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PHARMACOLOGICAL STUDIES ON VERNONIA AMYGDALINA (DEL) AND TITHONIA DIVERSIFOLIA (GRAY)

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SUMMARY

The plants examined contained carbohydrates and /or glucosides, catechol tannins, flavonoid glycosides, sterols and/or triaterpenes, oxidose enzyme present or absent, lactones and /or esters and volatile substance.

Because of the therapeutic application of these plants in Folk's medicine, it was decided to study the pharmacological properties of the *V. amygdalina* and *T. diversifolia*. They seem to possess the following properties, intestinal and uterine stimulant actions, hypotensive, depressing the coronary outflow, antidiabetic action, antitumour, but have no effect on renal outflow.

INTRODUCTION

Vernonia amygdalina (Del.) and *Tithonia diversifolia* (Gray) are two genera of the family compositae. They are grown in tropical and subtropical countries including Ethiopia and Egypt (Genus Vernonia and different applications as purgative (Dymock et al., 1981), for kidney disorders (Caius and Mhaskar, 1923), stomachic and good for hicough (Nadkarni, 1927 and Kirtikar and Basu, 1933), anthelmintic (Chopra, 1933 and 1934), anti asthmatic (Majumdar, 1943), carminative (Chopra et al., 1966) and recently as antitumour agent (Kupchan et al., 1969).

Some species of the genus Tithonia showed activity against lymphatic leukemia (Pal et al., 1976).

These therapeutic applications stimulated us to study these plants (*V. amygdalina* (Del) and *T. diversifolia* (Gray) especially the literature survey did not reveal any previous work on the chemical and pharmacological asepcts.

MATERIAL AND METHODS

A sufficient amount of plant were collected from El-Zohoria garden, as well as from plants cultivated in the experimental station of Medicinal plants, Faculty of Pharmacy, Cairo University, at Giza. The species were identified by Miss Badia Hassan Diwan, Agricultural Engineer, at the Orman garden for performing the phytochemical and pharmacological studies of plants.

Alcoholic evtract:

Known weight of the dry samples of plants were extracted in a Soxhelt apparatus, using 70 % ethyl alcohol till exhaustion. The alcohol was then evaporated on a water both leaving a vicid extract. The alcohol was then evaporated on a water both leaving a vicid extract. The results were calculated per 100 mg. dry sample.

Thin-layer chromatography (TLC) and column chromatography (Doss, 1974) for studying preliminary photochemical investigation.

Insulin solution:

One ml. insulin solution (Merck containing 20 units) was diluted 10 times with distilled water.

Tumour system:

Ehrlich's ascites carcinoma (EAC). Resistant to Endoxon, supplied from Amsterdam through the courtesey of Dr. G. Klein (Antoni Van Leeu Wenhoeschins Het Nefer lands kan kerinstitute Amsterdam), were used in this study.

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Media and Solutions:

To medium 199, is prepared according to the formula given by Morgan et al. (1950 and 1955).

Phosphate balance solution (PES), is obtained by penso and Baldncci (1963).

The alcoholic extracts from the plants were dissolved in Ringer's solution for studying their pharmacological characteristics as follows:

1- On the arterial blood pressure in dogs:

Thirty mongrel dogs of the body weight range 10-20 kg were used. They were divided into 5 groups of 6 dogs each. They were anaesthetised with pentobarbition (Nembutal) 30 mg/kg and prepared for Kymographic blood pressure using Starling Boradic Kymograph (Jackson, 1917).

2- On the renal blood flow:

Dogs were anaesthetises in the same manner as above and the direct colleciton technique for renal blood flow described by Blalock and Mason (1963) and modified by Hassan (1977) was employed.

3- On the isolated frog's heart:

The effect of the extracts were studied on the isolated frog's heart using 40 heart divided into 4 groups by Sollman and Barlow technique (1926).

4- On the intestinal and uterine motility:

Sixty experiments were performed using the glass jarbath apparatus of 50 ml capacity organ bath. Pieces of rabbits intestine, horns of rat uterus were suspended into inner vessel of the apparatus.

