

Effect of Alcoholic Extract of *Wedelia Chinensis* on Cold Immobilization Stress Induced Lipid Peroxidation in Rats

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Summary

Ethanollic extract of the *Wedelia chinensis* was studied on cold immobilization induced lipid peroxidation in albino rats. Administration of its extract at a dose of 500 mg/kg b. w. significantly inhibited cold immobilization stress induced increases in lipid peroxidation in the liver and brain of albino rat. The results of the present investigations suggest the potential use of the plant for decreasing anxiety and stress in many emotional and physical disorders.

Keywords: *Wedelia chinensis*, cold immobilization stress, lipid peroxidation.

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Introduction

An exponential rise in world population coupled with rapid industrial growth has a direct impact on environment and society thus making man easily vulnerable to stress conditions. These, in-turn, causes disturbances in the normal physiological functioning of the body by way of increased free radical generation culminating in hypertension, neurosis, immune suppression and other physical and mental disorders(1). Global search is on, for the development of an effective antistress drug from natural source which could effectively tone up the disturbed physiological functioning of the subjects affected by such stress problem. Many marketed formulations claim to possess antistress action, but still many herbs which have claims to be general tonics need to be investigated and their claims be authenticated. In recent era there is great thrust on screening of herbs for their antistress activity.

Wedelia chinensis Merrill (Asteraceae) is a small much branched annual herb, commonly known as “Pilabhamgara” or “Bhringraj” in Hindi and is a reputed herbal medicine in both Ayurvedic and Unani system of medicine (2). The herb contains wedelolactone and demethylwedelolactone (Coumestans derivatives) possessing potent anti-hepatotoxic effect and is incorporated as a major ingredient in a number of developed potent anti-hepatotoxic phytopharmaceuticals formulations. It is useful in the treatment of osteoporosis of knee and also possesses anti-inflammatory activity (3,4). The antioxidant (5) and wound healing activity(6)of this plant is reported by us. The role of free radicals in the pathogenesis of neurological disorder is well documented in literature. As the drug traditionally used as a tonic and possess significant antioxidant activity, considering all these fact here our aim is to screen the drug for antistress activity, by studying on cold immobilization induced lipid peroxidation in the liver and brain of albino rat.

Experimental:

Plant materials:

Wedelia chinensis was procure from the Plant Physiology Division, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, (M.P.) and authenticated by Dr. Anjula Pandey, taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, Pusa Campus, New Delhi. Voucher specimens (Vide access no. NHCP/NBPGR/2007/99/2225 dated 22/08/2007) was retained in our laboratory for further reference.

Plant extract:

The plant material was dried under shade reduced to moderately coarse powder and was extracted successively with petroleum ether (60-80°C) and ethanol using soxhlet apparatus. The ethanolic extract was dried under vacuum (yield 9.47%) and then suspended in 20% (v/v) propylene glycol –water to give a concentration of 500 mg/ml. Preliminary phytochemical screening was carried out on the *W. chinensis* extract to assess the presence of various phytoconstituents viz. steroids, alkaloids, glycosides, flavonoids, saponin and carbohydrates.

Chemicals and reagents:

All the chemicals and reagents used in the study were of analytical grade. Potassium chloride was obtained from Merck Limited, India. The chemicals used for lipid peroxidation parameters were obtained from Sisco Research Laboratories, India. Kits used for the estimation of malonyl dialdehyde content were purchased from Centronic GmbH, Germany.

Animals:

The Institutional Animal Ethics Committee, (IAEC) review the protocol and approved the use of animals for the studies, **(Ethical clearance number: 711/02/a/CPCSEA)**.

Wistar albino rats of both sexes (150±20 g b. w.) were used for the present studies. They were housed in clean polypropylene cages (3 in each cage) and maintained under standard laboratory condition at an ambient temperature 25±2°C with 55-64% relative humidity and 12 h light -dark cycle. They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libidum*.

Acute toxicity studies

Acute toxicity study was performed for the extract according to the acute toxic classic methods as per OECD guidelines (7). Wistar albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract were administered orally 500 mg/kg b. w. and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the dose administered was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 2000 mg/kg.

Assessment of cold immobilization stress induced lipid peroxidation in rats:

The animals were divided in to three groups of seven rats each. Group I, served as a control and received no treatment. The rats of group II were administered 1 ml of vehicle once daily for 16 days. The animals of group III were given 1 ml of suspension of the alcoholic extract of *W. chinensis* (500 mg/ kg/ p. o.) once daily for 16 days.

At the end of the 16 days, the rats of group II and III were individually immobilized in Plexiglas restrainer and kept at 4°C for 3 h. Thereafter all the animals were killed by decapitation, their brain and liver quickly dissected out, the tissue washed with potassium chloride (1.15% w/v) to make a 10% homogenate of the tissue. The homogenate was centrifuged and the malonyl dialdehyde content (an intermediate of lipid peroxidation) was determined in the supernatant using the spectrophotometric procedure (8).

Statistical analysis:

The data represent *mean ± S.E.M.* Results were analyzed statistically by one-way ANOVA followed by Student's 't' test. The minimum level of significance was set at $P < 0.001$ compared to control. The entire statistics were estimated by using **Sigma Stat 3.5™**, statistical software.

Results and Discussion

Cold immobilization stress significantly increased lipid peroxidation in the brain and liver tissue, the increases being relatively more pronounced in brain tissue than in the liver. On the other hand, in the *W. chinensis* treated groups of rats the brain and liver lipid peroxidation did not increase following cold immobilization stress ($P < 0.001$, Table-I). Such an increase in lipid peroxidation is believed to be caused by stimulation of the mixed function oxidase system resulting from hormonal changes induced by stress (9).

Since administration of ethanolic extract of *W. chinensis* effectively prevented the cold immobilization stress induced increase in brain and liver lipid peroxidation, it might possibly be useful in preventing a number of stress disorders which are now on the increase.

Table 1: Effect of *Wedelia chinensis* on cold immobilization stress induced brain and liver lipid peroxidation

Group	Lipid peroxidation (MDA nmol/g tissues wet weight)	
	Brain	Liver
I. Normal Control (7)	154.56±12.44	358.47±23.11
II. Restraint Stress Control (7)	1056.39±67.33**	743.44±28.99**
III. Restraint Stress + <i>W.chinensis</i> treatment (7)	169.33±16.22*	456.89±19.56*

Values are expressed as Mean ± S.E.M

Figures in parentheses indicate the number of observations

* $p < 0.001$ compared to control

** $p < 0.001$ compared with normal control.

Conclusion

Based on the above results obtained it may be concluded that the ethanolic extract of *Wedelia chinensis*, significantly inhibited cold immobilization stress induced increases in lipid peroxidation in the liver and brain of albino rat.

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