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Evaluation of analgesic activity of methanolic root extracts of *Trapa bispinosa roxb*

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Abstract
India has one of the richest plant medical traditions in the world. It is a tradition that is of remarkable contemporary relevance for ensuring health security to the teeming millions. There are estimated to be around 25,000 effective plant- based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually.

Chinese herbal medicines constitute multibillion dollar industries worldwide and more than 1500 herbals are sold as dietary supplements or ethnic traditional medicines. Another example of the use of an herbal preparation in modern medicine is the foxglove plant. This herb had been in use since 1775. At present, the millions of heart patients know the powdered leaf of this plant as the cardiac stimulant, digitalis. There are over 750,000 plants on earth. Relatively speaking, only a very few of the healing herbs have been studied scientifically. Because modern pharmacology looks for one active ingredient and seeks to isolate it to the exclusion of all the others, most of the research that is done on plants continues to focus on identifying and isolating active ingredients, rather than studying the medicinal properties of whole plants. Herbalists, however, consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown. Algesic substances are released by damaged tissues in localized area. This phenomenon is known as peripheral sensitization. As healing starts, nerve ending of the polymodal C-fibers show increased sensitivity to stimuli. Thus any stimuli in a winter area than the initial site of injury become painful. This process is a result of increased sensitization of nerve endings and is known as central sensitization.

Keywords: Algesic substances, arachidonic acid, Aβ-fibers, nociceptive, skeletal muscle

Introduction
The plant kingdom is the most important treasure of the nature and it serves as the important source of drugs. Drugs of natural origin are relatively safer and even cheaper. More than 65% of total world population depend on herbal and traditional drugs. The science of herbal and traditional drugs has been evolved along with the civilization. As the scientific knowledge accumulated, human beings have started deviating from the usage of herbal drugs to drugs of synthetic origin. All these drugs of synthetic origin are chemical molecules and when they are administered to human, they react with one or other endogenous substance to elicit their response. But along with the correction of the pathophysiological conditions these drug molecules also produce adverse reactions by interacting with other endogenous substances . Status of Herbal Medicine in India India has rich tradition of herbal medicine as evident from Ayurvedic, which could not have flourished for two thousand years without any scientific basis. Ayurveda which literally means Knowledge (Veda) of life (Ayur) had its beginning in Atharvaveda (Circa 1500-1000 BC). Vegetable products dominated Indian Materia Medica which made extensive use of bark, leaves, flower, fruit, root, tubers and juices. Charak, Sushruta and Vagbhata described 700 herbal drugs with their properties and clinical effects. Based on clinical effects 50 categories of drugs have been described such as appetizers, digestive stimulant, laxatives, antidiarrhoea, antihaemorrhoid, antiemetic, antipyretic, antiinflammatory, antipruritic, antiasthmatic, antiepileptic, antihelminthic,

haemopoeitic, haemostatic, analgesic, sedative, promoter of strength, complexion, voice, increases the sperm count, breast milk secretion, fracture and wound healing and destroyer of kidney stones. Throughout the history from the Bible, Koran, Vedas and other old texts, the medicinal benefits of herbs are quoted. Herbs have a variety of uses including Culinary, Medicinal or in some General usage differs between culinary herbs and Medicinal herbs. In medicinal or spiritual use any of the parts of the plant might be considered herbs, including leaves, roots, flowers, seeds, resin, root bark, inner bark, berries and sometimes the Pericarp or other portions of the plants.

A great deal of research is currently being focused worldwide on various herbs and traditional medicine in the hope that new cures for illness and disease can be found. In the early 19th century when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Often herbs may be used together because the combination is more effective and may have fewer side effects.

Plant Constituents

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multiple of compounds like glycosides, alkaloids, volatile oils, tannins, etc., that exert a physiological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism.

Traditional Herbal Medicine (THM)

THM is a practice of protecting and restoring health that existed before the relatively recent arrival of modern medicine. According to WHO, up to 80% of people living in developing countries still rely primarily on traditional medicine for their health care.

Traditional Unani Medicine (TUM)

TUM originated in ancient Greece around 400BC. Hippocrates, also known as the founder of allopathic medicine, is considered to be first unani physician. Unani treatments for restoring equilibrium and normal body functions involve the prescribing of herbal and mineral medicines, a specific diet.

