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Adaptogenic activity of *Plumbago zeylanica* Linn and *Plumbago rosea*: An experimental study using swiss albino mice by swimming endurance test

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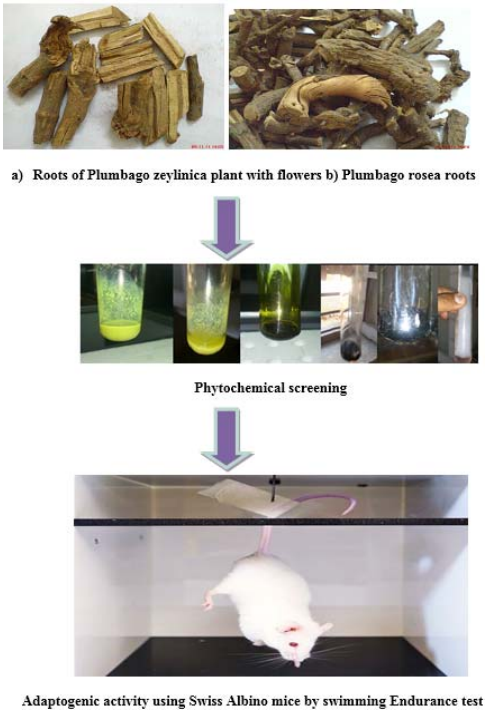
Abstract
The present study was designed to evaluate the adaptogenic activity of hydroethanolic extract of roots of Plumbago *zeylanica* and *Plumbago rosea* on Swiss Albino mice using swimming endurance test. The *Plumbago zeylanica* (Linn.) *and Plumbago rosea* (Linn.) extract were found to be safe 2000mg/kg of body weight. Therefore 1/5 and 1/10th cut off dose for *Plumbago zeylanica* (Linn.) and *Plumbago rosea* (Linn.) extract are 100 and 200 mg/kg were selected for present study **(**OECD 2000). The swimming time of mice was 289.7 s (S-100), 363.0 s(S-200) and 312.8 s (S-100), 396.7 s (S-200) for *Plumbago zeylanica* extract S and *Plumbago rosea* extract a respectively and the same was 224.3 s and 478.0 s for control and standard (Korean Ginseng).
The results of phytochemical screening reveals that the major constituents of Chitrak extract are flavonoids, phenolics compound, alkaloids, anthraquinones, glycosides and naphthoquinone compounds which may be responsible for the antioxidant, memory enhancement and adaptogenic activity. Adaptogens have not only specific therapeutic effects in some stress induced and stress related disorders, but will also have an impact on the quality of life of patients.

Graphical abstract



Keywords: Adaptogenic, hydroethanolic extracts, phytochemical screening, therapeutic effect, Swimming endurance.

Introduction
The term ‘adaptogen’ was originally used for various pharmacologically active compounds that could elicit a state of non-specifically raised resistance in mammals allowing them to counteract stressors and to adapt to exceptional strain.

Later this term was also applied to a group of plants that exert adaptogenic properties. Adaptogens are thought to reinforce the non-specific adaptive capacity to withstand situations of stress, to encourage a faster response towards stress conditions and to facilitate a more rapid return to normality [1]. The concept of Adaptogens was first introduced by the toxicologist N.V. Lazarev in 1947 to describe compounds which could increase "the state of non-specific resistance" in stress.

At the end of the 1960s Brekhman and Dardimov proposed that adaptogens are innocuous agents, nonspecifically increasing resistance against physically, chemically, biologically and psychologically noxious factors ("stressors"), normalizing effect independent of the nature of pathologic state [2]. Stress can be illustrated as a non-specific response of the body known to alter the physiological homeostasis of the organism resulting in various neuronal, endocrine and visceral dysfunctions. The ability to develop and maintain resistance against a variety of stressors (stress producing agents) encountered in human life is crucial for survival. The desire to control the stress has led to the origin of the concept of Adaptogens [3]. The stress response can be regarded as a positive adaptive process which comprises a set of functional and behavioral reactions to combat the challenging situations [4]. As a pharmacotherapeutic group adaptogens were recently defined as herbal preparations that increase attention and endurance in fatigue, and reduced stress-induced impairments and disorders related to the neuro-endocrine and immune systems.

Term Adaptogen is often applied to plants, even when the criteria of an adaptogen are not met with such as the important and significant general adaptive effect on stress involving the whole organism and its main organ and functions [5]. In the present study, opportunity has been taken to evaluate the adaptogenic activity using hydroethanolic extract of roots of the plants *Plumbago zeylanica* and *Plumbago rosea.*

According to the report of WHO, approximately 450 million people suffer from mental or behavioural disorders like stress. This amounts to 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020 [6].

