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Cytogenetic Studies on Some Native Egyptian Plants in Male Rats

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Abstract: The valuable use of medicinal plants in Egyptian folk medicine long time ago has encouraged researchers to solve some of health problems. Lotus (Fam. Fabaceae) and Deutzia (Fam. Saxifragaceae) species have been reported to improve fertility of female mammals through hormonal like effect (Deutzia) or due to phenolic constitutents (Lotus), however, no attention was paid to investigate their possible effects in males. In the present study, these two plant species which are growing in Egypt (*Lotus corniculatus* and *Deutzia scabra*) were investigated in males with the objective of developing potential non traditional safe food source with the minimal genotoxic effect. A total number of 50 male rats was treated with *L. corniculatus* at doses of 160 and 320 mg/100g.b.wt and *Deutzia scabra* at doses of 80 and 160 mg/g.b.wt for 30 and 60 days to study the effect on the bone marrow cells and spermatocyts. Results showed that the used dose and durations of both *L. corniculatus* and *D. scabra* are safe whereas they did not induce mutagenitic activity in rat bone marrow cells or spermatocytes. It was concluded that oral administration of *Lotus cornicuatus* and *Deutzia scabra* native Egyptian plants did not have any genotoxic activity on somatic and germ cells and they can be added in the ration of farm animals without any hazardous effect.

Key word: Lotus · Deutzia · Chromosome · Bone marrow · Spermatocytes · Rats

INTRODUCTION

Herbal medicine has been used throughout history, but has recently undergone a renaissance, despite the first documented use of medicinal plants was found in early Egyptian and Asian cultures.

More than two thousands species of medicinal and aromatic plants are growing in Egypt, which create a base of raw materials exploited for drug production [1,2].

The valuable use of medicinal plants in Egypt in folk medicine has encouraged the researchers to solve some of the health problems including infertility [3-5].

Lotus species (*L. reticus*) have a depressant effect on the central nervous system, while some of their constituents are used as contraceptive, for treatment of alimentary tract disorders and gastric ulcers (*L. tetragonolobus*). Temperate forages such as *L. corniculatus* and *L. pedunclatu* containing condensed tannins and have nutritional benefits including improvements in weight, wool growth, milk production and composition, ovulation rate and increased efficiency of feed utilization [6,7].

The present study was designed to investigate the possibility of using some native Egyptian plants as non traditional feed additives from the cytogenetic point of view.

MATERIAL AND METHODS

Plant Material: *L. corniculatus* aerial parts were collected from canal banks, Fayoum governorate, Egypt. *D. scabra* leaves were collected from Giza Zoo and kindly indentified at Department of Photochemistry and Plant Systematic, National Research Centre (NRC), Dokki, Egypt. A voucher specimen has been deposited at the herbarium of the NCR. The material was separated by plant parts, dried (~ 45°C) and ground.

Extraction: The ground aerial parts (1 kg) of *L. corniculatus* and *D. scabra* (500 g) were exhaustively extracted at room temperature with ethanol-water (3:1) mixture [8,9].

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Table 1: Exploratory trials for determination of tolerated Dose of 70% ethanolic extract of Lotus corniculatus

Groups	Dose mg/100 g b.wt.	No. of dead animals
0	0	0
1	50	0
2	100	0
3	200	0
4	400	0
5	800	0
6	1600	0
7	3200	0

Table 2: Exploratory trials for determination of tolerated Dose of 70% ethanolic extract of Deutzia scabra

Groups	Dose mg/100 g b.wt.	No. of dead animals
0	0	0
1	50	0
2	100	0
3	200	0
4	400	0
5	800	0
6	1600	0

Determination of the Maximum Tolerated Dose: Tolerated dose of ethanol extracts were determined as described by Carrol [10]. For this purpose, eight groups of each 5 male Albino rats weighing 120-140g., were used for each dose. The required doses of tested ethanolic extracts of *Lotus Cornicuatus* and *Deutzia Scabra* (Tables 1 and 2) were administered orally with a glass syringe fitted with a feeding needle. All groups of rats were kept under observation for 24 hours.

Experimental Animals: A total number of 50 healthy male rats, (*Rattus rattus*), 3-5 months old and weighing between 175-250g, was obtained from the Animal House, National Research Center. Rats were housed in steel cages and maintained under standard conditions of light with free access to standard diet and tap water.

Treatment Protocol: Rats were divided into five groups, each of 10 animals.

