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EMBRYOTOXIC EFFECT OF MELILOTUS SICULUS (Turra) IN RATES

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SUMMARY

The present work was conducted to study the embryotoxic effects of meliletus siculus seed extract. Examination of both uterine horns on the 20th day of gestation, after administration of the extract in doses of 50 and 100 mg/100 g b.wt. revealed foetal resorption of 50% and 65.93% respectively. Foetal weigt and foetal length were both reduced to 25.3±0.70 mm and 23.65 ±0.08 mm, and 3.77±0.10 g and 2.92 ± 0.15g respectively, compared to the control group.

Skeletal examination showed the following malformations: Skull, 10% and 13.3% Vertebral column, 16.6% and 8.3%; Ribs, 13.3% and 25%; Sternebrae, 8.3% and 10%; Limbs, 53.3% and 66.6 in dams receving 50 & 100mg respectively.

The respective visceral malformation were: Brain malformation, 10% and 12.5%; Palate, 7.5% and 15%; Heart, 12.5% and 22.5%; Lung, 0% and 0%: Liver, 31.25% and 37.5%, Kidney, 27.5% and 27.5%.

Evaluation of these results showed a definite embryotoxic effect from the alcoholic extract of Melilotus siculus seeds in ratss. it is therefore, suggested that the inclusion of the seeds of Melilotus siculus in feed-stuffs of pregnant animals, should be avoided.

INTRODUCTION

Melilotus siculus (Turra) known in arabic as Handaqug" is an annual erect herb belonging to the family leguminosa (subfamily papilionoidiae). It was found to grow in arab contries. Ibn El-bitar (1980) & El-Antaki (1923), reported that Handaqug was used in the treatment of skin diseases and rheumatic pains. Biely & Kitt (1965) mentioned that the herb had a diuretic action and was used as emmeragouge, they mentioned also that the seeds had aphrodisia effect. The aqueous extract of leaves and seeds cured feverish patients and used in treatment of scorpion bite.

Lack of knowledge regarding the teratogenic effect of Melilotus siculus suggests its investigation. The purpose of this present work is therefore to study the embryotoxic effects of melilotus siculus on albino wistar rats.

MATERIAL AND METHODS

Melilotus siculus seeds were direct, finaly powdered and their alcoholic extract prepared by continous extract of the powder in a soxhlet apparatus.

As the requiered dose of the extract in animals is relatively large, one intermediate dose of 50 and a higher dose of 100 g b.wt. were used. The techniques used in this work, are based on the principles for testing drugs for teratogenicity published by the W.H.O. (1967).

Virgin female Wistar rats, ranging from 90-100 days old and weighing 150-200 g were selected in the pro-oestrous phase, as seen by vaginal smear examiantion, and were placed overnight with male Wistar rats. The presence of free spermatozoa, in a smear of the vaginal contents in the morning indicated day 0 of pregnancy.

The pregnant animals were divided into 3 groups, each of 50 animals. Each animal in each group received a dose of Melilotus siculus seed extract, administered orally by a metal stomach tube, at 12.00 A.M. every day, from the 6th to the 15th day of gestation. The control animal received a

dose of saline by the same route. All animals were fed on adequate breeding diet. Pregnant females were sacrificed by cervical dislocation of the 20th day of gestation.

The uterus was opened and foetal swellings and sites of resorption in both uterine horns were carefully counted. Living foetuses were freed from surrounding membranes and inspected for external anomalies. Half of the foetuses were than placed in Bouin's fluid for subsequent measuring and sectioning for visceral examination (Wilson 1965). The others were placed in 95% ethyl alcohol for further clearing procedures and staining of skeletal elements with alizarin red (Dawson, 1926; Kotb, 1973).

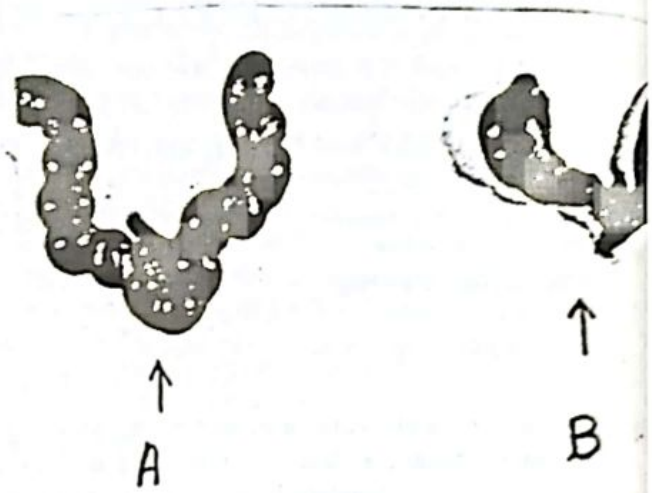

Fig 1: Rat uterus showing foetal resorption after oral administration of alcoholic extract of Melilotus siculus (100 mg/gm b.wt.) to pregnant rats daily from the 6th to the 15th day of gestation (control: left side).