Materials and Methods

Collection and identification of plant material

The roots of Plumbago zeylanica were collected from the SIPS herbal garden and Plumbago rosea (Linn.) roots were bought from the Gwalior market. The roots of Plumbago zeylanica and Plumbago rosea (Linn.) were authenticated by Dr. A.K. Jain, principal of Sagar Institute of Pharmaceutical Sciences (SIPS) Sagar (M.P). The roots of Plumbago zeylanica and Plumbago rosea (Linn.) were washed with water at the ambient temperature and dried under shed. They were preserved in the individual air tight containers after grinding to the requisite level

Preparation of Extract: The 100 g. of dried and grounded herb was macerated with 50% hydroethanolic solution and was macerated for 72 hours. Maceration was carried out with occasional shaking during the day time. Then the extract was filtered and the marc was pressed and the resultant extract was filtered and mixed in the earlier lot. Then it was distilled to recover the solvent (first ethanol at 55-60^{0}C, then water at 100^{0}C). The slightly moist extract was placed in a pre-weighed china dish and heated to get dry mass. Percentage yield of *Plumbago zeylanica* and *Plumbago rosea* extract were found to be 19.8% and 22.1%w/w.

Preliminary phytochemical analysis: The preliminary phytochemical studies were performed for testing different chemical constituents present in *Plumbago zeylanica* and *Plumbago rosea* extract.

Selection of animals: The experiment was carried out on Swiss Albino mice of the either sex weighing between 25±2g, and six-eight week. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2 °C relative humidity 44 –56% and light and dark cycles of 12:12 hours, mice were housed in groups of 6 per cage. They were fed with standard pellet diet supplied by Hindustan Lever Ltd. Kolkata and water *ad libitum.* Paddy husk was provided as bedding material, which was changed every day. The cages were maintained clean.

All the animal experiments for Extracts A and S were approved by the institutional ethics committee (IAEC) of Pinnacle Biomedical Research Institute, Bhopal (M.P.), India and conducted in accordance with the guidelines of the CPCSEA (Reg. No. no. 1283/c/09/CPCSEA). Protocol approval reference number is PBRI/IAEC/11/PN-165.

Acute Oral Toxicity: Acute oral toxicity was performed as per OECD-423 guidelines. Albino mice weighing 25 ± 2 g selected by random sampling technique were used in the study. The animals were fasted overnight, provided only water after which extract was administered orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed. If mortality was observed in 1 or 2 animals among 3 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2,000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsion and mortality for 24 hours [7].

Adaptogenic activity (swimming endurance test)

The mice in group of 6 were given vehicle or *Plumbago zeylanica* (PZ) Extract S/ *Plumbago rosea* (PR) Extract A (100 & 200 mg/kg p.o. for 7 days. The standard group was administered Korean ginseng extract 100 mg/kg (p.o) for 7 days. On the 8th day, the animals were allowed to swim till exhausted in a propylene tank of dimension 24 cm x 17 cm x 14 cm, filled with water to a height of 10 cm. The end point was taken when the animals drowned and 'swimming time' for each animal was noted. The mean swimming time for each group was calculated and the data was statistically analysed [8].

Statistical analysis: Durations of swimming (in seconds) were expressed as mean±standard error of mean (S.E.M.) an analyzed by ANOVA followed by Dunnett's test. Differences between groups were considered significant at $p < 0.01$ level and $p < 0.001$ was considered extremely significant.

Results

The results of phytochemical screening reveals that the major constituents of Chitrak extract are flavonoids, phenolics compound, alkaloids, anthraquinones, glycosides and naphthoquinone compounds (Table 1) which may be responsible for the antioxidant, memory enhancement and adaptogenic activity.

The *Plumbago zeylanica* (Linn.) *and Plumbago rosea* (Linn.) extract were found to be safe 2000mg/kg of body weight. Therefore 1/5 and 1/10th cut off dose for *Plumbago zeylanica (Linn.) and Plumbago rosea* (Linn.) extract are 100 and 200 mg/kg were selected in the present study **(**OECD 2000). The swimming time of mice was 289.7 s (S-100), 363.0 s(S-200) and 312.8 s (S-100), 396.7 s (S-200) for *Plumbago zeylanica* extract S and *Plumbago rosea* extract A respectively and the same was 224.3 s and 478.0 s for control and standard (Korean Ginseng) (Table 2 and 3; Fig 1, 2 and 3).