Traditional Ayurvedic Medicine (TAM)

TAM originated in India around 5000 BC with the publications of “Rigveda” and “Atharvanaveda” that contain hymns on disease and herbal treatments. The term Ayurveda means “Science of Life” a medicinal science where in health is achieved body-mind matrix usually involve the prescribing of herbal medicines, specific diet and physical activity routines, among other therapies including massage and various purification treatments.

Material and Method

Collection of Plant material

The roots parts of *Trapa bispinosa roxb*. were collected from local area of Gwalior Madhya Pradesh, in the month of August 2024. Plant material was collected as per standard procedure. Infected parts were carefully discarded from plant sample. The samples were thoroughly wash with water to remove foreign organic matter, debris and dried in the shade. Plant materials were authenticated by Department of Botany, Ayurvedic college in Gwalior. The dried plant materials were powdered by using pulveriser and sieve no.40 and kept in airtight container until used. The label stating name, part of plant date, collection site, weight etc. was appropriately pasted to respective samples.

Preparation of extract

For the preparation of extract 100gm of dried coarse powdered roots were charged in to the soxhlet‟s apparatus (hot extraction) and extracted successively with petroleum ether (600 - 800C), chloroform, ethyl acetate & methanol, in order of their increasing polarity. The successive methanolic extract (deep brown colour) was filtered & dried under reduced pressure to get a solid mass free from the solvent. The yield was 5.9% with respect to dry starting material with characteristic odour & greasy consistency.

Animals used

Adult albino rats weighing between 150-200 gm of either both sexes were used for the studies. The animals were housed under standard laboratory conditions at room temperature with relative humidity of 70-80%. They were fed with standard commercial diet and water ad libitum. Prior to the experiment, the animals were fasted for 12 h with water given ad libitum and weighed.

Study design

The rats were randomly assigned to four groups of six animals each for the two different experimental models. The first group served as negative control receiving normal saline (10 mL/kg). The second and third groups served as positive control and were given standard drugs, morphine sulfate and sodium salicylate, respectively (10 mg/kg each). The methanol extract of the *Trapa bispinosa roxb* was given at a dose of 20 mg/kg to the last group. All treatments were administered intraperitoneally.

Evalution of analgesic activity

Tail-Flick Test

Antinociceptive (analgesic) activity of the extract was evaluated by the tail-flick method described [9]. About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 50∘ C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$\text{MPA} = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15\ \text{Sec} - \text{reaction time for saline}} \times 100$$

Hot Plate Test

Evaluation of analgesic activity of the extract was also carried out using hot plate method [10]. The rats were placed on a hot plate maintained at 55∘ C within the restrainer. The reaction time (in seconds) or latency period was determined

as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{45 \text{ sec} - \text{reaction time for saline}} \times 100$$

Statistical analysis

Data were presented as mean±standard error mean (SEM). The results were analyzed using Statistical Package for the Social Sciences (SPSS) version 16. Statistical significance was determined by Student's *t*-test and *P* value less than 0.05 was considered as significant

Result-

Preliminary Phytochemical screening: As a part of the preclinical study, the methanolic extract of roots of *Trapa bispinosa roxb* was subjected to qualitative chemical test and confirmed the presence of alkaloids, steroids, flavanoids, glycosides, tannins and phenolic compounds.