- Group 1: included 10 rats given vehicle (sterile distilled water) alone for 60 days to serve as vehicle treated control.
- Group 2: included 5 rats given *L. corniculatus* extract at the dose of 160 mg/g body weight for 30 days and another 5 rats given the same dose for 60 days.
- Group 3: included 5 rats given *L. corniculatus* extract at the dose of 320 mg/g body weight, for 30 days and another 5 rats given the same dose for 60 days.
- Group 4: included 5 rats given Deutzia extract at the dose of 80 mg/g body weight, for 30 days and another 5 rats given the same dose for 60 days.
- Group 5: included 5 rats given Deutzia extract at the dose of 160 mg/100 g body b.wt for 30 days and another 5 rats given the same dose for 60 days.

Scarification Schedule: Scarification was done 30 and 60 days after administration. Rats were sacrificed under light ether anesthesia, the testes and bone marrow were obtained to carry out cytogenetic studies.

Genotoxic Study: Bone marrow (Somatic cells) and spermatocytes (Germ Cells) chromosomes from rats of all groups were prepared according to methods of Yosida and Amona [11] and Evans [12], respectively. Slides were stained with 70% Gimsa stain and microscopically examined.

Statistical Analysis: Data are expressed as mean ±S.E and analyzed for statistical significance using the procedure of Mitron and Tsokos [13].

RESULTS

Genotoxic Effect of *Loutus corniculatus* and *Deutzia scabra*: Tables 3 and 4 show the effect of oral administration of tested extracts of *Loutus corniculatus* (160 and 320 mg/100g b.wt) and *Deutzia scabra (*80 and 160 mg/ 100g b.wt*)* in male rats for 30 or 60 days, respectively. Results indicated that both plants are safe whereas, they induce neither toxic nor lethal effects.

Bone Marrow Cells: The observed structural abnormalities in bone marrow cells in treated rats were illustrated in tables 3 and 4. Both plants induced no significant structural aberrations (gap, break, deletion, centromeric attenuation and endomitosis) in bone marrow as compared with the control group.

Spermatocytes: Tables 5 and 6 show the incidence of abnormalities detected in spermatocytes of rats administered *L. conrniculatus* and *D. Scabra* For 30 or 60 days. It was noted that all types of meiotic aberrations (X-Y univalent, autosomal univalent and chain aberrations) are not significantly differ in treated and non treated rats.

Table 3: Effect of 160 and 320 mg/ 100g.wt. Of 70% ethanolic extract of L. corniculatus on rats bone marrow

		Structural aberrations						
Treatments		Normal	Gap	Break	Deletion	C.A	Endomitosis	Total aberration
Interaction	T1×Cont.	94.00±0.89	0.80±0.49	0.40±0.40	2.00±0.00	1.60±0.40	1.20±0.49	6.00±0.89
	T1×L	91.60±0.75	1.20±0.49	0.80±0.49	2.00±0.00	3.20±0.49	1.20±0.49	8.40±0.75
	T1×H	90.80±0.49	0.40±0.40	0.40±0.40	2.40±0.40	4.00±0.00	2.00±0.00	9.20±0.49
	T2×Cont.	94.00±0.89	0.80±0.49	0.40±0.40	2.00±0.00	1.60±0.40	1.20±0.49	6.00±0.89
	T2×L	90.80±0.49	0.40±0.40	0.40±0.40	1.60±0.40	4.00±0.00	2.80±0.49	9.20±0.49
	T2×H	88.80±0.80	0.00±0.00	0.80±0.49	2.40±0.40	4.80±0.49	3.20±0.49	11.20±0.80
LSD at 5%		N.S	N.S	N.S	N.S	N.S	N.S	N.S

T1: After the tirst month T2: After the Second month
L: Low doses (160 and 80mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
H: High doses (320 and 160 mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
Con: control group
N.S: Non-Significant

Table 4: Effect of 80 and 160 mg/ 100g.b.wt. of 70% ethanolic extract of D. scabra on rats bone marrow

		Structural aberrations						
Treatments		Normal	Gap	Break	Deletion	C.A	Endomitosis	Total aberration
Interaction	T1×Cont.	94.00±0.89	0.80±0.49	0.40±0.40	2.00±0.00	1.60±0.40	1.20±0.49	6.00±0.89
	T1×L	92.40±0.75	1.20±0.49	0.80±0.49	2.00±0.00	2.40±0.40	1.20±0.49	7.60±0.75
	T1×H	91.60±0.75	1.20±0.49	0.80±0.49	2.00±0.00	3.20±0.49	1.20±0.49	8.40±0.75
	T2×Cont.	94.00±0.89	0.80±0.49	0.40±0.40	2.00±0.00	1.60±0.40	1.20±0.49	6.00±0.89
	T2×L	90.40±0.98	1.20±0.49	1.20±0.49	2.00±0.00	3.60±0.40	1.60±0.40	9.60±0.98
	T2×H	90.00±0.63	0.80±0.49	1.20±0.49	2.40±0.40	4.00±0.00	1.60±0.40	10.00±0.63
LSD at 5%		N.S	N.S	N.S	N.S	N.S	N.S	N.S