Table 1: Preliminary phytochemical investigations

S. No.	Phytoconstituents	Hydroethanolic extract-1	Hydroethanolic extract-2
1.	Alkaloids	Present	Present
2.	Flavonoids	Present	Present
3.	Tannins	Present	Present
4.	Saponins	Present	Present
5.	Glycosides	Present	Present
6.	Phenols	Present	Present
7.	Reducing Sugars	Present	Present
8.	Proteins	Present	Present
9.	Phytosterols	Present	Present
10.	Triterpenoids	Present	Present

Table 2: Effect of *Plumbago zeylanica* extract S on duration of swimming(s)

Treatment (dose: mg/kg p.o. for 7 days)	Duration of swimming (s) (Mean ± SEM)
Control (Vehicle)	224.3 ± 8.56
S (100)	289.7 ± 9.96
S (200)	363.0 ± 11.83***a***
Standard (Korean ginseng -100)	478.0 ± 21.28

N = 6, *p< 0.01 ***p< 0.001, a***= significant result comparing with negative control group. One way ANOVA followed by Dunnett's test

Table 3: Effect of *Plumbago rosea* extract A on duration of swimming (s)

Treatment (dose: mg/kg p.o. for 7 days)	Duration of swimming (s) (Mean ± SEM)
Control(Vehicle)	224.3 ± 8.56
A (100)	312.8 ± 8.44**
A (200)	396.7 ± 8.62**a***
Standard (Korean ginseng -100)	478.0 ± 21.28***

N = 6, *p< 0.01 ***p< 0.001, a***= significant result comparing with negative control group. One way ANOVA followed by Dunnett's test

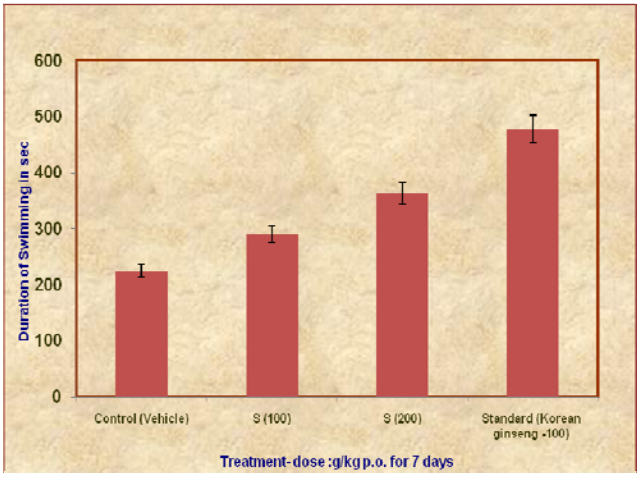


Fig 1: Effect of *P. zeylanica* extract (S) on duration of swimming

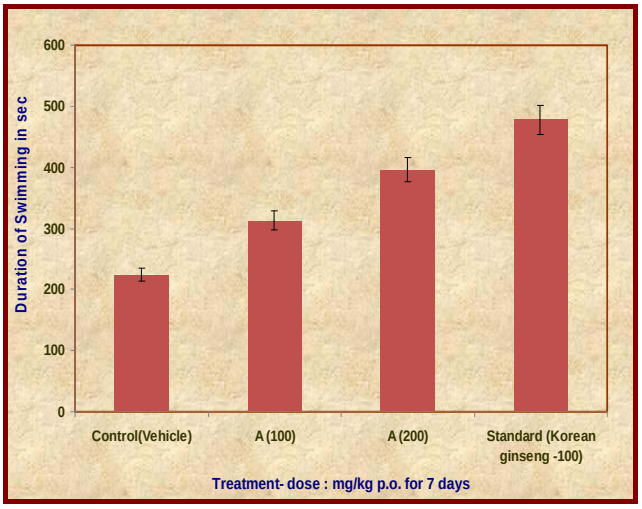


Fig 2: Effect of *P. rosea* extract on duration of swimming

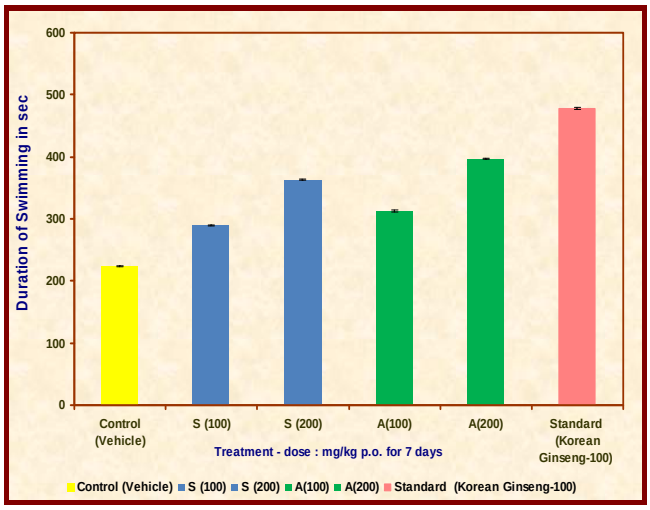


Fig 3: Effect of *P. zeylanica* and *P. rosea* extract on duration of swimming

Conclusion

The increased swimming time has been observed in mice, pre-treated with Extracts S and A with enhanced physical performance in Swim endurance test, significantly (***p<0.001) longer than untreated (control) group and thus confirming its adaptogenic nature. Korean Ginseng (100 mg) was used as standard**.** Hence the swimming endurance test exhibited significantly increase in swimming time with the extract of *Plumbago rosea* A.

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