RESULTS AND DISCUSSION

Teratogenicity of the alcoholic extract of melilotus siculus seeds was determined in this investigation by examination of morphological, visceral and skeletal malformations of 20 day old foetuses following oral administration of the extract to pregnant rats between the 6th and the 15th day of gestation.

The alcoholic extract of melilotus siculus seeds in doses of 50 and 100 mg/100 g b.wt., caused foetal resorption of 20% and 25.28% respectively, and a foetal mortality of 50% and 65.9% respectively. The results for resorption, mortality and viability of the foetuses were significant at $P<0.05$.(Fig. 1). Investigations by Nicholson (1954) revealed that a daily ration of 100 g of Melilotus siculus seeds produced poisoning in sheep. Symptoms of acute poisoning were; inappetance, hyperpyrexia, dyspnoea, trembling, staggering and haematuria, usually followed by death. moreover, Nowaski and Wezyk (1960) reported on melilotus siculus

Table (1): Morphological malformations in rat embryos after oral administration of the alcoholic extract of Melilotus siculus seeds to pregnant rats.

Drug	Dose mg/ 100 g body weight	Number of implantation sites	Foetuses						Foetal weight in gms Mean±S.E.	Foetal length in mm Mean±S.E.
			Resorbed		Dead		Viable			
			No.	%	No.	%	No.	%		
Control	--	180	10	5.56	5	2.78	165	91.66	3.88±0.75	28.8±0.43
Alchoholic Extract of Melitotus siculus seeds	50	200	40	20	100	50	60	30	3.77±0.10	25.8±0.70
	100	182	46	25.28	120	65.93	16	8.79	2.92±0.15	23.65±0.08

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)
The figures for resorbed, dead and viable foetuses were not significant at $P < 0.05$

poisoning in horses. Similarly, marchewski. (1955) reported on an outbreak of Melilotus siculus poisoning in pigs.

In the the current investigation, the length and body weight of examined foetuses were significantly decreases, as their mean values were 25.3±0.70 mm and 23.65±0.08 mm, and 3.77± 0.10g and 2.92±0.15g respectively, compared to 28.8±0.43mm and 3.88±0.75g in the control group (Table 1).

Oral adminstration of the alcoholic extract of Melilotus siculus seeds to pregnant rats produced the following visceral organ malformations: 12.5% and 27.5% cardiomegaly and petechiae in the heart; 31.25% and 37.5% hepatic demage; 27.5% and 27.5% petechiae of the renal pelvis respectively. (Table 2) (Fig. 2).

Supportive literature published by Imara (1952), said that Melilotus siculus especially the yellow ones, are very toxic to sheep and other animals, and that the toxic principle has certain effects on the heart and liver, Khoter (1960) stated that acute poisoning, with nervous sypmptoms affected 300 out of 389 sheep 3 days after feeding on Melilotus siculus that had been stored in a rick and threshed before feeding. Fifty-six animals died after an illness lasting 2 days. Petechiae were observed in the heart, abomasum, intestine and kidney. The liver was yellow with red spots.

The skeletal malformations recorded were; incomplete ossification of the skull, 10% and 13.3%; absence of one or more of the coccygeal vertebrae, 16.63% and 8.3%; absence of sternebrae 8.3% and 10%; absence of some metatarsal and metacarpal bones 53.3% and 66.6% for dams receving 50 & 100 mg respectively (Table 3) and (Fig. 3).

In conclusion it is advisable to exclude the seeds of Melilotus siculus from the feeds of pregnant

Fig 2: Rat liver showing foetal necrosis of cell liver and loss architecture of liver cells of rat, Electromicrograph (x 600).

Table (2): Visceral malformations in rat embryos after oral administration of the alcoholic extract of Melilotus siculus seeds to pregnant rats.

Drug	Dose mg/ 100 g body weight	Number of foetuses examined	Malformations											
			Brain		Palate		Heart		Lung		Liver		Kidney	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	--	80	--	--	--	--	--	--	--	--	--	--	--	--
Alchoholic Extract of Melitotus siculus seeds	50	80	8	10	6	7.5	10	12.5	0	0	25	31.25	22	27.5
	100	80	10	12.5	12	15	18	22.5	0	0	30	37.5	22	27.5

Table (3) : Skeletal malformations in rat embryos after oral administration of the alchoholic extract of Melilotus siculus seeds to pregnant rats.

Drug	Dose mg/ 100 g body weight	Malformations										
		Number of foetuses examined	Skull		Vertebral column		Ribs		Sternebrae		Limbs	
			No.	%	No.	%	No.	%	No.	%	No.	%
Control	--	60	--	--	--	--	--	--	--	--	--	--
Alchoholic Extract of Melitotus siculus seeds	50	60	6	10	10	16.6	8	13.3	5	8.3	32	53.3
	100	60	8	13.3	5	8.3	15	25	6	10	40	66.6

Fig 3: Absence of some coccygeal uertebrae and some phalonges after oral administration of alcoholic extract of Melilotus siculus (100 mg/gm b.wt.) to pregnant rats daily from 6th to the 15th of gestation (control, right side

animals. This investigation was carried out on rats, but it must be assumed in the absence of published literature, that the effects would be similar in other mammals.

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