Arch. Pharm. Res. **12**(1), 22-25 (1989)

Pharmacological Evaluation of the Glycosidated Phenylpropanoids Containing Fraction from *Orobanche crenata*

O.A. El-Shabrawy, F.R. Melek,* M. Ibrahim* and A.S. Radwan*
*Pharmacology Department and *Natural Products Department, National Research Center, Dokki, Cairo, Egypt.*
(Received November 3, 1988)

Abstract □ Ethylacetate fraction from *Orobanche crenata,* contained two phenylpropanoid glycosides, exhibited some pharmacological properties. It was found to be non-toxic to rats in oral doses up to 500mg/100gm body weight. In large doses, it lowered the arterial blood pressure of anaethetised rats, and produced significant analgesic effect in mice and diuretic effect in rats. It further showed smooth muscle relaxant and antispasmodic effects in the isolated rabbit intestine and guinea-pig ileum respectively.

Keywords □ *Orobanche crenata,* toxicity, hypotensive, analgesic, diuretic.

Orobanche crenata is a parasitic herb attacking leguminoseae especially *Vicia faba.* Several *Orobanche* species were found to have some pharmacological properties as diuretic, hypotensive and analgesic effects. The alkaloidal fraction from one of these species was reported to be a powerful hypotensive agent in dogs.[1)]

In a clinical study, it was reported that, out of 250 patients with renal colic, 130 showed improvement in 1-2 days on treatment with the aqueous *O. crenata* extract.[2)] The extract was also found to produce rapid discharge of calculi in some patients suffering from renal calculi.

As a part of our continuous programe[3)] to evaluate the natural products with biological activity from Egyptian plants, we have studied some pharmacological properties of the glycosidic fraction obtained from *O. crenata.* This fraction was obtained by solvent partition (ethyl acetate) of the aqueous alcoholic extract. The chemical examination of this isolate proved to contain two phenylpropanoid glycosides, Their strucuture determinaton is still in progress and will be the subject of another report.

MATERIAL AND METHODS

Plant material

Orobanche crenata plant was obtained from the *Vicia faba* fields, air dried and finely powdered. The powdered plant material was first extracted with chloroform to remove the non-polar material. The dried marc was then soaked in aqueous ethanol (70%) at room temperature for three days with occasional stirring. The slurry filtered through Buchner funnel. The extraction process was repeated one more time with the same solvent. The combined filtrates were concentrated on a rotary evaporator under reduced pressure at 40° until the ethanol was removed. The aqueous layer was partitioned several times successively in a separatory funnel with chloroform and then ethyl acetate. The total chloroform extracts were combined and left aside for further investigation. The combined ethyl acetate extracts were dried with anhydrous sodium sulphate and evaporated to dryness under reduced pressure to yield 18gm of glycosidic fraction. The glycosidic fraction was dissolved in distilled water and filtered, and the filtrate was used in the pharmacological testes.

Chromatographic and chemical investigation

TLC for glycosides was conducted on cellulose plate with EtoOA/MeOH/H_2O/Me_2CO/$CHCl_3$ (60: 11:11:5:1.5; R_f=0.56 and 0.63) and on silica gel(10: 2:3; R_f=0.72 and 0.8). Spots were detected by its bright blue flurescence under UVL 365 nm (turned to yellow with NH_3) and spraying with Valinine-Sulphuric acid followed by heating at 100° for 5-10 minutes. TLC for caffeic acid was performed on cellulose plate with 15% HOAc. NMR spectrum was obtained at 90 MHz using varian EM-390 spec-

trometer.

Acid hydrolysis

A solution of 100 mg from ethyl acetate fraction in 4N HCl (20ml) was refluxed for 1 hour. H_2O was added and the mixture extracted with chloroform. Caffeic acid was detected in the chloroform by Co-chromatography against authentic sample. The aquous layer was nutralized with $BaCO_3$, the precipitate filtered off and the filtrate evaporated in vacuo, giving residue in which the sugars were identified by PC with a developing BuOH/benzen/pyridine/H_2O (5:1:3:1.5).

Acute toxic effect

Male and female albino rats (150-200gm) were given the glycosidic fraction orally in different dose levels. Treated animals were observed for any toxic symptomes and the mortality rate was recorded in the first 24 hours after oral administration.

Effect on the arterial blood pressure in rats

This was investigated according to the method described by Mcleod *et al.*[4] Rats were anaethetized using urethane, the trachea, one common caroted artery and the external jagular vein were canulated. Blood pressure was recorded using Harvard pressure transducer and biographic recorder 2120. Teasted doses were intravenously injected 2-4 times and the mean effect was calculated.

Analgesic activity

This was investigated using the hot plate method described by Janssen and Jageneau[5]. Mice were divided into groups, and the extract was orally given to the animals in different doses. The reaction time was calculated from the time the mouse reaches the hot plate until it licks its feet or jumps out of the cylinder.

