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IN VIVO CENTRAL NERVOUS SYSTEM LOCOMOTOR ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF THE *TRIBULUS TERRESTRIS (Linn)* LEAF EXTRACTS

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ABSTRACT

Tribulus species is among the medicinal plants that are used frequently by south Indian traditional practitioners for its fascinating biological activities. Our study, based on evaluation of the *in-vivo* central nervous system stimulant or depressant locomotor activity of the petroleum ether, chloroform and ethanol leaf extracts of *Tribulus terrestris Linn.* Among that the ethanolic leaf extract at the dose of 100mg/kg only exhibited considerably significant central nervous system stimulant activity. The phytochemical investigation showed that alkaloids, saponins and glycosides are present as main active constituents.

KEYWORDS

Tribulus species, Active constituents, Actophotometer, Central nervous system stimulant and Central nervous system depressant.

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INTRODUCTION

A large group of plants used in medicine or veterinary practice for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavanoids, glycosides, vitamins, tannins and coumarin compounds and which are biologically active in relation to the causative agents of various diseases. More than 30,000 tons of raw materials from approximately 220 species of medicinal plants are used annually by the supervision of physician[1]. Amoung that tribulus species are used as famous

traditional medicines[2]. The earlier studies showed that flavanoids, alkaloids[3] tribulusterine and perlolyrine, tannins, saponins include furostanol saponin[4], spinostanol saponins[5] of tigogenin, ncogitogenin, hecogenin, neohecogenin, methyl prototribestin, tribestin, diosgenin[6], chlongenin, ruscogenin, sarsasapegenin glycosides including protodioscin and protogracillin phytochemical constituents are present in entire part of tribulus species. The earlier works on the leaves of tribulus species exhibited the antimicrobial, anti-inflammatory activity[7], diuretic activity, aphrodisiac activity [8,9,10], anti-urolithic activity, anxiolytic[11], anti-tumor[12], anti-oxidant[13], wound healing[14], immuno-modulatory activity, anti-diabetic activity, hypolipidemic activity, activity in cardiac disorders such as hepato protective activity, , analgesic activity, antispasmodic activity, anticancer activity, anti-bacterial activity[15], anti-cancer[16], proliferative, antihypertensive[17], anthelmintic activity, larvicidal activity and anti-cariogenic activitiy. The antidepressant activity also reported for the *tribulus terrestris* seeds and flowers. But still there is no evidence in the literature survey for the antidepressant central nervous system activities on the leaf extracts. So it was decided to investigate various phytochemical constituents using different polar solvents, followed by *in-vivo* central nervous system stimulant or depressant locomotor activity are evaluated by using the leaf extracts of the *tribulus terrestris*.

MATERIALS AND METHODS

Phytochemical Investigation of the leaves of *Tribulus terrestris* preparation of extracts using different solvent systems of increasing polarity. The dried leaves of *Tribulus terrestris* were dried in the shade. Then the shade dried leaves were powdered to get a coarse powder. About 500gm of powder was extracted first with the petroleum ether by continuous hot percolation using soxhelt apparatus. The extractions were continued for 48 hours. The petroleum ether extract was filtered and concentrated to a dry mass by using vacuum distillation. A yellowish residue was obtained. The marc left after the ether extract was taken and subsequently extracted with chloroform upto72 hours. The chloroform extract was then filtered and concentrated to get a dry mass. A dark brown residue was obtained. The marc left after the chloroform extraction were dried extracted with alcohol. The extraction was continued up to 72 hours. The alcohol extract was filtered and concentrated by vacuum distillation. A dark brownish waxy resides was obtained. The extracts obtained by the above methods were subjected to qualitative tests for the identification of various plant constituents. The results are displayed in Table No.1.

