Neuropharmacological evaluation of *Centaurium erythraea* extract.

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Abstract : *The review of literature clearly indicates that there is a paucity of literature on the scientific studies carried out on Centaurium erythraea. Although there are several claims made about this plant there is lack of proper scientific study. This experimental study was undertaken to studysome of the neuropharmacological actions of the extract particularly on Irwin schedule, gross behavior (blind screening), locomotion, motor coordination and catalepsy. The ethanolic extract of Centaurium erythracea significantly reduced locomotion in low doses whereas at higher doses it reduces both locomotion and rearing which clearly indicates the action of the drug as dopaminergic D1/D2 antagonistor or D3 agonist at low doses. At higher doses it may have direct action on 5 HT1A receptor. The centaurium erythrae was found to reduce haloperidol induced catalepsy in higher dose which may be due to D2 agonistic effect and/or may modulate by serotonin release.*

***Centaurium erythraea* –whole plant**

Keywords: Neuropharmacological, Locomotion, Catalepsy, Rearing, motor coordination, Phytochemical evaluation.

Abbreviations:

% - Percentage

5-HT - 5 Hydroxytryptamine

Ca2+ - Calcium

CNS- Central Nervous System

D_{1-} Dopamine D1 Neuron

D_2 Dopamine D1 Neuron

CPG - Central pattern generators

K^+ - Potassium

$FeCl_3$ - Ferric Chloride

IAEC- Institutional Animal Ethical Committee

CPCSEA-Committee for the purpose of Control and Supervision of experiments on Animals
NA- Noradrenaline

DA- Dopamine

SD-Standard Deviation

SEM- Standard Error of Mean

ANOVA- Analysis of Variance

i.p - intraperitoneal

Introduction

Introduction: The review of literature clearly indicates that there is a paucity of literature on the scientific studies carried out on ***Centaurium erythraea***. The objective of the presented study was to study the effect of the extract on gross behavior (blind screening), both qualitatively and quantitatively, and to study the effect of the extract in models of locomotion, motor coordination and catalepsy.

Locomotion is a fundamental and essential feature of most terrestrial animals including humans. Locomotion is controlled by 1.Supraspinal control, 2.Sensory feedback and 3. Spinal Central Pattern Generators (CPG). Daly & Waddington, 1993; Water et al ,1993)

Motor coordination is used to refer to the coordination of movements usually between different subsequent parts of the same movement or movements of several limbs. Motor coordination arises from a complex coordination between muscles and neural circuitry. Both gross and fine motor activity is controlled and coordinated by the central nervous system. (Amos 2001)

Catalepsy is a nervous condition characterized by muscular rigidity and fixity of posture regardless of external stimuli as well as decreased sensitivity to pain. Several neuroleptics can induce catalepsy by blockage of dopamine receptors (D2) and reduced dopaminergic transmission. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics eg haloperidol is a robust behavioural model to study the nigrostriatal function and its modulation by cholinergic, 5-HTand other neurotransmitters. (Silva SR et al, 1995, Pires JGP et al ;2003) Somani RS et al ; 1999) . Catalepsy is also modified by drugs that modulate the serotonin release. (Farde et al, 1992)

Materials and Methods

Animals:

Albino Wistar rats (180-220 g) of either sex are used in the study. Protocol was approved by the Institutional Ethical Committee (IAEC) (PBRI/IAEC/2009/PN 16) and all experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (CPCSEA no: 1283/c09/CPSCEA)

Plant Name*:* *Centaurium erythraea*

The plant material was collected and authentication was obtained from Mcgliari University, Italy with Voucher specimen no.929/A Herbarium CAG (Cagliari, Italy).

Extraction of Centaurium erythraea (whole plant) :

The weighed quantity of shade dried plant material (1kg) was macerated with ethanol (70 % v/v) and the extract was concentrated under reduced pressure to yield a solid residue (10.25 %w/w). The extract was kept in refrigerator and dissolved in water before use.

