COMPARATIVE ANTI-ALLODYNIC EFFECTS AND TOXICITY STUDIES FOR THE HERBAL *WRIGHTIA TOMENTOSA* LEAF & BARK IN SWISS ALBINO MICE

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Summary

Ethanol bark and leaf extract of Wrightia tomentosa has been shown to exhibit anti-allodynic activity in swiss albino mice. The aim of this study was to evaluate the comparative anti-allodynic effects of both the leaf and bark extract of the plant along with acute oral toxicity study. Acute toxicity studies were carried out by using organization of Economic Co-operation and development (OECD) guidelines 423, using minimum number of male wistar rats with defined doses (5,50,300,2000 mg/kg body weight) of extracts. The extracts treated animals did not show any mortality and observable signs of toxicity. From the toxicity studies, 1/5th of dose was selected as maximum dose for testing of anti-allodynic activity. Comparative anti-allodynic activity of ethanolic leaf and bark extracts were determined, with six groups of six albino mice each by tail immersion method. After administration of test drugs in two different doses, the reaction time was measured at 0, 30, 60, 120 and 180 minutes. The highest reaction time was considered as maximum anti-allodynic effect, which was exhibited by the bark

extract (400 mg/kg) after 120 minutes of administration with a value of 14.67 ± 4.17 seconds in comparison with standard drug aspirin (8.33 ± 1.72). The current studies generally focus on induced pain in animal models that are designed to evaluate anti-allodynic effects for humans in near future.

Key words: Wrightia tomentosa, Anti-allodynic effect, Toxicity testing, Leaf, Bark.

Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage **[1].** Chronic pain is not very satisfactorily managed with opioids. The hyper excitable sensory neuron bombards the spinal cord leading to increased excitability and synaptic alterations in the dorsal horn. Such changes appear to be important in chronic inflammatory and neuropathic pain states **[2].** In the effort to discover better anti-allodynic plant drugs for chronic pain, renowed attention is being paid to synaptic transmission in nociception and sensory processing.

Toxic effects are greatly variable in nature, target organ, and mechanism of action. A better understanding of their characteristics can improve assessment of the associated health hazards. It can also facilitate the development of rational preventive and therapeutic measures. In general, the effects of a toxicant elicited in laboratory animals can be expected to occur in humans under appropriate conditions.

Consequently, studies in laboratory animals have become the main source of toxicological data. On the positive side to animal studies, one should note that it is usually feasible in animal studies to employ one or more relatively high doses that will induce overt signs of toxicity. These signs will help to pinpoint the target organ and the specific effect, which can then be critically examined in animals treated with lower doses. The use of such high doses can also partly obviate the need for placing very large numbers of animals in the studies **[3].**

Herbal medicines are used throughout in developed and developing countries as home remedies, over the counter drug products and raw materials for the Pharmaceutical industry, and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing quality. The world health assembly-in resolutions, has emphasized the need to ensure the quality of herbal medicine by using modern control techniques and applying suitable standards **(4).** This article utilizes the method of OECD guidelines to support the development of national standards local market conditions, with due regard to existing national legislation and national and regional norms.

Wrightia tomentosa Roem.&Schult belonging to the family Apocynaceae is a small deciduous tree up to 12m high found throughout the warmer parts of India, ascending to an altitude of 600m in the Himalayas and to 1,200m in the Nilgiris. The bark is greyish yellow to rust –coloured, corky with light coloured specks, leaves elliptic, often tomentose,7.5 –15.0 cm long **[5].**

The bark and root–bark are believed to be useful in snake–bite and Scorpion –Stings **[6].** A novel isoflavone, wrightiadione isolated from the plant possess cytotoxic activity against the murine P 388 lymphocytic leukemia cell line **[7].** The butanol extract of Wrightia tomentosa bark and leaf were also reported to possess antibacterial properties **[8]**. The ethanolic bark extract of Wrightia tomentosa was found to possess maximum antihyperglycemic activity in streptozotocin induced diabetic rats **[9]**.The objective of the present investigation is to assess the comparative anti-allodynic activity of ethanolic leaf and bark extract of this plant along with acute oral toxicity study.

