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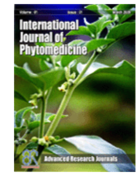

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Studies on antitussive effect of *Tectona grandis* roots using a cough model induced by sulfur dioxide gas in guinea pigs.

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Abstract

Objectives: Anti-tussive drugs are among the most widely used medications world wide; however no new class of drugs has been introduced into the market by keeping this point in mind. This attempt is made by testing a root of a well known timber tree Tectona grandis.

Materials and methods: Here in, we have analyzed the polar compounds of Tectona grandis roots using chemical and biological methods. In this study, in vivo antitussive activity of root extracts (methanol and water) of Tectona grandis Linn. f. (Verbenaceae) was evaluated using a cough model induced by sulfur dioxide gas in rats.

Results and Discussion: Treatment with aqueous extract and methanol extract at 500 mg/kg p.o. dose level showed more anti-tussive effect as compared with the dose level of 250mg/kg p.o. Differences between means were assessed by one way analysis of variance (ANOVA),followed by Dunnett's test using sigma stat software. Both the extracts (methanol and water) significantly ($P<0.05$) suppressed the asthmas at the dose level of 500mg/kg.Phytochemical tests showed that methanol and water extracts tested positive for carbohydrates, reducing sugars, alkaloids, glycosides, flavonoids, sterols and saponins.

Conclusion: This study provides a scientific basis on ethno medical uses of this plant. Further exploration in drug development of anti-tussives from Tectona grandis can focus on the purity activity relationships (PAR studies).

Keywords - Antitussive, Tectona grandis, Rhizomes and Methanol extract.

Introduction

Cough is an essential defensive reflex and is also an ordinary symptom of diseases such as asthma, chronic obstructive pulmonary disease (COPD) and lung cancer. Control of cough remains a major unmet medical need and, although the centrally acting opioids have remained the anti-tussive drug of choice for long period of time, they possess many unnecessary side-effects such as sedation and gastrointestinal symptoms [1]. Cough may be caused by several mechanisms including; mechanical stimulation of normal afferent system, chemicals, inflammatory mediators, and neurotransmitter stimulation of normal afferent system [2]. Although the modulating effects of inflammatory mediators and neurotransmitters on the cough reflex are likely to be vital but it needs further investigations [3, 4].

Plant and plant extracts have been used since the dawn of civilization by man. The use of ethnobotanical preparations for various reasons justified or not, is still continued by various cultures around the world [5]**.** Cough is the most common symptom of respiratory tract disorders. Pharmacological modulation of pathological cough is still unsatisfactory despite the intensive research resulting in large number of publications on experimental results. Opioid anti-tussives are still widely used in the treatment of dry cough during catarrhs of the respiratory system. However, their use brings about side effects such as increase of the mucus viscosity, decrease of expectoration, hypotension and constipation. Moreover long term treatment with opiate agonists may lead to drug dependence. Therefore in recent years much effort has been made to search for natural active plant components with diminished adverse effects [6]**.** One approach to discover newer antiasthmatic agents is to search for their presence in natural sources. Tectona grandis Linn. f. (Verbenaceae) is well known for its high grade timber. Roots are useful in the treatment of urinary system related troubles. According to Unani-system of medicine, the oil from flower is hair promoter and useful in scabies. Wood is good for headache, biliousness, burning sensation and pain and liver related troubles. It allays thirst and possesses anthelmintic and expectorant properties [7]. Khan and Mlungwana isolated a new compound 5-hydroxylapachol along with the known constituents' lapachol, dehydrolapachone, methyl quinizarin and squalene from the root heart wood of T. grandis. Both compounds were found to be cytotoxic to artemia saline (brine shrimp) with a LC_{50} of 5 ppm. Lapachol was also reported to have anti-ulcer activity [8]. Lack of scientific data with respect to the pharmacological properties of the root of Tectona grandis Linn. f. encouraged for the evaluation of antitussive activity.

Materials and methods

Sample Collection

Tectona grandis Linn. f. (Verbenaceae) roots were collected from, Matki village, Saharanpur (U.P.) India and authenticated by Dr. Prashant chaddha, Scientist, Botanical Survey of India (BSI), Dehradun (U.K.), India, on 07-01-2008 with letter Ref. No. BSI/NC- 9 ()/2007-08/Tech/1011. Authentic sample was deposited in the department of pharmacognosy of the institute.

Processing of Sample

The fresh roots were dried at room temperature (25-35 ^{O}C) for 20-25 days. The dried roots were powdered in a grinder and weighed before used for calculating the yield.

Preparation of root extracts and qualitative analysis

The dried powdered material of T. grandis was extracted using soxhlet apparatus with methanol and water in a sequence of polarity. The extracts were concentrated under vacuum to obtain semi-solid mass and qualitative phytochemical tests showed that methanol and water extracts tested positive for carbohydrates, reducing sugars, alkaloids, glycosides, flavonoids, sterols and saponins. All the extracts were stored in a clean glass bottles for further pharmacological studies.