Drugs Used:

Diazepam (Calmpose Manufactured by Ranbaxy Laboratories Ltd, Mumbai), **Haloperidol** (Serenase manufactured by RPG Life sciences Ankleshwar Gujarat), **Pheniramine maleate** (Avil Manufactured by Unimark Remedies Ltd.Vadodara , Gujarat & marketed by Aventis Mumbai, **Water for Injection**. (Nirlife)

Determination of LD50 using OECD guidelines: LD50 was determined using 'Up & Down method ' according to OECD guidelines. A dose of 2000mg/kg i.p was observed carefully for any sign of toxicity.

Phytochemical Evaluation:

Chemical Tests for Carbohydrates: Various colour reactions and identification tests may be carried out to identify the optically active compounds. These include Charring test, Molisch Test, Iodine Test , Barford Test, Seliwanoff test Fehlings solution test , Benedict test, Tollens test, Bials test and Osazone test. Starch may be identified by Molisch test, and Lugols iodine test.

Chemical Tests for lipids: Solubility in polar and non polar solvents, Sudan IV test, Grease spot test, Emulsification test may be used for detection of fats and oils.

Chemical Tests for Proteins and Amino Acids: Presence of proteins and amino acids is detected by Biuret Test, Millons Test , Ninhydrin Test, Lead sulphide test and Xanthoprotein test.

Chemical Tests for Alkaloids: Presence of alkaloids was ascertained by Dragenderoff's test, Mayer's Test, Hager's test, Wagner's test, Tannic acid test.

Chemical Tests for Steroids and Triterpenoid glycosides: Presence of steroid moiety can be detected by Libermann Bruchard test, Salkovaski test, Antimony trichloride test, Trichloroacetic acid test, Tetranitro methane test, Zimmermann test.

Chemical Test for cardiac glycosides: Keller Killiani test, legal test, Baljet test , 3,5 dinitro benzoic acid test may be used in detection of cardiac glycosides.

Chemical Tests for Coumarin glycosides: $FeCl_3$ test and fluorescence test may be used for detection of coumarin glycosides.

Chemical tests for anthraquinone glycosides: Borntrager's test Modified Borntragers test are used.

Chemical Test for Saponin glycosides: Haemolysis test and Foam test are used in identification of Saponin glycosides.

Chemical Tests for Tannins: Tests for iron salts, Goldbeater's skin test Gelatin test Phenazone test, Test for catechins, Chlorogenic acid test, Vanniline HCl test, Bromine water test are used.

Effect of extract on gross behavior using Irwin schedule:

The Irwin schedule is a simple yet effective way of differentiating between useful and useless drugs (Turner, 1978).

The animals were divided into 3 groups of 5 each, vehicle, extract (100 mg/kg i.p) and 200 mg/kg i.p and gross behavior was observed post one hour of administration for one hour and scores were noted.

Effect of extract on locomotor activity using open field apparatus:

The Albino Wistar rats after administration of the standard, vehicle and extract were placed individually in the corner of an open field apparatus. It consisted of a wooden box with the floor divided into 16 equal squares with pencil. The rat was gently placed in one corner of the box and number of squares crossed by the rat in 5 min was counted as an index of locomotion. The number of rearing was also counted simultaneously for 5 min. The albino Wistar rats were divided into 4 groups of 5 animals each and administered standard, vehicle, extract (100 mg/kg ip) and extract (200 mg/kg ip) Activity was tested after 30 minutes.

Effect of extract on motor coordination using stumbling board:

Albino Wistar rats were selected and divided into 4 groups each consisting of 5 animals, for administration of standard drug (diazepam0.5 mg/kg ip), vehicle, extract 100 mg /kg ip and 200 mg/kg ip). Motor activity was assessed after 30 min by counting the number of stumbles.