Materials and Methods:

I Preparation of the extract

The leaves and bark of Wrightia tomentosa were collected from the hills of yercaud forest,Tamilnadu. The plant identity was confirmed **[10,11]** and a specimen voucher was made with the authentication of an acknowledged Botanist. The leaves & bark were dried under shade and then powdered. The powdered bark and leaves were extracted with ethanol by continuous hot extraction using soxhlet apparatus for 16 hrs separately. The extract was concentrated further to remove the solvent using Rotary Vacuum evaporator (Buchi Rota vapour, Switzerland) and dried on dessicator. On qualitative analysis **[12],** ethanolic leaf and bark extract showed the presence of alkaloids, flvonoids, gums & mucilages and fats and oils.

II. Toxicological evaluation

1. Acute oral toxicity study [13-15]

The procedure was followed by using OECD (Organization of Economic Co-operation and Development) guidelines 423 (Acute Toxic class method). The Acute Toxic class method **(16)** is a stepwise procedure with 3 animals of a single sex per step.

Depending on the mortality or moribund status of the animals, an average of 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimum number of animals while allowing for acceptable data based scientific conclusion. The method uses defind doses (5,50,300,2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

2. Experimental

Male wistar rats weighing 150-200 gm were used for the study. The starting dose level of extracts was 2000mg/kg body weight p.o. As most of the crude extracts possess LD_{50} value of more than 2000 mg/kg p.o., the starting dose was selected in such a manner described above. Dose Volume was administered as 0.1ml/10 gm body weight to the rat which were fasted overnight with water adlibitum. Food was withheld for a further 3-4 hours after administration of extracts and observed for various signs of toxicity. As a general rule, the animals should be observed for at least seven to fourteen days **(17)**.

Body weight of the rats before and after termination were noted and any changes in skin and fur, eyes end mucous membranes and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behaviour pattern were observed, and also sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

III Analgesic activity

1. Animals

Albino Swiss mice of either sex (20 - 25 gm) were used. The animals were maintained in a well ventilated room with 12 hours light /dark cycle in standard polypropylene cages under constant room temperature (26±3°C) and humidity (30-70%) with free access to food (balanced and prepared) and water throughout the experimental period.

2. Experimental

Comparative anti-allodynic activity of the ethanolic leaf and bark extracts were determined by tail immersion method [**18].** Prior to anti-allodynic experiment, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C-55.5°C. The animal which lifted the tail from hot water within 5 seconds was selected for the study **[19].** The selected mice were then divided in to six groups of six mice each. Group –III and IV received ethanolic bark extract in 1% w/v Sodium carboxy methyl cellulose (SCMC) in normal saline intraperitoneally at a dose of 200 and 400 mg/kg respectively.

Similarly, Groups–V and VI received ethanolic leaf extract in 1% w/v SCMC in normal saline intraperitoneally at a dose of 200 and 400 mg/kg respectively. Group-II received the standard drug, Aspirin (100 mg/kg) and Group –I received 1% w/v of Sodium carboxy methyl cellulose (10ml /kg) in normal saline in similar manner. After administration of the test drugs, the reaction time was measured at 0,30,60,120 and 180 minutes. The cut-off time was considered as 15 seconds. The highest reaction time is considered as maximum anti-allodynic potency. The results are depicted in table-1.

3. Statistical Analysis [20].

Results were expressed as mean ± S.D from six observations and analysed by one way ANOVA followed by Dunnett's test. The level of significance for the anti-allodynic experiment was $p<0.05$*, 0.01** and 0.001***.

Results

The extracts treated animals in acute toxicity testing did not show any mortality and there has been no considerable change in body weight before and after treatment, which in turn indicates no observable signs of toxicity. The LD_{50} cut off mg/kg body weight was observed as X-unclassified. $1/5^{th}$ of dose (maximum dose) tested for LD_{50} of individual extracts was considered for the testing of anti-allodynic activity. The extracts were administered p.o at doses of 200mg/kg and 400mg/kg of ethanol bark and ethanol leaf respectively in the anti-allodynic experiments.

In anti-allodynic studies, both the bark and leaf extract of ethanol from Wrightia tomentosa showed significant anti-allodynic activity at higher dose level (400mg/kg) after 30 minutes internal. Among the extracts tested, the bark extract of the plant exhibited maximum anti-allodynic activity after 120 minutes of administration with a value of 14.67 ± 4.17 seconds at the dose of 400 mg/kg in comparision with the standard drug aspirin having the tail withdrawal time of 8.33 ± 1.72 seconds after 120 minutes of drug administration. When compared with the control, the test drug, preferably, ethanolic bark extract (400mg/kg) showed significant anti-allodynic activity ($p<0.001$) against noxious stimuli. From the above pharmacological studies & statistical analysis, it is quite apparent that the ethanolic bark & leaf extract of Wrightia tomentosa possesses significant anti-allodynic effects.