Evaluation of the *In-Vivo* C-N-S Locomotor Activity

The Central nervous system stimulant or depressant property locomotor activity of the leaf extracts on the mice was evaluated by using digital actophotometer[18]. Adult albino mice of either sex weighting 20-28g were divided into control, standard and test groups of five animals into each group and numbered. About 0.5ml of 1% CMC suspension vehicle was administered for five days once daily before starting the experiment. The animals were fasted for six hours before experiment and they were allowed to adapt to the activity cage environment for at least 5 minutes before the experiment. The activity cage and the basal activity counts of each mouse were observed for 15 minutes 2 days before to start experiment. A count is recorded when the beam of light falling on the photo electric cell of actophotometer which connected in circuit with a counter is cut off by mice separately in an activity cage which enables movement of the mice across a light beam to be recorded as a locomotion count. The tasted extracts were administered orally by intragastric (stomach) tube at a close of 50mg/kg and 100mg /kg body weight in the form of suspension in 0.5ml of 1% CMC while two ether groups received amphetamine and chlorpromazine at a dose of 5mg/kg body weight as a standard drugs, also given in the form of suspension in 0.5ml of 1% CMC. The control group mice received with 0.5ml suspension of 1% CMC in water. The locomotors behavior was monitored and for a period of 15 minutes. The

difference in the number of counts for each group was recorded. The mean score for standard and test groups were compared the result with control groups. The percentage increased as decreased in locomotors activity was then calculated. The values were tabled with multiple comparison ANOVA test. The results are displayed in Table No.2.

RESULTS AND DISCUSSION

The phytochemical investigations studies showed that petroleum ether extract have alkaloids, fixed oil and fats. The chloroform extract have tannins, steroids, alkaloids, saponins, proteins and amino acids. The alcohol extract have carbohydrate, glycoside, steroids, saponins, proteins and amino acids. The *In-vivo* pharmacological screening data of the central nervous system locomotor evaluation displayed that petroleum ether extract locomotion count values are almost equal to control at the tested 50mg/kg and 100mg/kg dose concentration. So it concluded petroleum ether extract does not passes CNS stimulant or CNS depressant activity. The chloroform extract displayed slightly more locomotor counts when compared to control at the tested both concentrations. So it is possible to say that chloroform leaf extract have mild CNS stimulant property. The tested 50mg/kg and 100mg/kg concentrations of ethanol extracts depicted elevated locomotor counts compared to control. So these results clearly registered ethanol extracts have CNS stimulant activity. So it is possible to say that at 100mg/kg high concentration has some considerable CNS stimulant activity when compared to the standard drug amphetamine at the dose of 5mg/kg. All the extracts failed to give the CNS depressant properties when compared to the standard drug chlorpromazine.

Table No.1: Data showing the preliminary phytochemical screening of the *Tribulus terrestris* leaves extracts

S.No	Chemical Tests	Pet-ether Extract	Chloroform Extract	Alcohol Extract
1	**Test for carbohydrate**			
	Molisch Test	-	-	+
	Fehling'sTest	-	-	+
2	**Test for Glycoside**			
	Legal's test	-	-	+
	Baljet's test	-	-	+
	Borntrage's test	-	-	+
3	**Test for Proteins**			
	Biuret Test	-	+	-
	Ninhydrin Test	-	-	+
	Xantho Protein Test	-	-	-
	Millions Test	-	-	-
4	**Test for Tannins**- lead acetate test	-	+	-
5	**Test for steroids**			
	Salkowski Test	-	+	-
	Libernanburchard test	-	+	+
6	**Test for flavanoids**			
	Shinodoy Test	-	-	-
7	**Test for Triterpienes**			
	Chloro SO_3H Test	-	-	-
	Tricholoracetic acid test	-	-	-

8	**Test for Diterpienes** copper Acetate Test	-	-	-
9	**Test for Alkaloids** Mayer's Test Wagner's Test Gragendroff Test Hoyer's Test	 + + + +	 + + + +	 - - - -
10	**Test for Saponins**	-	+	+
11	**Test for Fixed oils and fats**	+	-	-

(+) – Present, (-) – Absent

Table No.2: *In-Vivo* Central Nervous System Locomotor Activity of the Leaf Extracts of *Tribulus Terrestris (Linn*)

S.No	Groups	Average number of movements in 15 minutes			
		50 mg/kg	**%**	**100mg/kg**	**%**
1	Pet ether extract	164.4±1.28^{NS}	98.0	168.±+3.04 ^{NS}	100.5
2	Chloroform extract	177.8±2.81^{NS}	106.0	198.6±2.58***	118.4
3	Ethanol extract	203.2±3.30***	121.2	232.2±2.30***	138.5
4	Chloropromazine	113.6±2.54*** (5mg/kg)	67.7	-	-
5	Amphetamine	274.6+2.73*** (5mg/kg)	163.8	-	-
6	Control	167.6±2.89	Nil	-	-

