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CENTRAL NERVOUS SYSTEM ACTIVITY OF HYDROALCOHALIC EXTRACT OF *THESPESIA POPULNEA* LEAVES

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ABSTRACT

Thespesia populnea is a reputed ever green tree belonging to the family malvaceae; commonly known as Indian tulip tree. The plant is distributed tropical regions and coastal forest in India. It is well known and all the parts are used in traditional system of medicine. In the present study of Hydroalcohalic extract Arial part of *Thespesia populnea* was screened for locomotor, Rota-rod, Anticonvulsant, anti-anxiety activity of Hydroalcohalic extract (200 mg/kg and 400 mg/kg p.o.) was determined. The present study deals with various pharmacognostical examinations like organoleptic or macroscopical characters, microscopical or anatomical studies. The scientific parameter is necessary to identify the exact plant material and to find its quality and purity, The plant has been used as astringent, antibacterial, hepatoprotective, haemostatic, anti-diarroheal and anti-inflammatory.

Keywords: *Thespesia populnea*, locomotor, rota-rod, anticonvulsant, anti-anxiety activity.

INTRODUCTION

The traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. In Germany, for example, where herbal products are sold as 'phytomedicines', they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the market place are marketed and regulated

as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria. These matters are covered extensively in Section 3 below. The pharmacological treatment of disease began long ago with the use of herbs[1]. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment [2].

The WHO regional office for the western pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines [3]. This group recognized the importance of herbal medicines to the health of many people throughout the world, stating: 'A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health. Most herbal medicines still need to be studied scientifically, although the experience obtained from their traditional use over the years should not be ignored. As there is not enough evidence produced by common scientific approaches to answer questions of safety and efficacy about most of the herbal medicines now in use,

MATERIALS AND METHOD

Selection of plant: - The plant selection on their availability and folk usage o the plant. The plant was chosen.

Collection of Plant Material: The Plant material of *Thespesia populnea* was collected from Ratibad Bhopal (M.P.), during the month of march 2021.

Authentication of plant: - The plant was identified And authenticated by Dr. Zia ul Hasan H.O.D. Department of Botany, Saifia Sciences College Bhopal (M.P.) and stored in the herbarium of the Institute and a specimen voucher no.512/Bot./Saf. /21 was assigned.

Defatting of plant material: - The shade-dried plant materials are coarsely powdered and fats and oil removed by soxhlation process with petroleum ether. The extraction proceeded until the substance was defatted.

Extraction by soxhlation process:- Accurately weight 60 gram of dried powdered of aerial portion (leaf) of *Thespesia populnea* were extracted with Hydroalcohalic solvent using a 48- hour soxhlation

procedure, filtered and dried with vaccum evaporator at 40^0C, and prepared extract was also subjected to colour, odour and consistency.

Determination of percentage yield of the extract: - The crude extract after the soxhalation extraction process, extract was further on vaccum evaporater dried extract of aerial part of *Thespesia populnea* was done by using solvent Hydroalcohalic (ethanol:water, 70:30 v/v). The percentage yield of extract were calculated 4.7 gm.

Quantitative phytochemical analysis

Estimation of Total polyphenol content (TPC)

The total polyphenol content of the extract was estimated using the Folin Ciocalteau reagent based assay. 5-50 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. The absorbance of the developed colour was recorded at 765 nm using a UV-Vis spectrophotometer. All determinations, for gallic acid as well as the plant extract, were carried out in triplicate. Data are represented as an average of the three determinations. Using these readings, a calibrated gallic acid standard curve was made. Based on the measured absorbance of the plant extract, the concentration of phenolics was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material) [4-5].

Estimation of Total flavonoids content (TFC)

Total flavonoid content was based on aluminium chloride method. The 10 mg quercetin was dissolved in 10 ml methanol and various aliquots of 5,10,15,20 and 25 µg/ml were prepared in methanol. And the 10 mg of dried extract of were dissolved in 10 ml methanol and filter. 3 ml (1 mg/ml) of this solution was used for the evaluation of flavonoid. In addition, 1 ml of 2 **%** $AlCl_3$ methanolic solution was added to 3 ml of extract or normal and allowed to stand at room temp. for 15 min. absorption was measured at 420 nm [5-6].