Evaluation analgesic activity

Tail-Flick Test: The results of the analgesic activity of the methanol extract of the roots parts of *Trapa bispinosa roxb*. are shown in Table 3. Rats treated with normal saline (negative control) did not show any significant difference in the reaction time on tail-flick throughout the 60 min observation. In comparison with the baseline values within the same treatment groups, the increase in reaction time at different time points significantly differed ($p< 0.05$) for morphine sulfate only. Duration of the reaction time in morphine sulfate and extract treated animals was significantly higher compared to saline treated animals, except for the extract group at 60 min. The highest reaction time for the extract treated group was 8.0 sec at 30 min, while it was 4.4 sec and 11.9 sec for saline and morphine sulfate groups, respectively. At all-time points, the tail-flick latency time differed significantly between the extract and morphine sulfate groups, being greater for the latter group. No significant difference in reaction time was observed between the extract and sodium salicylate. Observation in rats treated with sodium salicylate did not give any significant analgesic effect in comparison with baseline values, saline, or extract (except for 30 min after treatment). The analgesic effects of morphine sulfate, sodium salicylate, and extract could be seen from the maximum possible analgesia (MPA) graph (Figure 2). The analgesic effect of morphine sulfate was evident within 15 min following intraperitoneal administration. The MPA remained elevated during the observation period, reaching its peak at 60 min (83.0%). Likewise, the extract also showed analgesic activity beginning at 15 min, with the highest MPA at 30 min, and gradually decreased towards 60 min (34.2%). For sodium salicylate, the MPA exhibited similar trend, producing a peak at the same time point (22.0%). With reference to MPA value, the extract demonstrated stronger analgesic activity than sodium salicylate at all time points.

Table 1: Analgesic effect of methanol extract from the roots parts of *Trapa bispinosa roxb*. by tail-flick method in rats

Treatments	Reaction time in seconds (mean±SEM)				
	0 min	15 min	30 min	45 min	60 min
Control (normal saline)	4.25±0.57	4.50±0.34	4.42±0.45	4.58±0.44	5.17±0.80
Morphine sulfate	6.50±1.22	11.04±0.73∗ab	11.92±0.84∗ab	12.83±0.35∗ab	13.33±0.83∗ab
Sodium salicylate	4.13±0.54	5.29±0.57	6.75±0.62∗a	5.75±0.56	6.79±1.24
Roots of *Trapa bispinosa roxb*	6.67±0.85a	7.04±0.67a	8.04±0.73a	7.00±0.92a	6.67±0.86

All values by Student's *t*-test, significant at $P< 0.05$, and SEM = standard error mean. ∗$P< 0.05$ versus baseline of the respective treatment, a $P< 0.05$ treatment versus control, b $P< 0.05$ extract versus morphine sulfate, extract versus sodium salicylate was not significant at all time points.

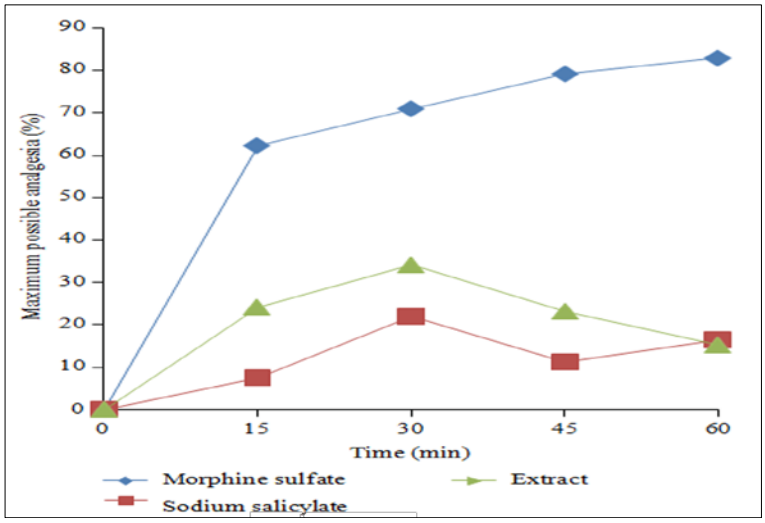

Fig 1: Maximum possible analgesia (MPA) (%) representing the effect of the methanol extract of *Trapa bispinosa roxb*. compared to morphine sulfate and sodium salicylate (positive control) administered into rats,evaluated by tail-flick method