Each average value represents the mean ± SEM (n=5). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective control. NS-Not Significant. (-) = Not tested

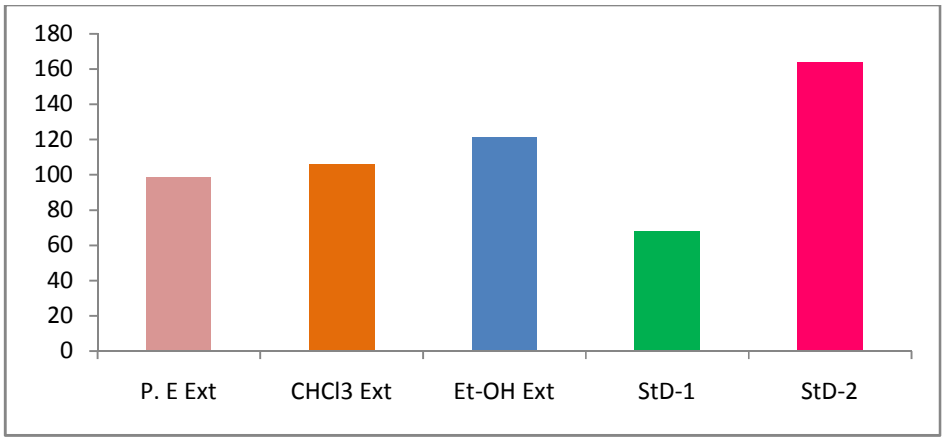

Figure No.1: The Central nervous system locomotor activity of the leaf extracts of *Tribulus terrestris* at 50mg/kg dose when compared to Chlorpromazine at 5mg/kg (StD-1).and amphetamine at 5mg/kg (StD-2)

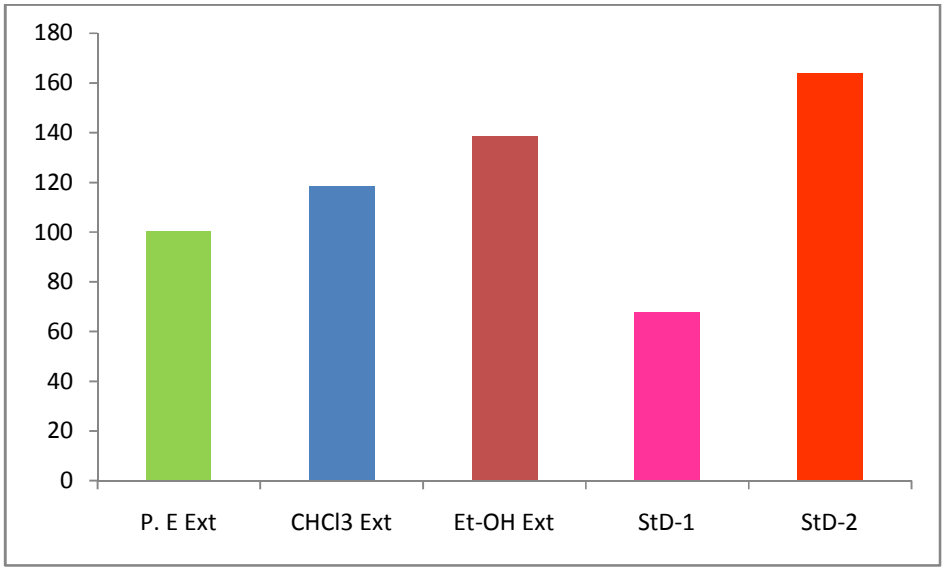

Figure No.2: The Central nervous system locomotor activity of the leaf extracts of *Tribulus terrestris* at 100mg/kg dose when compared to Chlorpromazine at 5mg/kg (StD-1) and amphetamine at 5mg/kg (StD-2)

CONCLUSION

The present study demonstrates the CNS locomotor effect of the petroleum ether, chloroform and ethanol extract of the plant Tribulus *terrestris Linn* leaves. The present studies suggest that *In-vivo* locomotor behavioral of the petroleum ether and chloroform extracts neither CNS depressant nor CNS stimulant properties. Meanwhile the results of this study confirmed that ethanol extract has hyper locomotion at the high dose so Tribulus *terrestris Linn* may be used to develop or as a lead plant material for a new target in antidepressant or CNS stimulant pharmaceutical dosage forms.

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