Diuretic activity

This was evaluated in rats, and food was removed 18 hours before the experiment was begun. At the beginning of the experiment the animals were given 50ml/kg tap water orally in which the calculated dose or doses were dissolved. Groups of three animals were used and put into diuresis cages, and the urine was collected and measured after 1,3,6 and 24 hours after treatment.

Effect on smooth muscles

The effect of the extract under investigation was assessed using the method of Magnus[6]. The percent effect of the tested extract on the peristaltic movements of the isolated perfused rabbit intestine was calculated. Each dose was tested 3,5 times and the mean percent effect was calculated.

Antispasmodic activity

The antispasmodic effect of the tested extract on the acetylcholine induced contractions using the isolated perfused guinea-pig iluem was performed according to the method of Foster *et al*[7]. The percent effect was calculated and each dose was tested 3-5 times and the mean percent effect was calculated.

RESULTS AND DISCUSSION

Recently, ethyl acetate fraction from alcoholic aqueous extract of *Orobanche* species was shown to possess some pharmacological properties.[3] It was concluded that such properties were due to the occurrence of phenylpropanoid glycosides. This prompted us to examine the same conistituents and the pharmacological properties of their containing fraction in the Egyptian plant *Orobanche crenata,* following the same reported processing steps.[3]

Preliminary chromatographic, chemical and spectral studies of this fraction were undertaken in an attempt to identify the nature of its composition. TLC showed that it only contained two related compounds with similar colour performed under UVL and with spray reagents. Chemical hydrolysis with 4 N HCl yielded caffeic acid, glucose and rhamnose, thus confirming their glycosidated phenylpropanoid nature. ^{1}H-NMR spectrum exhibited signals for substituted aromatic residue, probably due to their aglycones, in addition to that of caffeic acid and sugar moieties.

Oral administration of the tested glycosidic fraction to rats showed no lethal effect in doses up to 500mg/100gm. No toxic symptomes were obtained on giving this dose level to rats. This finding is in agreement with that recently reported by Andary *et al.*[37] on using similar glycosidic fraction from *O. rapum* genisiae.

The tested fraction when intravenously injected to rats in dose up to 20mg/100gm was found to produce a temporary lowering of the arterial blood pressure of rats, this effect returned back to normal after period of time depending on the dose used. Higher doses produced a slight persistant lowering of the arterial blood pressure (Fig. 1). The hypotensive effect caused by the investigated glycosidic fraction was comparable to that produced from the

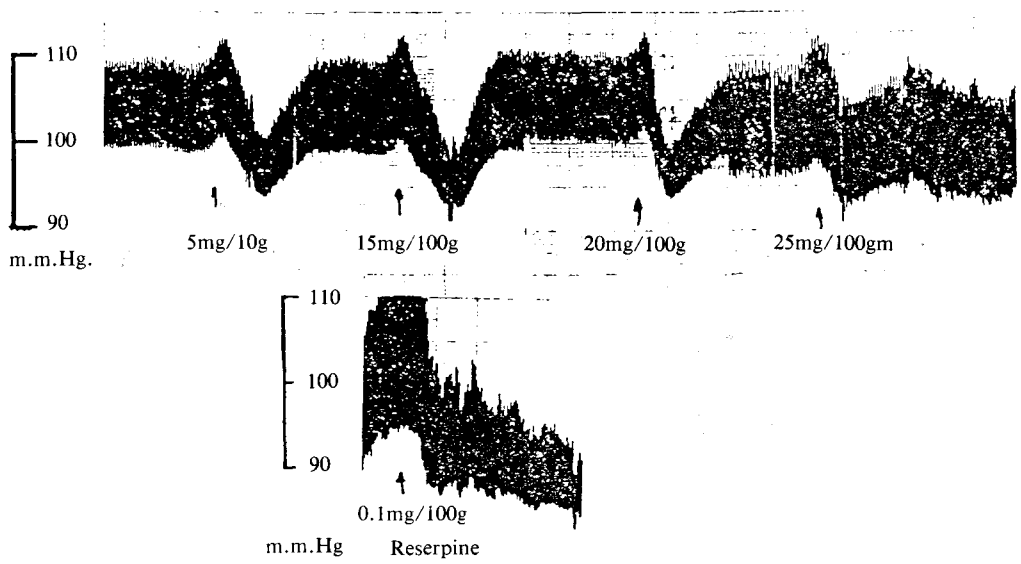

Fig. 1. Effect of the glycosidated phenylpropanoid containing fraction from *Orobanche crenata* and reserpine on the blood pressure of urethan anaethetised rats.