Antitussive activity

Animals Used

30 Guinea pigs of both sexes, weighing 400-500 g were procured from animal house, GRD (PG) IMT, Dehradun (U.K.) India. They were placed in cages with wire net- floars and kept in standard environment conditions with a standard rodent diet and water ad-libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout the experiment. The Guinea pigs were divided into six groups of 5 each. The first group was designated as control group, 2nd, 3rd, 4th and 5th groups were used as test groups and 6th group was the standard group.

Ethical approval

The animal experiment was carried out as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms, mentioned by the NIH guidelines, wide our college Regn No: 1145/a/07/CPCSEA, Dated; 02 Jan 2008 and project resolution no. 7.

Dose administration

The control group received vehicle (Saline 10 ml/Kg, p.o.); groups 2 and 3 were treated with methanol extract (250 and 500 mg/kg, p.o, respectively); groups 4 and 5 were treated with water extract (250 and 500 mg/kg, p.o., respectively); and group 6 with codeine sulphate (10mg/kg, p.o.), as a standard antiasthmatic agent.

Animal model

The effects against SO_2 induced cough were evaluated according to Miyagoshi, M. et al. (1986) [9] A burette containing concentrated sulfuric acid was fixed to a three necked flask containing aqueous saturated sodium hydrogen sulfite solution, and the acid was added to this solution to generate sulfur dioxide gas. After 30 minutes of gastric perfusion, the guinea pigs were placed in a special glass chamber and exposed to sulfur dioxide. The latent period of cough was recorded for 2 minutes. After 1 hr. administration of saline solution, and test drugs; the guinea pigs of all the groups were exposed (one by one) to So_2 for 30 seconds and then the frequency of the cough was observed for 5 minutes. The mean score for each group was calculated.

Results

Qualitative test results were compiled in table 1. Treatment with aqueous extract and methanol extract at 500 mg/kg p.o. dose level showed more anti-tussive effect as compared with the dose level of 250mg/kg p.o. All the data is summarized in table 2.

Orally administered methanol extract of Tectona grandis root produced 67.5% and 70.3% inhibition of So_2- induced cough at dose level of 250 and 500 mg/kg respectively and water extract produced 60% and 76.06 inhibition of So_2 induced cough at doses of 250 and 500 mg/kg, respectively, whereas, the standard antiasthmatic drug codeine phosphate (10mg/1kg, p.o.) showed 30% inhibition (Table 2). Measurement of coughing were also noted and summarized in table 3.

Table 1: Qualitative Chemical Analysis of Root extract of Tectona grandis Linn.f.

Test Performed	Color	Methanol extract	Water extract
Test for Alkaloids			
Mayer's Test	Cream	+	+
Hager's Test	Yellow	+	+
Wanger's Test	Red-Brown	+	+
Dragendroff's Test	Brown	+	+
Test for Carbohydrates			
a. Molisch Test	Purple	+	+
b. Fehling Solution	Brick red	+	+
Test for Cardiac Glycosides			
Legal's Test	Pink	+	+
Test for Saponin			
Foam Test		+	+
Test for Steroids			
Salkowski's Test	Deep red	+	+
Test for saponin			
Foam Test		+	+
Test for phenols/tannins			
Lead Acetate Test	White	-	-
1% Gelatin Test	White	-	-
Test for proteins and Amino Acids			
Ninhydrin Test	Purple	+	+

Table 2: Antiasthmatic effect of root extracts of Tectona grandis by So_2 induced cough method in guinea pigs.

Treatment	Dose (mg/kg/p.o)	↓Frequency of cough	Inhibition (%)
Control (Saline)	10	66±10.752	**-**
Methanol Extract	250	21.4 ±2.441*	**67.5**
	500	19.6±4.905*	**70.3**
Water Extract	250	26.4 ± 2.926 *	**60**
	500	15.8 ± 3.007**	**76.06**
Codeine Phosphate	10	46.2±3.153 ***	**30**

Values are mean + S.E.M., n = 5; in each group p*<.01, p<.001, p***<.1 Compared with control.**

Table.3 Determination of Frequency and It's scoring.

