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**ANTI-MUSCULAR ACTIVITY OF METHANOL AND ETHYL ACETATE
EXTRACT OF ODINA WOODIER LEAVES**

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Abstract

In continuation of our work on Odina woodier leaves extracts, the present study deals with the finding of the skeletal muscle relaxant activity of methanol and ethyl acetate extract of the leaves of Odina woodier using Rota-rod method using the standard Diazepam. Both the extracts were administrated orally at dose of 250mg/kg to Albino rats. The methanol and ethyl acetate extract significantly reduced the fall off time, and highly significant ($*P < 0.05$) at 30 minutes of duration. The ethyl acetate extract was found to have greater skeletal muscle relaxant activity (74 %) than the methanol extract (43.8%). Ethyl acetate extract have slightly less activity compared to the standard (81.6%). Thus, the muscle relaxant activity of Odina woodier leaves extracts may be due to the presence of different chemical compounds like alkaloids, tannins, steroids and flavanoids present in the extract.

Keywords: Diazepam, ethyl acetate extract, methanol extract, Odina woodier, skeletal muscle relaxant activity.

Introduction

Herbal medicines are employed in a wide variety of health related applications ranging from treatment of common colds to treatment of cancer [1]. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry.

Odina woodier was used in traditional medicine for leprous, obstinate ulcers, toothache, mouth sores and impotency. The paste of Odina woodier fruits are applied externally for bone fracture [2]. It was found to be effective in healing of wounds. It's bark is very astringent and in decoction is used for chronic ulcers. The bark is traditionally used

in particular in the treatment of cuts, other wounds, bruises (sprains and strains) and diarrhoea, aqueous extracts of the bark also being used as an abortive. The leaves have been reported to use in Elephantiasis of the legs [3]. The dried and powdered bark is found to use as tooth powder and anodyne [3]. In continuation of our work on Odina woodier leaves extracts [4, 5], in this paper the attention was focused on determination of anti-muscular activity of Odina woodier leaves

Materials and Methods

A. Materials Used

a) Collection of plant part

Odina woodier leaves were collected in the S.F.R.college campus, Sivakasi, Virudhunager district in Tamil Nadu. It was cleaned with running tap water to remove adhering elements, shadow dried and powdered in a domestic mixer.

b) Animals used

Adult Albino rats (Wistar strain) of either sex with weighing 100-150 g were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals were fed with commercially available rat pelleted diet. Water was allowed under strict hygienic conditions. The experimental protocols were duly approved by the Institutional Animal Ethical Committee (IAEC, Approval No: CPCSEA/5) of Sankaralingam Bhuvanewari(SB) college of Pharmaceutical Sciences, Anaikuttam, Sivakasi, Virudhunager District.

c) Drugs used

Drugs Diazepam (Dose 4 mg/kg, Ip. prepare a stock solution containing 0.4mg/ml of the drug and inject 1 ml/100g of body weight of the mouse. Diazepam is suspended in 1% w/v gum acacia or carboxymethylcellulose).

B. Methods Used

a) Plant extraction

The powdered leaves of Odina woodier were extracted by using ethanol, ethyl acetate, and methanol in soxhlet apparatus by using standard procedure[4], the distillates were collected and distilled separately to yield the concentrated

extracts. These extracts on cooling produces powdered mass. The powdered mass product was subjected to anti-muscular activity.

b) Determination of anti-muscular activity

The animals were weighed and numbered. Then the animals were placed one by one on the rotating rod. (If the rod is divided into several compartments, one can place more than one mouse at a time). A normal untreated mouse generally falls off within 3-5 minutes. Diazepam (4mg/kg) was injected to all the animals. After 30 minutes, the fall off time was noted and compared with that of standard before and after diazepam treatment [6,7].

i) Determination of acute toxicity (LD50) [8]

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals for next 14 days). Two set of healthy male rats (each set of 4 rats) were used for the experiment. First set animals were divided and fasted for 18 hrs, deprived from food, water withdrawn before 4 hrs of the dosing, body weights were noted before and after dosing with methanol and ethyl acetate extract of *Odina woodier* leaves (250mg/kg) orally. Individually animals were observed for 4 hrs to see any clinical symptoms, any change in behavior or mortality. 6 hrs post dosing again body weights were recorded. Behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors [8].

ii) Selection of dose for pharmacological screening

The methanol and ethyl acetate extract of *Odina woodier* leaves was found to be non-toxic up to the dose of 4000mg/kg and did not cause any death, therefore it is considered as safe. Hence 1/16th of this dose i.e. 250mg/kg body weight was used for the activity.

iii) Skeletal muscle relaxant activity [9,10,11]

Rats were divided into three groups consisting of four animals each. Group I served as control which received distilled water. Animals of group II received standard drug Diazepam at a dose of (4mg/kg, i.p.). Group III received the methanol extract orally at a dose of (250mg/kg). Group IV received the ethyl acetate extract orally at a dose of (250mg/kg). Animals remaining on Rota-Rod (16 rpm) 2min or more in low successive trials after the administration

of test material or control vehicle the same test of 30min for 2 hr. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and the treated rats was taken as an index of muscle relaxation.