5- On blood sugar level

Rats of both sexes, weighing 150 - 200 grams, were prepared for the test by withholding food 12 hours before the experiment. For *V. amygdalina*, three groups of animals (each consisting of 10 rats) were utilized. The first group received 0.5 mg of the alcoholic extract of the plant, the second received 1 mg of a diluted insulin solution (corresponding to 2 units). In each group the extract was injected I. P. For testing the effect of the alcoholic extract when administered by the oral route, 1 mg was given to each rat in a fourth group. For *T. diversifolia*, two groups of animals were utilized (each consisting of 10 rats) each was injected I. P. either 0.5 mg of the extract or 1 mg of the diluted insulin solution.

Samples of blood (0.1 ml) were obtained from the eyesocket of each rat with a micropipette before injection of test solution, and at hourly intervals for 5 hours after the injection. The analysis of blood glucose was carried out according to krawczynski method (1967), and the potency of alcoholic extract was compared with reference to insulin solution.

On the neoplastic cells:

a- Preliminary in vitro evaluation of the cytotoxic activity of extracts by Klein (1950).

The concentrations of alcoholic extract of V. *amygdalina* were used for the in vitro test were assigned as 1/10, 1/100 and 1/100.

The concentrations of alcoholic extract of T. diversifolia were used, 5, 10 and 25 ug to one ml of tumour cells suspension.

b- Determination of life span prolongation effect on tumour bearing animals by Lettre (1950) and Creech (1955).

Random-bred female swiss albino mice weighing 18 - 22 g, from the animal house of cancer institute, Cairo University, were used the line was maintained by successive intraperitoneal transplantation in random, female swiss albino mice.

The tumour bearing animal died by accumulaton of ascites in the abdomen, within three weeks after tumour inoculation. Distal metastasis is seldom observed and spontaneous regression is very rare seven groups of ascites tumour bearing

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Table (1): Preliminary phytochemical screening of different organs of plants

Constituents	V. amygdalina (del)			T. diversifolia (Gray)				
	Stem	Constituents	Fruits	Stems	Stem	Constituents	Fruits	Stems
Crystalline sublimate	-	-	-	-	-	-	-	-
Volatile substance	-	-	±	-	-	-	±	-
Carbohydrates and / or glycosides	+	+	+	+	+	+	+	+
Cardiac glucosides	=	=	-	=	-	-	-	-
Catechol tannins	+	+	+	+	+	+	+	+
Flavonoid aglycones	=	=	±	=	-	-	-	-
Flavonoid glycosides	±	±	±	±	±	±	+	+
Saponins	±	±	±	±	-	-	-	-
Sterols and / or triterpenes	+	+	+	+	+	+	+	+
Oxidase enzyme	+	+	+	+	-	-	-	-
Alkaloids	±	±	±	±	-	-	-	-
Lactone and / or esters	+	+	+	+	+	+	+	+