Effect of the extract on haloperidol - induced catalepsy in mice

Animals were divided into 4 groups as before and administred standard drug pheniramine maleate (10 mg/kg ip) , vehicle , extract (100 mg/kg ip) and (. 200 mg/kg ip). Catalepsy was measured at 15, 30, 60, 90, 120 and 180 min after administration of haloperidol at dose of 0.5 mg/kg ip using the Bar test. (Bazian et al, 1999)

RESULTS:

The results of phytochemical evaluation of extract are tabulated in Table1. The phytochemical evaluation of extract was carried out as described by Khandelwal, 2006. The following phytochemical tests were performed on the extract.

Table1. Phytochemical test results

Tests	Observations
Carbohydrates	+ve
Fats and Oils	+ve
Steroids	+ve
Volatile Oils	+ve
Cardiac glycosides	+ve
Anthraquinone Glycosides	+ve
Saponin Glycosides	+ve
Coumarin Glycosides	+ve
Flavanoids	+ve
Alkaloids	+ve
Tannins and Phenolic Compounds	+ve

Effect of the extract on gross behavior using Irwin Schedule

There were no significant and stable individual alterations in behaviours and no mortality was observed 24 hrs post experiment in either dose of extract.

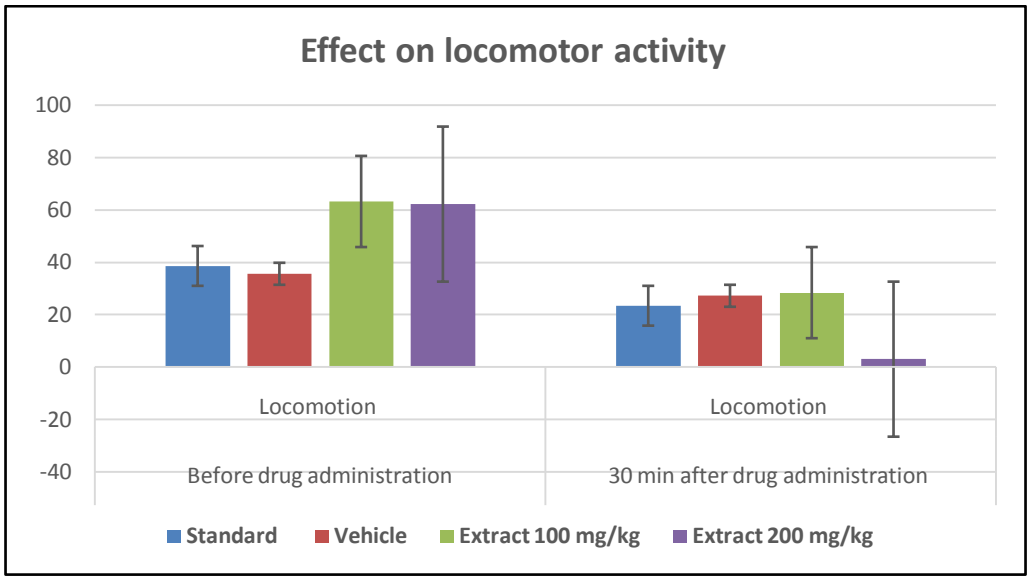
Effect of extract on locomotor activity using open field apparatus

In the vehicle treated rats there was a slight decrease in the locomotor activity after 30 min. This decrease however was not statistically significant. The standard drug Diazepam used in this study significantly reduced the locomotor activity (p=0.009 one way ANOVA). Similarly, the extract in both the doses significantly reduced the locomotor activity. The observations are given in Table 3.

Table 2: Effect of extract on locomotion and rearing.