Pharmacological Activity

Literature reveals that *Thespesia populnea* has been explored for its pharmacological activity

Animals

Swiss albino mice weighing between 25-35 gm are used in the experiments. The animals were placed randomly and allocated treatment group. All the experiments were performed between 9:30 to 16:30

hours to overcome diurnal and circadian variations. All the animals were housed at a temperature of 25±2^{0}C and in a relative humidity of 65±5%. A 12:12 light: day cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.) proposal number lAEC/Rips/2021/03 and were in accordance with the guidelines of the IAEC.

Acute oral toxicity study.

The acute oral toxicity study was conducted according to the OECD-423 (Acute toxic class method) guidelines. Six group of mice n=6 were administered orally for 7 days with HETP (50, 300, and 2000 mg/kg) and the animals were kept under observation for mortality and any behavioural changes.

Effect of HETP on locomotor activity of mice on Actophotometer.

Swiss albino mice weight 25-30 gm were taken and divided in groups each consisting of 6 animals. The first group was marked as control and second as standard group. Rest two groups were marked for different doses (200 and 400 mg/kg.p.o.) HETP. The was turned on checked to make sure that all the photocell are working for accurate recording and each mice was placed individually in the activity cage for 5 minute. Basal activity score of all the animals were noted. Diazepam (2 mg/kg) was injected. and after 30 minute placed each mouse in activity case for 5 minute. Note the score, the difference in the activity before and after diazepam treatment. Repeat the above procedure for different doses of hydroalcohalic extracts (200,400 mg/kg p.o.). Percentage change in motor activity was calculated [7].

Effect of HETP on muscle grip performance of mice on Rota-rod apparatus.

Swiss albino mice of about 30-35gm weight were taken and divided into 4 groups each consisting 6 animals. The first group was marked as control and second as standard group. Rest 2 group were marked for different doses (200 and 400 mg/kg. p.o.) of the HETP. Rota-road was turned on setting the speed of rotation at 22-25 rpm. The animals were placed singly one by one on rotating rod. The fall off time, when the mouse falls from the rotating rod was noted down.

The drug (diazepam,2 mg/kg i.p.) was injected to animal of second group and after 30 minute, the above mentioned parameter was observed. after that the same procedure was followed for the test group. Comparison was made between the fall-off time of all the animal [7].

Effect of HETP on parameter of anxiety on elevated plus- maze in mice.

Swiss albino mice of about 25-35gm were taken and divided into 4 groups each consisting of 6 animals. The first group was marked as control and second as standard. Rest of 2 groups were marked for different doses of (200 and 400 mg/kg. p.o.) HETP. Animals were placed individually at the centre of the plus maze with their head facing towards the open arm and their following behaviours were noted for five minutes. First preference of mice to open or enclosed arm. Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm). Average time of each animal spends in each arm (Average time = total duration in the arm/number of entries).

Standard drug (Diazepam 2 mg/kg.i.p.) and different doses of HETP (200 and 400 mg.kg. p.o.) was injected to the animals of 3rd and 4thgroup and after 30 minutes. The above mentioned parameters were observed. Comparison were made among the preferences of the animal to open/enclosed arm. average time spent in open arm and number of entries in open arm for each group [7].

Effect of HETP on MES induced convulsion in rat.

Swiss albino Rats of about 80-140 gm were taken for experiments. Animals were marked and divided in 5 groups. each group consisting of 6 animals. First group was marked control and second and third group were designated for standard drug treatment (Phenytoin 120 mg/kg and Phenobarbitone 45 mg/kg i.p.). Rest 2 group were marked for 2 different dose of HETP (200 and 400 mg.kg. p.o.) respectively. Care was taken to hold the animal properly. Corneal electrodes were placed on the cornea and a current of 150 mA was applied for a duration of 0.2 sec. Different stages of convulsions i.e. (a) tonic flexion. (b) tonie extensor phase. (c) clonic |convulsions. (d) stupor, and (e) recovery or death was noted after electric current application. The time (sec) spent by the animal in each phase of the convulsions was noted. The same procedure is repeated with all animals of the group.