+ = Postive ± = Traces - = Negative

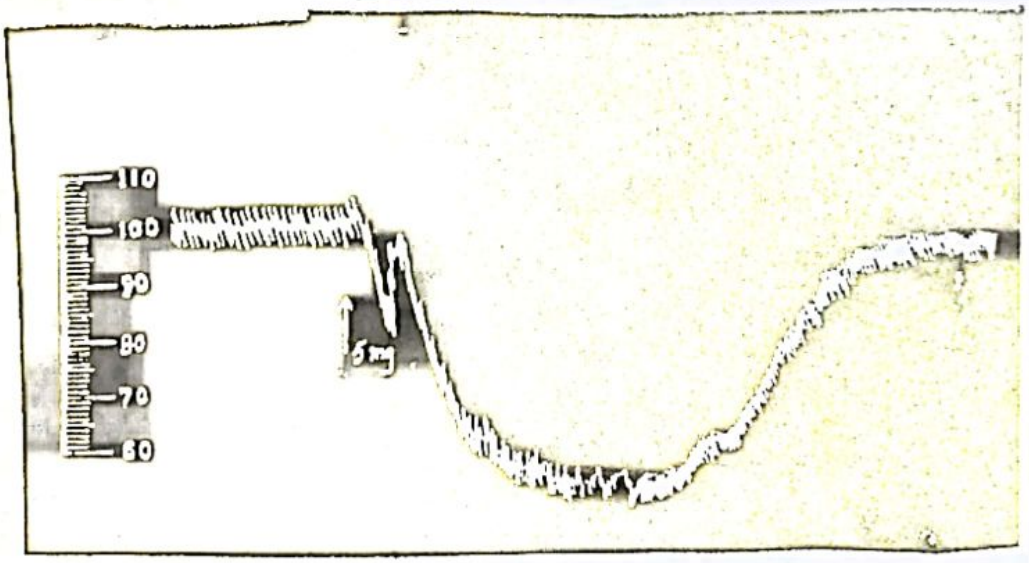

Fig. (1): Effect of `t. diversifolia alcoholic extract on systemic blood pressure of anaesthetised dogs in a dose of 5 mg / kg. B. wt.

mice (each group of 10 animals) were utilized for the test, (3 groups were used for *V. amygdalina* extract, first was used as control (untreated), second was injected by 0.1 / mg of extract every other day, the last was injected 0.2 mg of the extract in a single dose, and 4 groups were used for *T. diversifolia* extract, first group was untreated (control) while the other thre groups received different doses of the extract as mentioned in Tables (5 and 6).

RESULTS

Phytochemical studies:

Results could be summarized in Table (1).

1- The effect on blood pressure and renal blood flow:

When the tested drugs were injected i/v into the anaethetised dogs doses up to 5 mg /kg were without effect on the blood pressure, where as, dose of 10 mg / kg caused a fall in blood pressure (*T. diversifolia* is more effective than *V. amygdalina)* but retained its normal level after 10 - 50 minutes respectively (Fig. 1) on the other hand doses up to 10 mg / kg were without effect on renal circulation .

2- The effect on isolated frog's heart and coronary circulation:

It was clearly noticed that a dose of 0.25 mg/frog's heart was without any effect on heart rate and on coronary circulation, doses from 0.5 -

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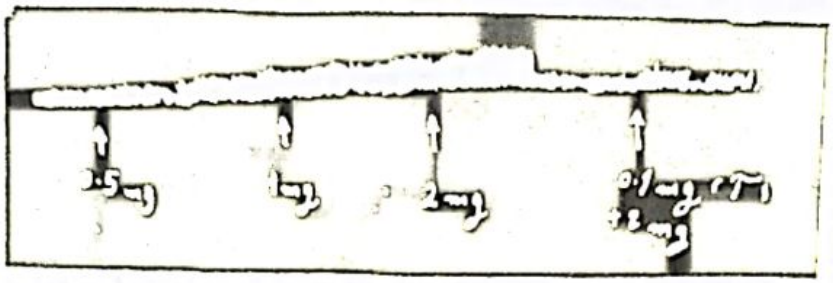

Fig. (2): Effect of V. amygdalina alcoholic extract on isolated amphibian heart.

2 mg / heart caused inhibition in amplitude and frequency of heart contraction and followed by transient cardiac arrest (Fig. 2), these doses showed severe reduction in coronary outflow. When trimetaphan was applied before the extract the depressant effacts could not be observed (Fig.2).

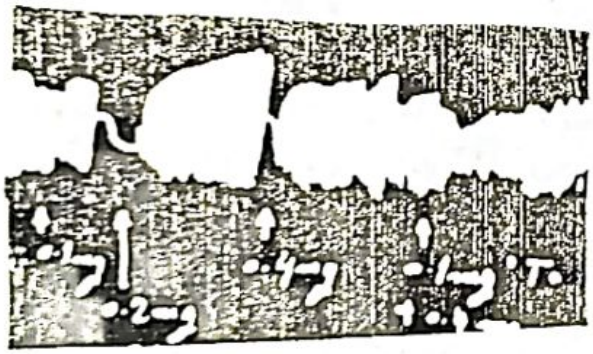

Fig. (3): Effect of V. amygdalina alcoholic extract on isolated rabbit's intestine.

intestine before the extract, abolished the stimulant effect (Fig. 3).