Hot Plate Test

The results of the analgesic effect of the methanol extract of the roots parts of *Trapa bispinosa roxb.* using hot plate method are presented in Table 4. The results showed that there was no significant difference on the thermal stimulus in rats treated with normal saline (negative control) throughout the 60 min observation. There was no increase in reaction time at all time points compared to baseline values (0 min) within the same treatment groups. In comparison to the saline treated animals, the significant increase in the reaction time to thermal pain was not detectable in both sodium salicylate and extract with the exception of morphine sulfate. However, the observation in morphine sulfate treated animals is only noted at 45 and 60 min. The reaction time was significantly different between the extract and morphine sulfate, being greater for morphine sulfate at 30, 45, and 60 min after treatment. No significant difference was observed between the extract and sodium salicylate. Figure 3, illustrates the analgesic effect of morphine, sodium salicylate, and extract using MPA. Morphine sulfate elicited significant analgesic activity within 15 min following administration as evidenced by the gradual increase throughout the observation period. At the peak of activity (45 min), morphine sulfate showed MPA of 84.7%. Rats treated with sodium salicylate exhibited analgesic activity at a slower interval, which began at 45 min (49.2%) and then declined. The MPA value for the extract did not show any analgesic effect in the first 30 min after treatment but increased at 45 min (14.8%) and declined thereafter. On the basis of these findings, tail-flick is a better method to evaluate analgesic activity compared to hot plate as no significant results were observed for all treatments using hot plate with the exception of morphine sulfate.

Table 2: Analgesic effect of methanol extract from the roots parts of *Trapa bispinosa roxb.* by hot plate method in rats

Treatments	Reaction time in seconds (mean±SEM)				
	0 min	**15 min**	**30 min**	**45 min**	**60 min**
Control (normal saline)	30.67±5.15	28.25±4.87	31.50±4.57	24.08±6.37	24.83±6.02
Morphine sulfate	30.75±5.64	37.54±5.55	41.58±3.22b	41.79±2.87ab	41.46±2.55ab
Sodium salicylate	32.04±4.59	27.92±4.04	31.42±2.61	34.38±2.08	31.73±1.46
Roots of *Trapa bispinosa roxb*	30.88±4.08	27.67±2.17	24.50±3.08	27.17±3.92	26.71±4.06

All values by Student's *t*-test, significant at $P< 0.05$, and SEM = standard error mean. $*P< 0.05$ versus baseline of the respective treatment, a $P< 0.05$ treatment versus control, b $P< 0.05$ extract versus morphine sulfate, extract versus sodium salicylate was not significant at all time points

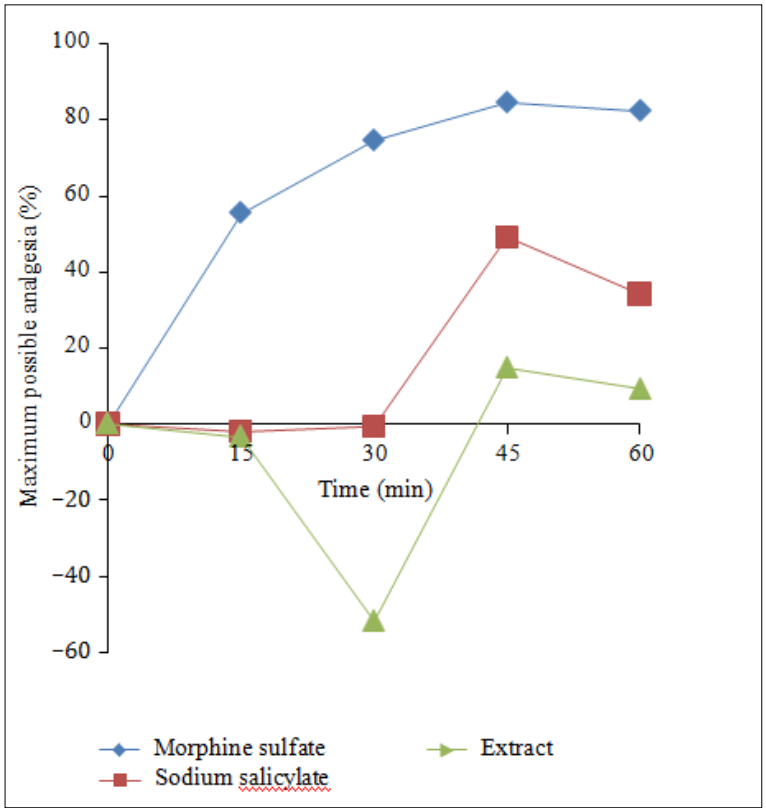

Fig 2: Maximum possible analgesia (MPA) (%) representing the effect of the methanol extract of *Trapa bispinosa roxb*. compared to morphine sulfate and sodium salicylate (positive control) administered into rats, evaluated by hot plate method.

