present investigation, the effect of hydro-alcoholic extract of *Ocimum sanctum* in swim endurance test using Swiss albino mouse model was carried out. The groups III A and III B having hydro-alcoholic extract of *O. sanctum* at concentrations of 100 and 150 mg/kg BW, gave the swimming survival time of 391.31±9.30 and 456.74±6.77 sec, respectively, proving potential adaptogenic effect of *Ocimum sanctum*. Desipramine (30 mg/kg BW) that was used as a standard drug gave the swimming survival time of 253.01 ± 4.21 sec (Table 2).

The swim endurance test clearly indicates that the extract have the properties to increase the physical endurance as well as the overall performance in animals. The enhanced swimming endurance in mice as compared to the normal animals may attribute to the steroids [17], which were

Table 1. Phytochemical analysis of *Ocimum sanctum* hydro-alcoholic extract

No.	Test	Constituents *Ocimum sanctum*
1	Alkaloids (Draggendorff's and Mayer's reagent)	-
2	Tannins (5 % $FeCl_3$)	+
3	Flavonoids (Shibata's reaction)	+
4	Saponins (Froth test)	-
5	Glycosides (Fehling's test)	+
6	Cardiac Glycosides (Keller-Killiani test)	+
7	Carbohydrates (Molisch's test)	+
8	Anthraquinones (Borntrager's test)	-
9	Steroids (Liebermann's test)	+
10	Terpenoidss	+
11	Proteins (Biuret test)	+
12	Gums/Mucilage	-

+ = Present; - = Absent

found in the hydro-alcoholic extract of *Ocimum sanctum*. Similarly ethanolic extract of rhizomes of *Zingiber officinale* has been investigated for anoxia stress tolerance test in Swiss albino mice model [18]. The animals were also subjected to acute physical stress (swimming endurance test) to gauge the anti-stress potential of the extract. The extract treated animals showed an increase in swimming endurance time and increase in anoxia tolerance time in physical and anoxia stress models, respectively. The results indicated that the plant extract has significant adaptogenic activity against a variety of biochemical and physiological perturbations in different stress models [18].

Effect of *Ocimum sanctum* extract on anoxic stress tolerance test

In the present investigation, the effect of *O. sanctum* extract on anoxic stress tolerance test in Swiss albino mouse model was exe-cuted. The duration of the tolerance was calculated in two time intervals, i.e., on the 7th and 14th day. Observations on both the time intervals reported that hydro-alcoholic extract of *O. sanctum* at 200 mg/kg BW, proved to enhance the duration of tolerance significantly on 7th and 14th day, and the duration was 52.45±1.01 and 57.18±1.25 min, respectively (Table 3). The group III B having hydro-alcoholic extract of *O. sanctum* at 150 mg/kg BW reported stress tolerance of 29.96±1.31 min on the 7th day and 35.11±1.38 min on the 14th day. The standard drug desipramine, when administered (30 mg/kg BW), reported a stress tolerance of 30.23±1.04 min on the 7th day and 33.09±1.05 min on the 14th day. The results show a gradual increase in the stress tolerance level (Table 3).

Anoxia serves as a significant stress stimulator. All the organs of the human body functioning through cellular respiration depend on the oxygen

Table 2. Effect of *Ocimum sanctum* extracts in swim endurance test

Group	Treatment group (mg/kg BW)	Swimming survival time (sec) Mean ± SEM
I	Normal saline (Control)	214.80 ± 7.76
II	Desipramine -30 (Standard drug)	253.01 ± 4.21
III A	OSE -100	391.31 ± 9.30
III B	OSE -150	456.74 ± 6.77
III C	OSE -200	517.21 ± 8.65

All values represent Mean ± SEM; OSE = *Ocimum sanctum* extract
* = 6 Swiss albino mice in each group

Table 3. Effect of *Ocimum sanctum* extract on anoxic stress tolerance test

Group*	Treatment group(mg/kg BW)	Duration of anoxic stress tolerance (min)Mean ± SEM	
		7th Day	14th Day
I	Normal saline (control)	22.16 ± 1.18	24.30 ± 1.12
II	Desipramine 30 (Standard drug)	30.23 ± 1.04	33.09 ± 1.05
III A	OSE-100	26.26 ± 1.06	32.10 ± 2.27
III B	OSE-150	29.96 ± 1.31	35.11 ± 1.38
III C	OSE-200	32.29 ± 1.17	37.81 ± 2.16

All values represent Mean ± SEM; OSE = *Ocimum sanctum* extract
* = 6 Swiss albino mice in each group

supply to their cells in adequate amount and time interval. Any kind of deficiency of this vital element will develop disorder in the metabolic mechanisms of body organs. The increase in adaptation during anoxic stress by any drug could be considered as major anti-stressor effect of that drug. Major cause of oxidative stress is change in production ratio of oxygen reactive species. Whereas human biological system promptly heals this deteriorating damage through the use of defense system. ROS imbalance led to the formation of various hazardous toxic products which may eventually result in the formation of toxic effect to any part of the body including cell, protein, lipid and 260 nm materials. Oxidative stress causes various chronic diseases in human body and it has been found that glycogen transfer from liver to myocardium could be a physical or hypoxia adaptation character of animals [19].