Antimuscular activity of Odina woodier leaves extracts in methanol and ethyl acetate were recorded using Diazepam as a standard and the percentage of activity were listed in **Table -I**.

Table-I: Anti-muscular activity of Odina woodier leaves extracts.

Group no	Body Weight (g)	Drug and dose	Fall off time		% of decrease in time (A-B)=(C/A)*100
			Before drug administration	After drug administration	
1	H-125	Diazepam (4mg/kg) ip	90	19	78.89
	B-115		59	5	91.53
	T-110		53	18	66.04
	C-125		44	03	93.18
					Mean
2	H-125	Methanol extract (250mg/kg)	66	46	30.30
	B-130		111	67	39.64
	T-125		164	68	58.54
	C-100		40	15	62.5
					Mean
3	H-115	Ethyl acetate extract (250mg/kg)	53	18	66.05
	B-150		73	21	71.23
	T-135		81	17	79.01
	C-130		53	11	79.25
					Mean

H - Animal's Head

B - Animal's Body

T - Animal's Tail

C - Animal's concentration

Statistical analysis

The data obtained for this investigation was subjected to statistical analysis. All results are expressed as Mean \pm SEM (standard error of mean); 4 animals in each group. All statistical comparison was made by Bonferroni's test after conducting one-way ANOVA.

Results

A preliminary acute toxicity study in Albino rats showed that the methanol and ethyl acetate extract of *Ocimum sanctum* was found to be non-toxic up to a dose of 4000mg/kg. Anti-muscular activity of methanol and ethyl acetate extract of *Ocimum sanctum* leaves were listed in **Table-II**

Table-II:

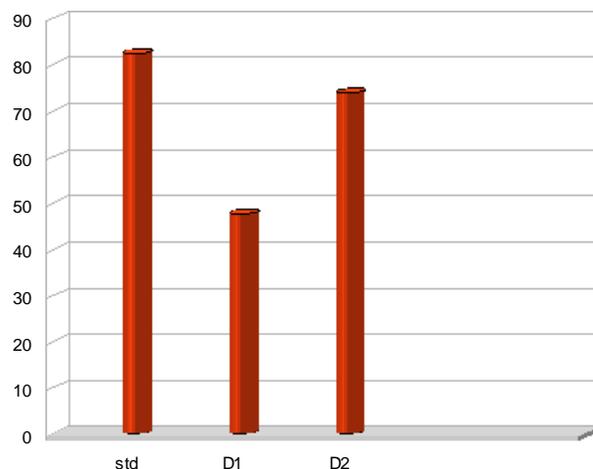
Drugs	Dose	Fall off time		%of decrease in time
		Before drug administration	After drug administration	
Diazepam	4 mg/kg ip	61.5 \pm 11.53	11.25 \pm 1.936	81.650
Methanol extract	250 mg/kg	95.25 \pm 31.43	49 \pm 4.041	43.836
Ethyl acetate extract	250 mg/kg	65 \pm 8.219	16.75 \pm 2.362	74.270
		* P < 0.05	P < 0.5	

Data are expressed as Mean \pm S.E.M, n=2 in each group Statistical analysis done by one way ANOVA followed by paired t-test.

T- Statistics(Table-III):

Parameter	Methanol extract (250 mg/kg)	Ethyl acetate extract (250 mg/kg)
t- values	5.223	0.761
p- values	< 0.05	< 0.5

Fig-I: Skeletal muscle relaxant activity of Odina woodier leaves extract.



% of change in activity

Drugs

Std- Diazepam; **D1-** Methanol extract; **D2-** Ethyl acetate extract

The results obtained from both the extract treated groups were compared with the standard group. A highly significant ($P < 0.05$) reduction in the motor coordination was observed in the test drug at 30min of duration.

Discussion

The result showed that the methanol and ethyl acetate extracts of Odina woodier leaves possess a significant skeletal muscle relaxant activity in experimental rats. At dose of 250mg/kg it showed highly significant skeletal muscle relaxant activity after 30 minutes of duration. The ethyl acetate extract was found to have greater skeletal muscle relaxant activity (74%) than the methanol extract (43.8%).

Conclusion

From our study, we conclude that the methanol and ethyl acetate extracts of Odina woodier leaves possessed significant skeletal muscle relaxant activity. The ethyl acetate extract was found to have greater skeletal muscle relaxant activity (74%) than the methanol extract (43.8%) and have slightly lesser activity compared to the standard (81.6%).

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