(4) The effect on isolated rat uterus:

The uteri, indifferent stages of estrus cycle, were not responsive to the plants extracts when used in doses up to 5 mg / 50 ml bath, and by increasing the dose there was an increase in response (Fig.4).

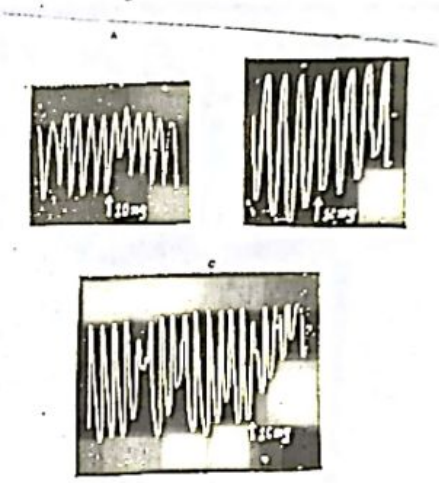

Fig. (4): Effect of V. amygdalina alcoholic extract in a dose of 10 mg on the uterine motility of rat add to 50 ml both.
a) Non oestrus.
b) Oestrus uterus
c) Early pregnant

(5) The effect on blood sugar level:

Results were recorded in Table (2).

Table (2): The effect of plants on blood sugar level

Plant	Extract	Blood glucose level in mg					
		Route of administration	Dose / mg	Before treatment		1 1 / 2 hr after treatment	% change from initial
V. amygdali	Alc. ext.	I.P.	0.5	58 ± 1.87	@	38 ± 2.84	34.4
	Insulin solution	I.P	1.0	72.2 ± 1.02	@	25 ± 0.98	66.25
	Alc.	Oral	1.0	66.5 ± 1.18	@	57 ± 1.15	14.3
aT. diversifolia	Alc. ext.	I.P.	0.5	0.5		35.83 ± 4.72	37.68
	Insulin solution	I.P.	1	1		25.5 ± 1.55	64.7

N. B: @ significant difference at $p < 0.05$

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Table (3): Percentage of stained tumour cells after incubation with different concentrations of V. amygdalina (Del) extract, for different periods of time.

Conc. of extract	Percentage of stained cells after exposure for		
	2 hours	4 hours	6 hours
1 / 10	Cytolysis	Cytolysis	Cytolysis
1 / 100	Cytolysis	Cytolysis	Cytolysis
1 / 1000	96, 95	100, 100	100, 100
	94, 95	100, 100	100, 100
M ± SE	95 ± 0.4	100 ± 0.0	100 ± 0.0

M ± SE = Mean value ± Standard error

Table (4): Effect of the extract of V. amygdalina on the life span of mice bearing EAC cells.

Dose / mg	Schedule	No. of animal	Valid No.	M ± SE	T / C
Control	-	10	10	15.6 ± 2.32	10
0.1	Three doses e. o. d.	10	9	17.0 ± 3.39	10
0.2	Single dose	10	10	15.0 ± 2.50	10

N. B:
e. o. d. = every other day.
M ± SE = mean survival time ± standard error.
T / C = Mean survival time of treated group / Mean survival time of control group

Table (5): Percentage of stained tumour cells after incubation with different concentrations of T. diversifolia extract, for different periods of time

Cone. of extract ug / ml of tumour cell suspension	Percentage of stained cells after exposure for	
	2 hours	4 hours
25	80, 82, 80, 78	100, 100, 100, 100
	M ± SE 80 ± 0.81	M ± SE 100 ± 0.0
10	45, 40, 35, 40	58, 60, 65, 57
	M ± SE 40 ± 2.04	M ± SE 60 ±2.08
5	19, 20, 23, 18	34, 30, 36, 32
	M ± SE 20 ± 1.08	M ± SE 33 ± 1.29