The standard drug and HETP injected to the animals of all respective groups. After 30 minutes the same current was applied for similar duration and time spent in different stages was noted. The reduction in time or abolition of tonic extensor phase of MES-convulsions for ever groups was noted [7].

RESULTS AND DISCUSSION:

The hydroalcoholic extract of *Thespesia populnea* show the presence of steroid, tannins and phenolic compounds, alkaloids, glycoside, carbohydrate. The results are shown in table 1 The Percentage yield of hydroalcoholic extract was (4.7gm). This study was conducted on several central nervous systems related experimental models e.g., locomotor activity, rota rod. elevated plus maze, and MES induced convulsion

to investigate the possible central effect of *thespesia populnea*. The classical models for screening CNS action providing information on depressant property of psychomotor performance anxiolytic and myorelaxant activity. There has been a considerable popular interest in the use of the natural remedies or herbal products to treat anxiety and depression. Recently several plants have been reported to possess anxiolytic effects in different animal models of anxiety. Various traditional herbal medicines have also been suggested to possess anxiolytic activity. The plant was found to be rich in steroidal and flavonoid content. The phytoconstituents which are responsible for many pharmacological activities.

Table 1: Qualitative analysis of *thespepsia populena* hydroalchoholic extract of presence of different phytoconstituents

S.NO.	TEST	OBSERVATION	INFERENCE
1	**Alkaloid**		
	Wagner's reagent	Reddish brown ppt	+ ve
	Dragendorff's reagent	Reddish brown ppt	+ ve
	Mayer's reagent	Cream colour ppt	+ ve
2	**Glycoside**		
	Keller Killiani test**.**	Appearance of reddish brown colored ring at the junction of two layers	+ ve
	Conc. sulphuric acid test	reddish color precipitate	+ ve
	Molish’s test	Formation of reddish-purple colored ring at the junction of two layers.	+ ve
3	**Steroid**		
	Solkowski Test	brown or red colored ring on the sulphuric acid layer given the confirmatory test.	+ ve
	Libermann Burchard’s Test	translucent green colour given the confirmatory test**.**	+ ve
4	**Carbohydrates**		
	Molisch Test	Formation of the red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates.	+ ve

	Benedict's Test	Depending on the concentration of the reducing sugar, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange, or red.	+ ve
5	**Phenolic compounds**		
	Ferric chloride test	Formation of blue, green or violet colour indicates the presence of phenolic compounds.	+ ve
	Lead acetate test	Formation of white precipitate indicates presence of phenolic	+ ve
	Dilute iodine solution test	Formation of transient red colour indicates the presence of phenolic compounds	+ ve

The total phenolic content for aqueous, hydroalcoholic extract was estimated by Folin Ciocalteu's method using gallic acid as standard. The gallic acid solution of concentration (10-100 ppm) conformed to Beer's Law at 750 nm with a regression co-efficient (R2) = 0.997.The plot has a slope (m) = 0.028 and intercept = 0.003. The equation of standard curve is y = 0.028x + 0.003 (Fig. 1).

The total flavonoid content for hydroalcoholic extract was measured with the aluminium chloride colorimetric assay using quercetin as standard. The quercetin solution of concentration (5-25 ppm) conformed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.999. The plot has a slope (m) = 0.043 and intercept = 0.013. The equation of standard curve is y = 0.043x + 0.013 (Fig. 2).

