

EFFECT OF ETHANOLIC DATURA LEAVES EXTRACT ON STERILANT OF WHITE NORWAY RAT, *RATTUS NORVEGICUS*

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ABSTRACT: *The effect of ethanolic Datura leave extract, Datura stramonium, was studied on the fertility of albino rat males and teratogenic effect on the pregnant female of white Norway rat, Rattus norvegicus. Results showed that LD₅₀ values were 116.70 mg/kg body weight. The results cleared that the treatment of male albino rats with sub-lethal doses (¹/₁₀ and ¹/₄ LD₅₀) of tested extract decreased the pregnancy rate and also reduced the weight of males testes. Also, the obtained data from the treated pregnant female rats with the same sub-lethal doses have potential harmful effects on the fetuses in addition to fetal retardation of embryonic formation and development inside the uterus of the pregnant females especially in the first week followed by the second and third weeks. The resorption effects were observed inside the uterus of the pregnant female when compared with those of untreated pregnant females. Also, the tested plant extract reduced the number of newly born individuals and the weight of fetuses.*

Key words: *Datura extract – sterilant- Rattus norvegicus.*

INTRODUCTION

In recent years, the emphasis on plant protection has definitely shifted from the dominant chemical pesticides to the integrated pest management (IPM), with a focus on biological control and other natural resources (Schmutterer, 1981). Many investigators suggested that the basic research must be directed to the discovery of new safe types of pest control agents in order to ensure high crop production (Afifi *et al.*, 1992; Oji *et al.*, 1994; Mourad 2010 and Abou- Hashem, 2012).

Plant extracts have been used as pesticides by humans since the time of the ancient romans, a practice that continues to the present time using many plant species known to have pesticidal properties (Fellows, 1979). The use of toxic plants is especially prevalent in the developing countries, where plants grown locally are cheaper than the synthetic chemical pesticides (EL-Gengaihi *et al.*, 1997).

Therefore, many plants, trees, shrubs, annuals, either wild or cultivated were surveyed for their plant protection activity. Many of them have been proved to be active (Hussein, 1991, Muley *et al.*, 2009, Stanic *et al.*, 2009 and Matic *et al.*, 2011).

Datura plants contain toxic Tropane alkaloids, which have caused poisoning and death in humans and animals (Cheeke and Shull, 1985). All parts of Datura plants contain dangerous levels of poison and may be fatal if ingested by humans or animals. The active ingredient are the Tropane alkaloids atropine, hyoscyamine and scopolamine which are classified as deliriant or anticholinergies (Preissel and Hans-Georg, 2002 and Tannis *et al.*, 2008).

The fertility of males rats and the weight of testes recorded as signs of the chemosterilant effect of any chemical compound (drug) (Ghanem *et al.*, 1995; and Mourad and Khidr, 2011). The increase in the resorption rate, the decrease in the litter size and the reduction in foetal weight are evidences for the teratogenic effects of any drug on female rats (Kim *et al.*, 2000).

The present work aims to study the effect of ethanolic Datura leaves extract as a sterilant for albino rats under laboratory conditions. .

MATERIALS AND METHODS

1- Tested plant:

The Datura plant leaves, *Datura stramonium* (Fam. : solanaceae) used in this

study were collected from Helwan district, Cairo Governorate. The plants were identified by the Department of Timber-trees Research, Horticultural Research Institute, Agricultural Research Center, Giza.

2- Plant preparation and extraction:

Leaves were collected and cleaned from dust debris. Clean leaves were dried under room temperature of $28 \pm 1^\circ\text{C}$ for at least one week. The dry leaves were ground in a grinder to a coarse powder. Extraction was carried out according to the method adopted by Freedman *et al.* (1979) with minor modification (where ground leaves were soaked in the chosen solvent instead of using Soxhlet procedure). Then 150g of plant material were extracted with about 750ml of ethyl alcohol 95%.

3- Tested animal:

The adult males, females and pregnant albino Norway rats, *Rattus norvegicus* (170-200 grams body weight) were used for laboratory experiments. Animals were caged individually and offered standard diet (65% crushed maize + 25% ground wheat + 5% sugar+ 5% corn oil) for a minimum of 15 days before the test and allowed free access to water. The unhealthy animals were excluded.

4- LD₅₀ determination:

The test was carried out with individually caged Albino rat, *Rattus norvegicus*. Ten rats were orally administrated with the tested dosages of 40, 60, 80, 100 and 150 mg/kg body weight from ethanolic Datura extract. Mortality percentages were recorded and LD₅₀ values were calculated by the moving-average interpolation method using standard tables and simplified formula given by Horn (1956).