N. B:
M ± SE = Mean value ± Standard error.

3- The effect on isolated rabbit's intestine:

When the solutions of the tested drugs were added to the organ bath of 50 ml capacity in

Table (6): Effect of the extract of T. diversifolia on the life span of mice bearing EAC cells

Dose mg / kg	Schedule	M ± SE	T / C	Long survivors 60 days
ontrol	---	19.8 ± 1.8	---	0 / 10
5 doses of 10 mg / kg	e. o. d.	32.6 ± 3.9	1.63	1 / 10
3 doses of 25 mg / kg	1, 5, 9	44.6 ± 10.3	2.25	6 / 10
Single dose of 100 mg / kg	Single	15.1 ± 9.1	0.76 toxic	2 / 10

N. B:
e. o. d. = every other day.
1, 5, 9 = day 1, 5 and 9 after tumour inoculation
M ± SE = mean survival time ± Standard error.
T / C = Mean survival time of treated group / Mean survival time of control group

doses less than 0.05 mg, they were wighout efects, whereas, higher doses stimulated the force of intestinal motility (Fig. 3). Trimetaphan when added to the bath in a dose 0.1 mg, stimulated the intestine before the extract, abolished the stimulant effect (Fig. 3).

(6) The effect on neoplastic cels:

Results could be summarized in Tables (3, 4, 5, 6).

DISCUSSION

Family compositae has received special interest in the field of pharmaceutical research, on account of its important species, which are reputed for their sesquiterpene lactones. These lactones have attracted, recently world attention for their antitumour activity (Kupchan et al., 1969 and Pal et al., 1976).

Among these species which are included in this investigation are *V. amygdalina* (Del.) and *T. diversifolia* (Gray). Moreover, V. amygdalina is praised in Egyptian folk medicine as effective antidiabetic. However, the literature lacks informations regarding their biological properties and possible therapeutic value. From the chemical point of view, preliminary phytochemical screening has been performed and showed the results presented in Table 1.

On studying the pharmacological effects of the

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extracts of plants, hopeful results were obtained. Its effects on the smooth muscles differs according to the type of the muscle. Thus, it stimulated the intestine, which may be due to ints carminative effect, and the uterine musculature. The stimulatory effects of these plants on the intestine was abolished by trimetaphane and this proves that extracts of both plants have ganglionic stimulant property. Moreover, its stimulant effect on uterine musculature may be due to various hormones existing at these stages.

It is interesting too, to notice in our present study that V. amygdalina and T. diversifolia have hypotenisve effect in anaesthetised dogs. In this respect, doses below 5 mg / kg body weight have no effect on the other hand, 5 mg / kg body weight produced a fall in blood pressure. This lowering in blood pressure prompted us to study on the effect of these plants on renal blood flow. Results denotes that they have no effect on the renal blood flow in tested doses, up to 10 mg/kg body weight.

The study on the effect of the drugs using the heart seems of importance. The drugs inhibited the cardiac contraction and this may be a factor which explains the fall of blood pressure. Doses of 0.25 mg/ heart were without any effect on coronary circulation whereas doses as big as 2 mg / heart caused inhibition in force and frequency, and moreover, they showed severe reduction in coronary outflow which may indicate the possible coronary contraction. When trimetaphan was applied before the extracts the depressant effect could not be observed this emphasized that the extracts of plants have a ganglionic stimulant property.

Blood sugar level of rats was determined before and after injection of extract. It was found that the alcoholic extract from *V. amygdalina* (Del), induced significant loweing of blood sugar level. The alcoholic extract when administrered orally caused only mild lowering of blood sugar level. This results may be due to blocking of adrenal gland by the drugs.