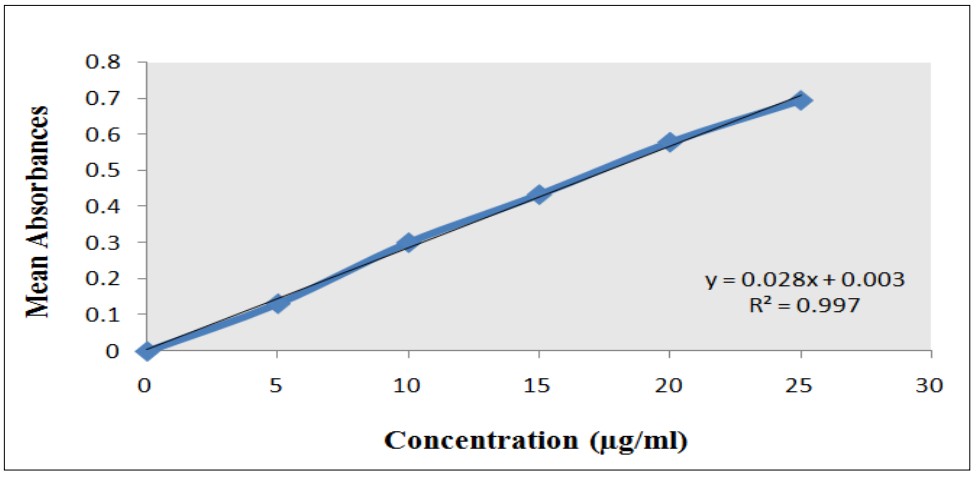

Fig. 1: Total phenolic content for standard gallic acid.

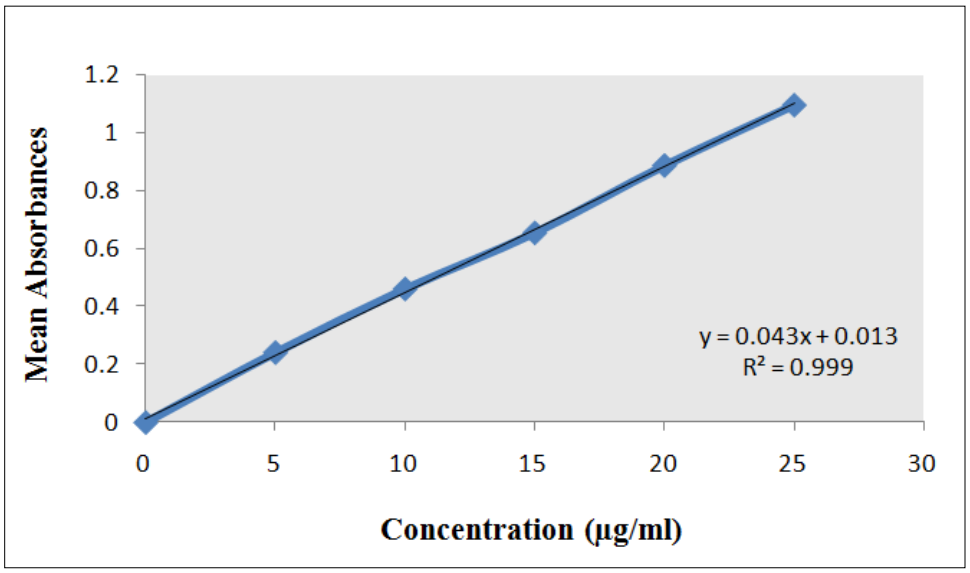

Fig. 2: Total flavonoid content for standard quercetin.

Table 2: Estimation of total phenols and flavonoid content

Extract	Total phenols content (GAE mg/100mg)	Total flavonoid content (QE mg/100mg)
Hydro alcoholic extract of *Thespesia populnea*	0.185	0.274

Locomotor activity is considered as an index of alertness and a decrease in it is indicative Sedative activity. The spesia populnea significantly decreased locomotor activity in all the tested doses that act as a centrally acting muscle relaxant interacting with specific receptors enhancing chemical and mission. Decrease in locomotion reveals depressant effect on GABAergc transmission due to increase in the concentration of GABA in brain [8]. The result are shown in table 3.

. The HETP CNS depressant the reduce grip strength and mice may fall from the rota-rod due loss of muscle or muscle coordination. HETP decreases the fall off time of mice from the rotating rod. Based on the exposure of animal to an elevated plus maze. The results are shown in table 4. The fear due to height induces anxiety in the animals when placed on the elevated plus maze (EPM). The animal being exposed to the new environment tends to avoid And tries prefer to stay in closed arm due to fear [9-10].

The ultimate manifestation of anxiety and fear in the animals is inhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic spent by the animal in the open arms[10]. an anxiolytic

effect expressed by an increased number of open arm entries and time spent in the EPM. Diazepam produced significant increase in open arm duration and also number of entries into the open arms. Plus maze model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances acting at the GABA benzodiazepine complex [11]. Current study data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce significant anxiolytic effects in a variety of anxiolytic screening procedures, including elevated plus-maze test procedures. In our finding the HETP treated. The result are shown in table 5.