T1: After the tirst month T2: After the Second month
L: Low doses (160 and 80mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
H: High doses (320 and 160 mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
Con: control group
N.S: Non-Significant

Table 5: Effect of 160 and 320 mg/ 100g.b.wt. of 70% ethanolic extract of L. corniculatus on rats bone spermatocytes

		Structural aberrations				
Treatments		Normal	Chain	Autosomal Univalent	Univalent X-Y	Total aberration
Interaction	T1×Cont.	96.40±0.40	0.00±0.00	1.20±0.49	2.40±0.40	3.60±0.40
	T1×L	95.60±0.40	0.40±0.40	1.20±0.49	2.80±0.49	4.40±0.40
	T1×H	94.80±0.80	0.40±0.40	2.00±0.00	2.80±0.49	5.20±0.80
	T2×Cont.	96.40±0.40	0.00±0.00	1.20±0.49	2.40±0.40	3.60±0.40
	T2×L	93.60±0.40	0.40±0.40	2.40±0.40	3.60±0.40	6.40±0.40
	T2×H	92.80±0.49	0.80±0.49	2.80±0.49	3.60±0.40	7.20±0.49
LSD at %		N.S	N.S	N.S	N.S	N.S

T1: After the tirst month T2: After the Second month
L: Low doses (160 and 80mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
H: High doses (320 and 160 mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
Con: control group
N.S: Non-Significant

Table 6: Effect of 80 and 160 mg/ 100g.b.wt. of 70% ethanolic extract of D. scabra on rats spermatocyte

		Structural aberrations				
Treatments		Normal	Chain	Autosomal Univalent	Univalent X-Y	Total aberration
Interaction	T1×Cont.	96.40±0.40	0.00±0.00	1.20±0.49	2.40±0.40	3.60±0.40
	T1×L	96.00±0.00	0.00±0.00	1.60±0.40	2.40±0.40	4.00±0.00
	T1×H	95.20±0.49	0.40±0.40	1.20±0.49	3.20±0.49	4.80±0.49
	T2×Cont.	96.40±0.40	0.00±0.00	1.20±0.49	2.40±0.40	3.60±0.40
	T2×L	94.00±0.00	0.40±0.40	2.00±0.00	3.60±0.40	6.00±0.00
	T2×H	94.00±0.63	0.40±0.40	2.00±0.00	3.60±0.40	6.00±0.63
LSD at 5%		N.S	N.S	N.S	N.S	N.S

T1: After the tirst month T2: After the Second month
L: Low doses (160 and 80mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
H: High doses (320 and 160 mg/100g.b.wt for *L. corniculatus* and *D.* Scabra respectively).
Con: control group
N.S: Non-Significant

DISCUSSION

In all countries, plants are used as traditional health nutritive agent in both man and animals. Moreover, some plants are known to have the ability to prevent some diseases.

Due to shortage of animal feeds, non traditional sources of feeds must be tried, but such sources should be of considerable nutritive value and safe.

The present investigations were carried out on two plant species that growing in Egypt (*Lotus corniculatus* and *Deutzia scabra*). *L. corniculatus* is perennial herb growing in Egypt (Nile bank, delta and wetlands) and Tropical Africa, while. *Deutria scabra* is strong shrub growing in Asia and Himalayan region [7].

The present results indicated that ethonalic extracts of *L. corniculatus* and *D. scabra* are safe whereas, they did not induce mutagenic activity in bone marrow cells or spermatocytes of rats. Similar results were obtained by Kadry *et al.* [14].

L. corniculatus and *D.scabra* contain several beneficial compounds, the efficacy of these compounds was related to the duration and the dose of administration, whereas for *L. corniculatus*, the maximum response occurred after oral administration for 60 days with dose of 320 mg/ 100g body weight. Possible reasons for this result may be related to the phenolic contents in *L. corniculatus* which have the ability to complex with proteins [15]. Moreover, it was reported that temperate forages including *L. corniculatus* and *L. pedunclatu* containing condensed tannins which induce nutritional benefits in the form of improved live body weight, wool growth, milk production and composition, ovulation rate as well as efficiency of feed utilization [6].

In conclusion, ethanolic extracts of *L. corniculatus* and *D. scabra*, orally administered for 30 or 60 days in rats induce no hazardous effect as monitored by the incidence of chromosomal aberration in somatic and gem cells. These beneficial results encourage the addition of these native Egyptian plants in the ration of farm animals.

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