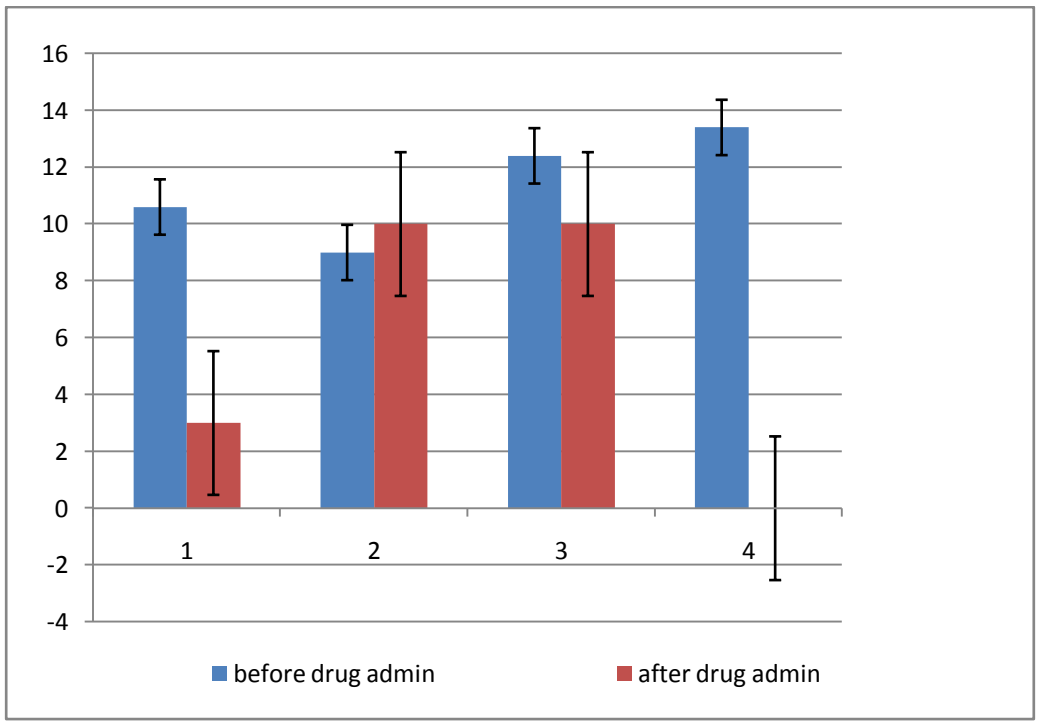
SEM	**Before drug administration**		**30 min after drug administration**	
	Locomotion	**Rearing**	**Locomotion**	**Rearing**
Standard	38.6 ±6.17	10.6 ± 2.92	23.4 ± 7.88	3.0 ± 0.75
Vehicle	35.6 ± 5.33	9.0 ± 2.21	27.2 ± 11.3	10.0 ± 1.58
Extract 100 mg/kg	63.2 ± 15.09	12.4 ± 5.78	28.4 ± 16.53	10.0 ± 3.52
Extract 200 mg/kg	62.2 ± 14.96	13.4 ± 4.20	3.0 ±2.99	0.0± 0.00*

Values are mean ± SD of 5 observations. * P< 0.05 compared to respective control.



Graph 1: Effect of extract on locomotor activity

Both the vehicle and ***Centaurium erythracea*** extract in dose of 100 mg/kg ip treated groups did not show any significant changes in the rearing behavior, whereas , the rats treated with standard and ***Centaurium erythraea*** extract in dose of 200 mg/kg i.p significantly reduced rearing.