Epilepsy is one of the most common serious neurological conditions. drugs that inhibit voltage-dependent Na+ channels, such as phenytoin. The effect of HETP on MES-induced convulsion in rats are tabulated in table 6. The tonus and and extension phase was decreased in dose dependent manner. Treated group change in duration of clonus phase in all the HETP treated group was non-significant compared to the control. The animals were recovered in vehicle treated. phenytoin, and all doses of HETP. The result are shown in table 6.

Table: -3: Effect of HETP on locomotor activity of mice on Actophotometer.

Groups	**Dose (mg/kg)**	**Locomotion Score (M±SEM) (Min.)**		**% Change in locomotor activity**
		Basal	After 30 min. drug administration	
Vehicle control	5 ml/kg/p.o.	192.5±12.50	-	-
Diazepam	2 mg/kg/i.p.	133.5±1.50	14±2.00***	92.72
HETP	200 mg/kg/p.o.	565.5±47.50	142.5±60.50^{ns}	25.97
HETP	400 mg/kg/p.o.	565±17.10	92±10.00 ^{ns}	52.20

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table 4: Effect of HETP on muscle grip performance of mice on Rota-rod apparatus.

Groups	Dose (mg/kg,)	Fall off time in Sec. (M±SEM)		% Change in fall off time
		Basal reaction time (M±SEM)	After 30 min. drug administration (M±SEM)	
Vehicle control	5 ml/kg/p.o.	1066±41.00	-	-
Diazepam	2 mg/kg/i.p.	708±20.40	66±16.00***	93.80
HETP	200 mg/kg/p.o.	863.5±50.50	458.5±12.50***	56.98
HETP	400 mg/kg/p.o.	408.5±10.50	354.5±19.12***	66.74

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table 5: Effect of HETP on parameter of anxiety on elevated plus- maze in mice.

Groups	**Dose (mg/kg,)**	**% preference to open arm**	**Total No. of entries (M±SEM)**	**% open arm entries**
Vehicle control	5 ml/kg/p.o.	41.01	12.17±2.15	28.84
Diazepam	2 mg/kg/i.p.	65.24	11.60±2.28**	53.64
HETP	200 mg/kg/p.o.	42.13	12.42±1.93 ns	29.71
HETP	400 mg/kg/p.o.	51.23	11.86±1.79 *	39.37

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table 6: Effect of HETP on MES induced convulsion on rat.

Group	**Dose mg/kg**	**Flexon phase in sec. (M±SEM)**	**Extensor phase in sec. (M±SEM)**	**Clonus phase in sec. (M±SEM)**	**Stuper phase in sec. (M±SEM)**	**Recovery/ Death**
Vehicle control	5 ml/kg/p.o.	08.54±0.81 ns	13.78±1.62 ns	17.12±2.3 ns	102±5.1 ns	Recovery
Phenytoin	120mg/kg/i.p.	Absent	Absent	16.10±3.98**	59.9±5.6**	Recovery
HETP	200 mg/kg/p.o.	10.00±2.30 ns	19.02±3.45 ns	29.23±4.01 ns	101±5.1 ns	Recovery
HETP	400 mg/kg/p.o.	07.12±1.12 ns	15.23±2.14**	19.45±2.10*	96.47±4.2**	Recovery

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc

CONCLUSION

pharmacological investigation of the plant *Thespesia populnea* produce depressant action on the CNS. hydroalcohalic extract of *Thespesia populnea* induce, act as hypnotic, also decrease anxiety, means act as anxiolytic agent due to hypnosis. *Thespesia populnea* also exert muscle relaxant and locomotor, anti-anxiety effect of mice. it is pharmacological safe with good bioavailability with least toxicity *Thespesia populnea*. This review gives some phytochemicals as well as the detailed pharmacological information of *Thespesia populnea*. The main focus on the pharmacological potentials of *Thespesia populnea* which is very helpful to researcher to add more about this valuable plant. Apart from this still there are few options to investigate the unexplored potential of plant based on its uses. The active constituent needs to be isolate and should be considered for further in-vivo or in-vitro studies to confirm the traditional claims and to explore the potential of development of drug.

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