Discussion

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness . Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli

which include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level. While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supraspinally organized response. In tail-flick model, the methanol extract of *Trapa bispinosa roxb*. exhibited significant analgesic activity by increasing the reaction time of the rats compared to control (saline treated rats) at all time points, except at 60 min. Sodium salicylate and morphine sulfate were used as reference drugs, which are considered mild and moderate to severe analgesics, respectively. In comparison with control, morphine produced the most significant antinociception effect during all observation times, followed by the extract, while no significant analgesic effect was observed for sodium salicylate. The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the reaction time of typical tail-withdrawal effect in rats. This method is also useful in differentiating central opioid-like analgesics from peripheral analgesics. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure . Therefore, the analgesic effect of the extract on this pain-state model indicates that it might be centrally acting. With reference to the MPA value, the analgesic effects of both the extract and morphine sulfate were evident within 15 min following intraperitoneal administration. However, the extract showed short-lived analgesia as the MPA gradually decreased after 30 min compared to morphine sulfate. The tail-flick latency of the extract at all time points was less than that of reference drug, morphine sulfate, which is a slow onset opioid with long duration of 4 Evidence- Based Complementary and A action. Although there was no significant analgesic effect between the reaction time of the extract and sodium salicylate, the extract exhibited a non-significant trend of higher reaction time compared to sodium salicylate. Both treatments produced comparable reaction times, suggesting that the *Trapa bispinosa roxb*. could be a better natural alternative for mild pain relief. The methanol extract from of *Trapa bispinosa roxb*. failed to increase the reaction time of the rats on hot plate method in this study. The difference in the mean reaction time of the extract and the control groups was not statistically significant during all observation times. Analgesia in morphine sulfate treated rats was only detectable at 45 and 60 min. No significant analgesic effect was observed between sodium salicylate and control and the extract tested. Hot plate method produces two measureable behavioural components in response to thermal pain, with regard to their reaction times. Responses such as paw licking and jumping in rats are considered to be supraspinally integrated. Thus, the failure of the extract to inhibit these behaviours on hot plate method indicates that it might not be acting at supraspinal level.

Taken together, tail-flick is a better method to evaluate analgesic activity compared to hot plate as no significant results were observed for all treatments using hot plate with the exception of morphine sulfate. This observation is in agreement with findings of which reported that the extract of *Trapa bispinosa roxb*. produced a significant elongation of the tail-flick reaction time but not in hot plate method. Tail-flick and hot plate are two of the several methods available for evaluating central analgesic activity. Although both methods employed thermal stimuli, the tail flick response indicates spinally mediated reflex while the paw-licking hot plate response is due to complex supraspinally integrated behaviour. Findings from this study demonstrated that the methanol extract prolonged the reaction time in the tail-flick method but showed an apparent lack of effect in the hot plate method. This might indicate higher sensitivity of the spinally mediated reflex response in the tail-flick method. However, intra-animal variation may also contribute to the lack of effect in hot plate method. Unlike the typical tail-withdrawal reflex in rats, problem arises in the hot plate method as the rats have to learn what nociceptive response they need to show in order to stop the thermal stimulus. Taken together, the differences in sensitivity of both methods as wellas the mechanism involved may explain the analgesic effects observed in this study.

Conclusion

In conclusion, the methanol extract of roots parts of *Trapa bispinosa roxb* displayed analgesic activity and supported the traditional use of this plant in pain relief. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its analgesic properties. To identify analgesic activity of *Trapa bispinosa roxb*. using roots part of this plant with methanolic solvent. Nature has been a source of medicinal agents for thousands of year and an impressive number of modern drug have been isolated from natural sources. The side effects of the currently available analgesic drugs pose a major problem during their clinical uses. Therefore, the development of newer and more potent analgesic drugs with lesser side effects is necessary. Many medicinal plant are found useful in treating analgesic disease. This study will describe role of *Trapa bispinosa roxb*. in analgesic disease.

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