The extract of *V. amygdalina* (Del.) had a strong cytotoxic action on FAC cells. Thus after 2 hours incubation with the lowest concentration of extract (1 / 100) 95 % of cells lost their viability. Also we found that the extract of *T. diversifolia* had a marked cytotoxic effect on EAC cells.Thus using a concentration of 5 - 10 and 25 ug / ml of extract respectively 20, 40 and 80 % of cells lost their viability after two hours contact, and 33, 60 and 100 % of EAC cells, after 4 hours contact.

The extract of *V. amygdalina* (Del.) exerted only border line activity in vivo against EAC cells. Thus T / C value for 0.2 mg extract is 0.96 and for 0.1 mg extract is 1.1 Also, it was found that a single dose of 100 mg / kg of animal from the extract, was toxic to animals. Meanwhile, when a dose of 10 mg / kg was injected 5 times, e. o. d., only one out of 10 treated mice was cured by therapy. A dose of Tithonia 25 mg / kg administered on the day 1,5 and after tumour inoculation induced significant increase in the life span and 6 out of 10 animals were cured by the therapy. This shows that the extract of *T. diversifolia* (Gray) is a good candidate for further evaluation of anticancer activity against wide spectrum of experimental tumours. These results due to inhibitory activity against carcinoma. These findings agree with Kupchan et al., (1969) and Pa et al., (1976).

REFERENCES

Black, A. and Mason, M. F. Amer. J. (1936): Physiol., 11, 328 .

Caius, J. F. and Mhaskar, K. S., (1923): "Ind. J. Med. Res", 11, 353 .

Chopra, R. N., (1933): Endogenous Drugs of India, 409, The Atpress, Calcatta.

Chopra, R. N., Ghosh, N. N., and Mukerji, A. R., (1934): Indian J. Med. Res., 22, 183.

Chopra, R. N., Nayer, S. L . and Chopra, I. C., "Gbssary (1966): of Indian Mesicinal plants", C. S. I. R. 254, New Delhi.

Creech, H. J., Hanscka, T. S., (1955): Hankwitz, Jr, R. F. Littleton B. J. and Andre, J., Cancer Res. (Suppl. 3). 47.

Doss, S. L. "A pharmacognostical (1974): study of *Beoumontia* grandifora (Wall.)., Family Aocynaceae", A Thesis (M. Ph. Sx.) submitted to the Fac. of Pharmacy, Cairo UNiv.

Dymock, W., Warden, J. H. and Hooper, D., "Pharmacographia (1981): Indica", Vol. 2, 241, Trubner and Co., London .

Hassan, A. B.; M. D. (1972): Thesis presented to the Fac. Vet. Med., Cairo Univ.

Jackson, D., E. (1917): Experimental pharmacology, Page 99.

الممسوحه ضوئيا بـ CamScanner

Kupchan, S. M., Hamingway, R. J., Karim, A. and Werner, D. J. (1969): Org. Chem., 34, *34*, 3908.
Krawczynsri, F., Osinski, T. (1967): "Laboratory metody diaynesstyczme", 283, PZWL, Warszawa, Polska., (Polish).
Kirtikar, K. R. and Basu, B.D., (1933): Vol. 11, 2nd ed., 1325, Lalit Mohan, Basu, Leader Road, Allahabad.
Lettre, H., Z. Krebsforsch, 57, 1, (1950).
Majudar, D. N., Indian J. Pharm., 5, 61 (1943).
Morgan, J. F., Mortan, H. J. and Parker, R. C., (1950): proc. Soc. Exp. Biol. Med., 73, 1.
Morgan, J. F., Camphell, M. E. and Mortan, H. J., J. (1955).
Nadkarni, K. N., Indian Materia Medica, 883 Bombay (1927).
Pal, R., Kulshreshtha, D. K. and Rastogi, R. P., J. Pharma. Sc., 65 (6), 918 (1976).
Penso, G. and Balducci, D., (1963): Tissue culture in Biological Research, Elsevier Publishing Company, Amsterdam, London, New York.
Soliman, J. and Barlow, O. W., (1926): "J. Pharmacol. Exp. There", 27, 236.

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