Table I. The analgesic effect of the glycosidated phenylpropanoids containing fraction from *Orobanche crenata*

Treatment	Dose mg/100 gm B.wt	Reaction time in minutes (Mean ± S.E.) 0	10	20	30	45	60	120
Cont.	0.0	16.10 ±0.01	16.30 ±0.09	14.91 ±0.10	15.30 ±0.12	14.10 ±1.10	16.10 ±1.32	15.80 ±1.13
Orobanche	50	17.83 ±0.57	22.00** ±0.93	22.60** ±0.94	19.00** ±0.51	16.00 ±1.00	16.50 ±1.18	16.13 ±0.80
Orobanche	100	14.33 ±3.03	22.83** ±1.40	29.66** ±2.82	27.33** ±1.67	33.66** ±1.71	31.50** ±1.52	22.66** ±0.84
Para-cetamol	50	16.00 ±1.30	26.11** ±0.20	26.10** ±0.20	28.36** ±0.33	28.40** ±0.66	28.00** ±0.30	26.39** ±1.40

* $p<0.05$, ** $p<0.01$

Table II. Diuretic activity of the glycosidated phenylpropanoids containing fraction from *Orobanche crenata*.

Treatment	Dose mg/100gm B.wt	Mean urine volume ± S.E. 1H.	3H.	6H.	24H.
Control		0	2.67 ±0.22	6.25 ±0.05	11.17 ±0.11
Orobanche	100	0	3.27* ±0.05	6.95** ±0.05	12.85** ±0.07
Orobanche	200	0.37** ±0.05	3.95** ±0.05	8.25** ±0.12	14.25** ±0.20

* $p<0.05$, ** $p<0.01$

alkaloidal fraction obtained from *O. aegyptiaca* investigated by Sharaf and Youssef[1] and similar to that induced by the glycosidated phenylpropanoids isolated from other *Orobanche* species[3].

The glycosidic fraction was found to produce potent analgesic effect especially on using large doses. This analgesic effect was also observed on studying similar isolates from other *Orobanche* species[3], results are shown in Table I.

Oral administration of the tested glycosides in rats was found to produce highly significant diuretic effect especially with high doses. The results are listed in Table II.

The glycosides were also found to produce

Table III. Effect of the glycosidated phenylpropanoid containing fraction from *Orobanche crenata* on smooth muscle and its antispasmodic effect

Effect on smooth muscles		Antispasmod	
Dose**	inhibition % ± S.E.	Dose	inhibition % ± S.E.
50	31.89 ± 6.8	200	22.82 ± 2.1
80	38.41 ± 10.1	400	55.62 ± 3.7
100	44.20 ± 4.3	600	67.28 ± 10.1
150	51.20 ± 10.3	800	94.28 ± 6.4
200	95.41 ± 2.7	—	—

* After submaximal dose of acetylcholine. ** mg/50ml *bath.*

smooth muscle relaxant effect, which proved to be dose dependent (Table III). The tested material was found to have a potent antispasmodic effect when tested on the isolated perfused guinea-pig ileum. after submaximal dose of acetylcholine (Table III). This is the first time to reveal diuretic and antispasmodic properties for the glycosidic phenylpropanoids isolated from Orobancheaceae plants. The significant diuretic, smooth muscle relaxant and antispasmodic effects may account for rapid relief of renal colic symptoms and discharge of calculi, observed in some patients after treatment with total aqueous extract of *O. crenata* previously reported by Ibrahim.[2)]

In general the observed biological effects of the glycosidic fraction obtained from *O. crenata* were found to be highly significant in high doses, but smaller doses were less effective.

LITERATURE CITED

1. Sharaf, A. and Youssef, M.: Pharmacological investigation in *Orobanche egyptiaca* with a special study on its hypotensive action. *Qual. Plant. Mater. Veg.* **XX,** 269 (1971).
2. Ibrahim, D.F.: Preliminary studies on the physiological and pharmacological actions of *Orobanche crenata. J. Egyt. Med. Ass.* **51,** 939 (1968).
3. Andary, C., Privat, G., Cheullet, P., Orzales, H., Serrana, J. and Boucord, H.: Chemical and pharmacological study of heteroside esters of caffeic acid isolated from *Orobanche rapumgenistae. Farmaco. Ed. Sc.* **35,** 3 (1980).
4. Mcleod, J.I. and the staff of the Department of Pharmacology Univ. of Edinburgh: *Pharmacological experiments on intact preparations,* E&S Livingstone, Edinburgh and London (1970).
5. Janssen, P.A. and Jageneau, A.H.: Series of potent analgesic-d-2,2-diphenyl 1-3-methylmorpholine-butyrpyrrolidine and related basic amides (II) Comparative analgesic activity, acute toxicity and tolerance development in rats for R 875, morphine, pithidine and methadone. *J. Pharma. Pharmac.* **9,** 381 (1957).
6. Magnus, R.: *Pharmacological experiments on isolated preparations* preface by Perry, M.L.W. 2nd Ed E&S Livingstone, Edinburgh and London (1970).
7. Foster, B.H., Niklas, H. and Lutz, S.: Antispasmodic effects of some medicinal plants. *Planta Medica.* **40,** 309 (1980).