Discussion

Some herbal drugs may be directly used, without any toxicity testing. However, when an extract or active fraction of such drug is used it is better to evaluate possible toxicity. Although it is the normal practice to determine the

LD_{50} value, now it is acceptable to limit the study to an acute toxicity test using multiple doses including reasonably high doses of the drug **(21).**

Table: 1

Comparative anti-allodynic effects of ethanolic extract of the bark & leaves of Wrightia tomentosa Roem & Schult by Tail immersion method in mice.

Group	Treatment	Dose	Basal reaction time (sec)	Reaction time (sec)			
				30 min	60 min	120min	180min
I	1%w/v Sodium carboxy methyl cellulose	10 ml/kg	2.83 ± 0.69	3.0 ± 0.63	2.83 ± 0.52	2.67 ± 0.52	2.83 ± 0.69
II	Aspirin	100 mg/kg	2.83 ± 0.69	6.83 ± 0.75***	9.67 ± 1.63***	8.33 ± 1.72***	7.0 ± 1.78***
III	Ethanol bark extract	200 mg/kg	2.83 ± 0.69	3.66 ± 0.89 NS	7.67 ± 1.03**	8.5 ± 0.54***	5.33 ± 0.52*
IV	Ethanol bark extract	400 mg/kg	2.83 ± 0.69	4.14 ± 0.51*	7.67 ± 1.03**	14.67 ± 4.17***	8.66 ± 4.62***
V	Ethanol leaf extract	200 mg/kg	2.83 ± 0.69	3.33 ± 0.82 NS	5.33 ± 0.76*	4.67 ± 1.2*	4.67 ± 0.82*
VI	Ethanol leaf extract	400 mg/kg	2.83 ± 0.69	4.16 ± 0.61*	6.33 ± 1.96*	7.33 ± 2.06***	5.83 ± 1.72*

Values are mean ± S.D of six animals in each group. Comparison were made between control Vs drug treated groups. One way ANOVA followed by Dunnett's t-test is performed. The BRT is compared with reaction time after 30 min, 60 min, 120 min & 180 minutes.

NS-Not Significant;
***signified p<0.01;*
**Signified p<0.05;*
**** signified p<0.001*

In this connection, the test drug, that is, both the leaf and bark extract of ethanol was also subjected to an acute toxicity testing and it was tested up to a high concentration of 2000mg/kg, orally (ten times more than the anti-allodynic dose, evaluated in the present study). Even at this dose, both the leaf and bark extract does not produce signs of toxicity or treatment-related adverse effects in the tests for anti-allodynic activity. This suggests that its short term use for this purpose is apparently safe.

As preliminary phytochemical results indicated, it could be suggested that the higher anti-allodynic effects of the bark extracts may be due to their content of flavonoids predominantly present in it. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, hesperidin and bioflavonoids produced significant antinociceptive and anti-inflammatory activities **(22-25).** Both the bark and leaf extract at higher doses (400mg/kg) exhibits significant anti-allodynic effects against noxious stimuli. The thermal methods generally require higher anti-allodynic dose rates to prevent a pain response than other methods, and the duration of analgesia is shorter than for mechanical methods. Therefore it is suggested that drug dosages based on thermal assessment may be more clinically relevant than dosages based on other techniques (as evidenced from Table.1).

Anti-allodynic effects have been demonstrated in some of the experimental models of pain in rats. Among the models tested, Cholecystokinin (CCK) appears to have an anti-opioid effect, and CCK receptor blockers have been shown to have anti-allodynic activity in rats **(26).**

N-methyl-D-aspartate (NMDA) glutamate receptor antagonist drug, ketamine is more widely used as an anaesthetic agent, but shows significant analgesic properties when used alone or in combination **(27).** As the anti-allodynic activity of most extracts in tail immersion test was not inhibited by naloxone, the ethanol bark & leaf extracts may not act via opioid receptors and may exert their activity via a peripheral mechanism. Hence the possibility exists to counteract or interrupt those changes only through targeted antagonism of neuro transmitters. The current studies generally focus on induced pain in animal models that are designed to evaluate anti-allodynic effect for humans in near future.

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