Treatment	S.No.	Running one Ground	Sniffing	Coughing	Paws Raising	Total Score
Control Group	1.	1x5=5	2x5=10	5x3=15	1x24=24	54
	2.	1x8=5	2x4=8	5x16=80	1x12=12	108
	3.	1x4=4	2x6=12	5x5=25	1x17=17	58
	4.	1x6=6	2x2=4	5x4=20	1x18=18	48
	5.	1x2=2	2x7=14	5x6=30	1x16=16	62
CH_3OH Extract (250 mg /kg)	1.	1x2=2	2x1=2	5x0=0	1x12=12	16
	2.	1x3=3	2x2=4	5x0=0	1x23=23	30
	3.	1x1=1	2x2=4	5x2=10	1x5=5	20
	4.	1x4=4	2x3=6	5x1=5	1x7=7	23
	5.	1x2=2	2x0=0	5x2=10	1x6=6	18
CH_3OH Extract (500 mg /kg)	1.	1x0=0	2x0=0	5x0=0	1x2=2	2
	2.	1x1=1	2x1=2	5x2=10	1x4=4	17
	3.	1x2=2	2x1=2	5x2=1	1x14=4	28
	4.	1x2=2	2x3=6	5x0=0	1x21=21	29
	5.	1x3=3	2x2=4	5x1=5	1x10=10	22
H_2O Extract (250 mg /kg)	1.	1x1=1	2x1=2	5x0=0	1x21=21	24
	2.	1x0=0	2x0=0	5x1=5	1x29=29	34
	3.	1x3=3	2x2=4	5x0=0	1x17=17	24
	4.	1x2=2	2x2=4	5x2=10	1x16=16	32
	5.	1x2=2	2x1=2	5x1=5	1x9=9	18
H_2O Extract (500 mg /kg)	1.	1x0=0	2x2=4	5x1=15	1x15=15	24
	2.	1x2=2	2x0=0	5x0=80	1x8=8	10
	3.	1x0=0	2x1=2	5x0=25	1x8=8	10
	4.	1x1=1	2x1=2	5x1=20	1x5=5	13
	5.	1x2=2	2x2=4	5x0=30	1x16=16	22
Codeine Phosphate	1.	1x2=2	2x4=8	5x2=10	1x22=22	42
	2.	1x0=0	2x2=4	5x3=15	1x30=30	49
	3.	1x2=2	2x3=6	5x3=15	1x28=28	51
	4.	1x4=4	2x3=6	5x2=10	1x33=33	53
	5.	1x3=3	2x4=8	5x4=20	1x25=25	56

The observed antiasthmatic effect were dose dependent for both the extract and codeine phosphate. Both concentration of methanol and water extract caused significant reduction in frequency of cough compared to control ($P<0.01$ to $P<0.001$). However, the antitussive effect of aqueous extract (500 mg/kg) was more significant ($P<0.001$) as compared to the codeine sulphate ($P<0.1$).

Discussion

Folkloric treatment of asthma of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. Through trial and error down the ages, many plants are found to have therapeutic values and toxicity. Such empirical and indigenous knowledge have passed from one generation to the next generation either by word of mouth or written records. The applications and benefits of traditional medicines are often limited by the lack of sensitive analytical tools to weed out the undesirable and by the heterogeneous nature of natural products. Ethno pharmacological studies today can help to document, verify and refine such knowledge to the extent that the herbs can be better utilized for health maintenance and disease treatment [10]. After screening of root extracts of T. grandis has revealed that the root possesses potent antitussive effect in the animal models. Treatment with aqueous extract and methanol extract at 500 mg/kg p.o. dose level showed more anti-tussive effect as compared with the dose level of 250mg/kg p.o. (Table 2). Methanol extract produced 67.5% and 70.3% inhibition of So_2- induced cough at dose level of 250 and 500 mg/kg respectively while water extract produced 60% and 76.06 inhibition of SO_2 induced cough at doses of 250 and 500 mg/kg, respectively, whereas, the standard antiasthmatic drug codeine phosphate (10mg/1kg, p.o.) showed 30% inhibition (Table 2). Measurements of coughing were also noted and summarized in table 3. The observed antiasthmatic effects were dose dependent for both the extract and codeine phosphate. More studies are required to achieve the proper role of Tectona grandis Linn. f. extract to find out more specific biochemical, pharmacological and molecular aspects of the targeted molecules within that may have the broadest implication to society. Further work on the types of phytoconstituents isolation of bioactive compound can reveal the exact potential of the plant to inhibit several types of pharmacological evaluation and encourage in developing a novel new drugs in future. The mechanism behind the anti-tussive activity of the compounds present in the roots is poorly understood. However, it may be due to thinning, protective effects on mucous and other mechanisms. Further studies in this area are in progress.

Conclusions

The findings of this study highlight several novel and important aspects of T. grandis derived extract with regard to their anti-tussive properties. Water soluble compounds could be isolated in pure form through various modern methods including chromatography. The results obtained in this study provide support for the use of Tectona grandis in the treatment of cough. This study indicated that the antitussive effect of T. grandis was comparable to that of codeine. Furthermore, work relating to the isolation and characterization of the active constituents as well as the evaluation of mechanism of antitussive effect of the drug needs to be explored.

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