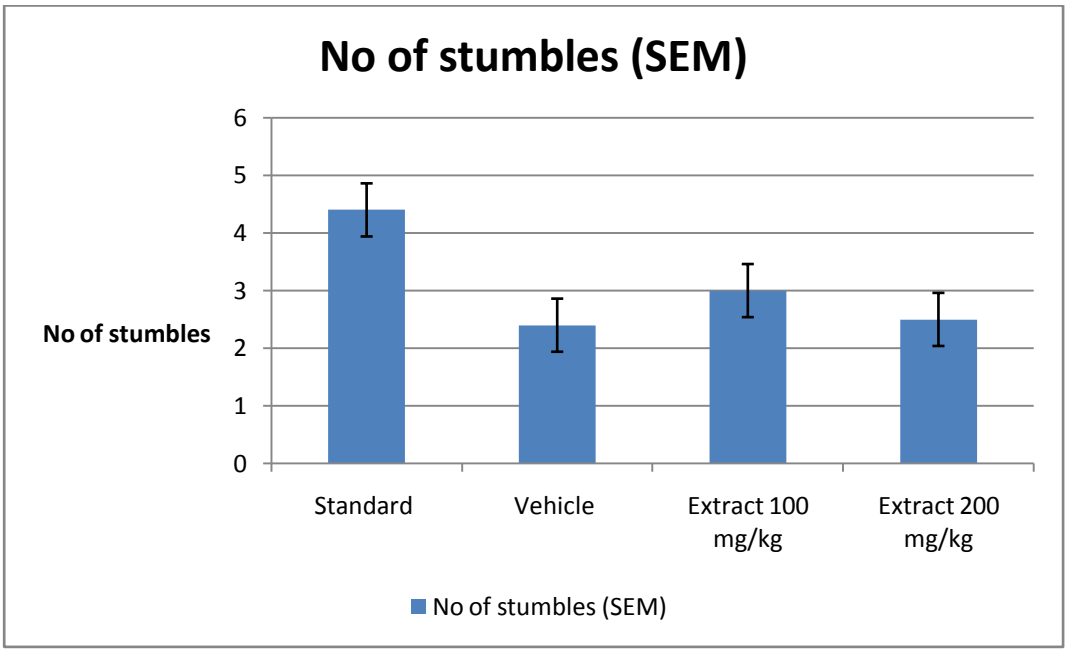
Graph 2: Effect on rearing

Effect of extract on motor coordination:

Results of motor coordination test are presented in table 4. It was found that no significant changes in motor coordination were observed by either dose of extract compared to vehicle and standard treated group in the time span of experiment.

Table 3. Effect of the extract on motor coordination

SEM	No of stumbles (SEM)
Standard	4.4 ± 0.60
Vehicle	2.4 ± 0.24
Extract 100 mg/kg	3.0 ± 0.95
Extract 200 mg/kg	2.5 ± 1.05



Graph 3:- Effect of the extract on motor coordination using stumbling board

Effect of the extract on haloperidol –induced catalepsy in mice

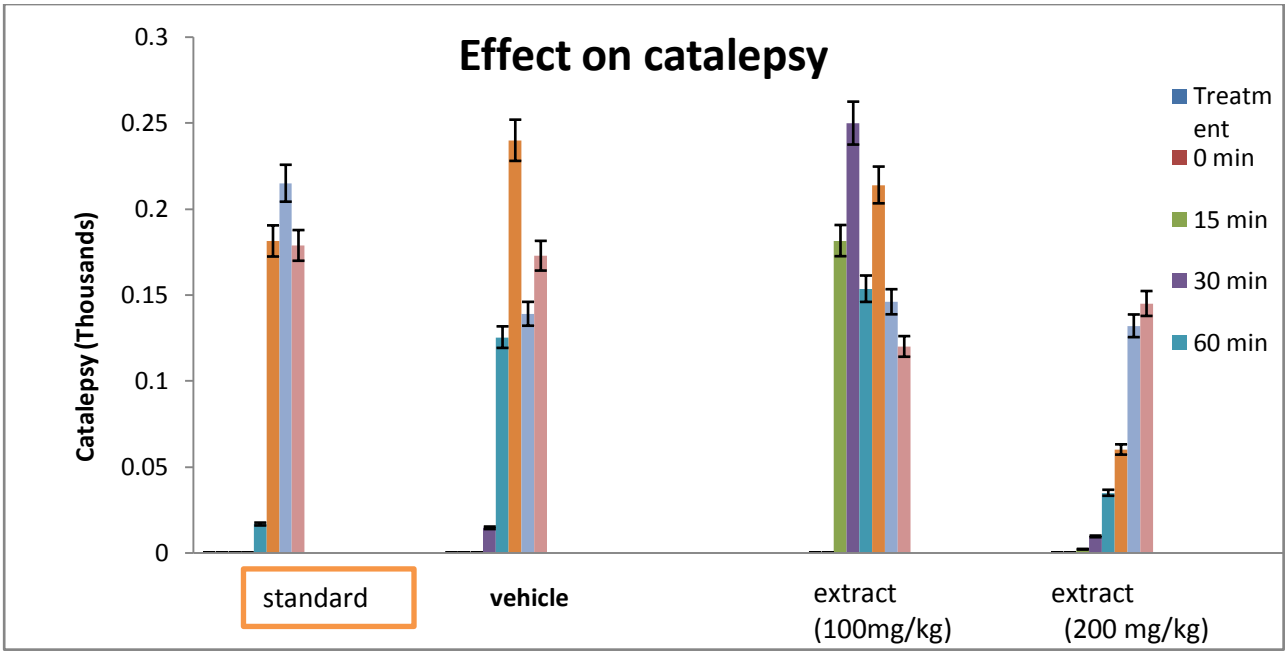
Results are presented in Table 5. Vehicle treated rats exhibited time dependant changes in duration of catalepsy and maximum catalepsy (240 + 27.5) was observed 90 min after haloperidol. The duration of catalepsy decreased gradually.