Mechanism for the possible mode of action

Adaptogens increase body capacity to fight against stress condition and provide ability to respond to stress stimuli through activation of mediators [13]. Hypothalamic-pituitary-adrenal axis modulation is thought to be major mechanistic action of such adaptogens. Moreover, adaptogens modulate functional ability during stressful condition as well as stimulate production of adrenocorticotropic hormone and corticosteroid acutely [20,21]. Under situations of chronic stress, adaptogens (hydro-alcoholic *Ocimum sanctum* extract) may act by re-establishing the functioning of the axis, stopping the liberation of stress hormone by the negative feedback mechanism (Fig. 1). Adaptogens exhibit their role in hypothalamic-pituitary-adrenal axis through promotion of specific stress modulators, suggesting that *Ocimum sanctum* extract, may also provide a significant contribution in the cell life [22].

Due to the improved cognitive performance, adaptogens have ability to modulate cholinergic and other neurotransmission systems as reported previously [23]. Also it has been stated that the modulation of the dopaminergic, monoaminergic and serotonergic systems are significant plant tar-

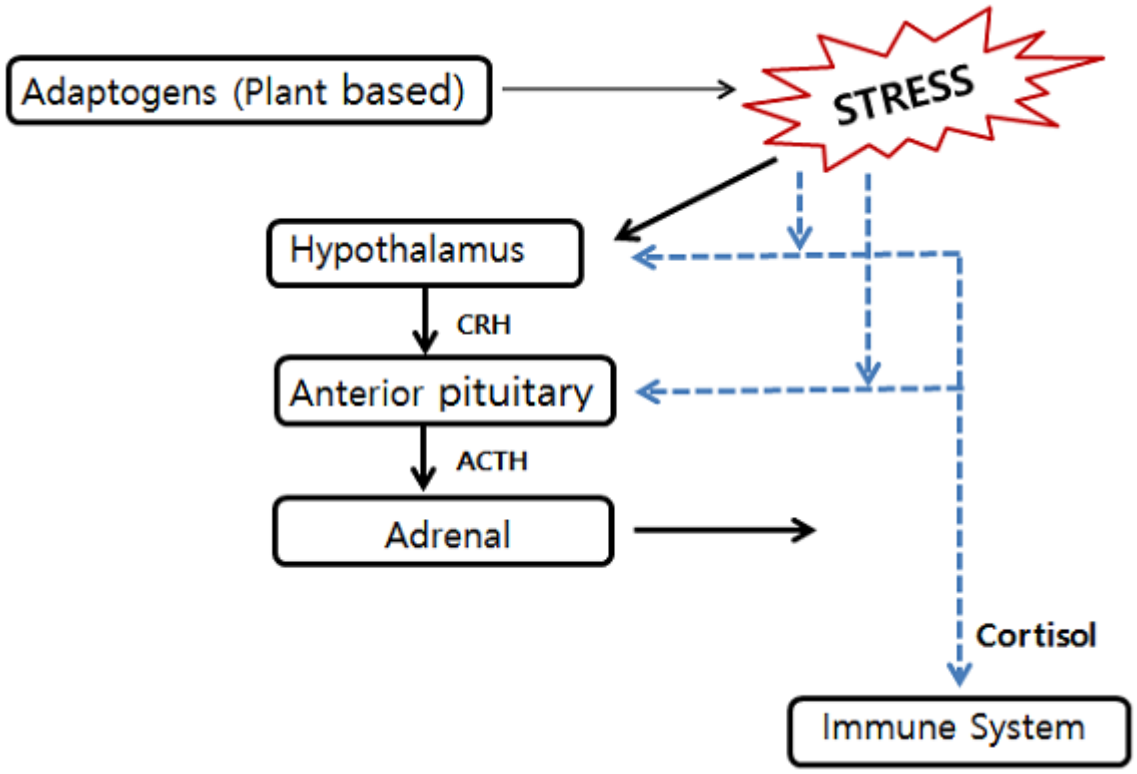

Fig. 1. Response to stress by hypothalamus in presence of plant based adaptogens. Thick lines: stimulation; Dotted lines: inhibition

gets as well as in impotency [24]. Other action mechanisms contributing to adaptogen modulation incorporate genic-transcription, and protein synthesis. Various phytochemicals including phenolics and flavonoids have been found to exhibit adaptogenic property [15].

Conclusion

This study shown that the extract derived from *O. sanctum* increased convulsion mean time, confirming its efficacy as an anti-stress agent as well as significant pharmacological agent. The efficacy of the extract-treated animals in overcoming the adversary effects of drug-induced effects for balancing and adaptogenic efficacy of the hydro-alcoholic *O. sanctum* extract. Thus, from the results obtained, it can be concluded that *O. sanctum* extract having therapeutic potential could be served as an effective anti-stress candidate. Further research to confirm mechanistic action of *O. sanctum* extract through its efficacy to treat stress related disorders is warranted.

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