The ***Centaurium erythracea*** extract in dose of 100 mg/kg i.p. preponed the peak effect from 90 min to 30 min whereas the higher dose of extract significantly reduced the duration of catalepsy throughout the experiment. In rats treated with Centaurium erythracea extract in dose of 100 mg/kg i.p, the change in duration of catalepsy was not significant.

Table 4:- Effect of extract on haloperidol induced catalepsy

Treatment	0 min	15 min	30 min	60 min	90 min	120 min	180 min
Standard	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.6 ± 4.98	181.4 ± 29.9	215 ± 43.91	178.8 ± 54.82
Vehicle	0.0 ± 0.0	0.0 ± 0.0	14.4 ± 5.55	125.4 ± 47.09	240.0 ± 27.52	139 ± 43.60	172.8 ± 33.48
Extract 100 mg/kg	0.0 ± 0.0	181.6 ± 72.38	250 ± 63.13	153.6 ± 54.71	214.0 ± 24.37	146 ± 48.45	120 ± 47.35
Extract 200 mg/kg	0.0 ± 0.0	2.0 ± 0.60	9.4 ± 2.78	34.8 ± 10.90	60 ± 20.21	132.0 ± 47.39	145 ± 63.76

N=5, * p<0.05 compared to the vehicle treated group.



Graph 4 :- Effect of extract on haloperidol induced catalepsy

Discussion:

Very little information is available on the traditional uses of this plant. Very few systematic pharmacological studies have been reported on this plant. Therefore Irwin schedule was used to assess the effect of this plant extract on gross behavior. The ethanolic extract of ***Centaurium erythraea*** was found to be safe upto the dose of 2000 mg/kg i.p. The other studies were performed using the doses 100 and 200 mg/kg i.p. ie $1/10^{th}$ and $1/5^{th}$ of this dose. Both the doses were devoid of any striking effect on the gross behavior.

Conclusion: The present study reports some neuropharmacological activities of ethanolic extract of Centaurium erythraea. The pharmacological activities are attributed to the whole extract of Centaurium erthrae. Results indicated that the ethanolic extract of Centaurium erythracea significantly reduced locomotion in both doses whereas at higher doses it reduces both locomotion and rearing which clearly indicates the action of the drug as dopaminergic D1/D2 antagonist or D3 agonist while in higher dose it may have direct action on 5 HT 1 A receptor. The centaurium erythrae was found to reduce haloperidol induced catalepsy in higher dose which may be due to D2 agonistic effect and/or may modulate by serotonin release.

Since the extract has shown good activities in the animal models studied and is a potential one, further studies need to be carried out. The further studies shall include fractionation of the extract using different solvents and evaluating activities of the fractions. Studies can also be planned to separate the extract on the basis of its phytoconstituents. Mechanistic approach shall be used in further studies.

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