5- Effect of Datura leaves extract on the fertility of albino rat males:

This test was carried out in individually caged males of albino rat. Five males were

used for each treatment, plus another group left without treatment as a control. The tested males were starved for 12 hrs., then the rats were orally administrated with 1/10 and 1/4 LD₅₀ from ethanolic Datura extract. After each treatment, normal feeding of rats was continued. The male rats of each treatment were kept with mature females at a rate of 1 male (treated): 3 females (untreated) for 7 days, then removed to investigate the fertility of the treated male rats. Then males autopsied and the testes were weighed and compared with the control group. The females were left individually caged for 25 days, then the pregnant females were counted and the following fertility parameters were calculated as:

$$\text{Pregnancy\%} = \frac{\text{Number of pregnant females}}{\text{Total number of females}} \times 100$$

6- Teratogenic effect of Datura leaves extract on pregnant female rats:

The female which was in estrous phase, vaginal smear was taken to ensure the mating, if the sperms were present, this means that the gestation period had begun (Zero day) (Sherif *et al.*, 1989). Regular vaginal smear examination was done daily for about 6 days to follow up the pregnancy. The pregnant females of albino rats were divided into three groups as each group divided into 1st, 2nd and 3rd weeks of the gestation period. Thirty pregnant females rats were used for each treatment. The tested rats were individually caged and the dosages were given as orally administrated at 1/10 and 1/4 LD₅₀ from ethanolic Datura extract. The time of dosing was 2nd day during the 1st week of pregnancy, 9th day for the 2nd week of pregnancy and 16th day for the 3rd week of pregnancy. Control was concurrently conducted. The tested pregnant females were observed until birth. Number of newly born individuals, weight of fetuses, the gestation period and the number of delivered, resorbed and aborted females were recorded and compared with the control group.

RESULTS AND DISCUSSION

1- Toxicity of ethanolic Datura leaves extract to *Rattus norvegicus*:

The mortality percents and LD₅₀ of the tested plant extract, *Datura stramonium*, administered to white Norway rats, *Rattus norvegicus* are recorded in Table (1). From this table it was found that, only 10% mortality were observed within the group given 40 mg/kg body weight. The mortality percents increased with the increase of the tested doses, as follows: 60, 80, 100 and 150 mg/kg body weight caused 20, 30, 40 and 70% mortalities, respectively. The LD₅₀ and LD₉₀ values were 116.70 and 315.29 mg/kg b.w., and the slope of regression line was 2.67. Thus, ethanolic Datura extract could be considered as a moderately toxic compound against rats according to Loomis (1976). From previous results, it can be proposed that the ethanolic Datura leaves extract can be used as a useful potent rodenticidal baits for controlling rodents.

2- Effect of Datura leaves extract on the fertility of male albino rats:

Data presented in Table (2) showed that control male was able to mate the three-housed females, which became all pregnant after mating. Treatment of male albino rats with tested plant extract decreased the pregnancy rat. Minimal percent of pregnancy was noticed in rats treated with 1/4 LD₅₀ of ethanolic Datura extract showing 20.0% pregnancy. On the other hand, maximal pregnancy percent was obtained in females mated by males treated with 1/10 LD₅₀ of ethanolic Datura extract as 53.33% pregnancy. Testes of untreated male weighed 1.21g and obviously reduced to be 0.94 and 0.77g with 1/10 and 1/4 LD₅₀ from ethanolic Datura extract, respectively.

All treated males had epididymal lesions which blocking the transfer of sperms from testis to the epididymis, this may be the reason of reducing fertility of treated males with tested plant extract.

These results are in agree with those obtained by Prakash *et al.* (1985), Lohiya *et al.* (1994), Chaturvedi *et al.* (2003) and

Mourad and Kidr (2011). Whereas, Datura plant induces antiandrogenic and reversible infertility in male albino rats.

3- Teratogenic effect of Dature leaves extract on pregnancy and embryotoxicity:

The effect of administration sublethal doses 1/10 and 1/4 LD₅₀ of ethanolic Datura extract an albino rat females pregnancy and their embryotoxicity are shown in Tables (3 and 4).

Examination of tabulated data, showed that, the average number of untreated delivery females was 10 (100% pregnancy) gave 8 newly born with average weight of 5.75g during 21 days of gestation period.

The delivery females were reduced to 7, 8 and 80% by the treatment with 1/10 LD₅₀, 20, 30 and 40% by the treatment with 1/4 LD₅₀ during 1st, 2nd and 3rd week of pregnancy, respectively. Whereas, the maximum embryos resorption were occurred in case of 1/4 LD₅₀ with 70% resorption during 1st and 2nd week of pregnancy followed by 3rd week with 60% resorption. But the only abortion was occurred in case of 1/4 LD₅₀ with 10% abortion during 1st week of pregnancy.

The effect of tested plant extract were obvious on the number of newly born individuals, which reduced the newly born from 8 individuals in untreated females to 3.60, 4.20 and 6.40 individuals by the treatment with 1/10 LD₅₀, and to 2.0, 3.20 and 4.40 individuals by the treatment with 1/4 LD₅₀ during 1st, 2nd and 3rd week of gestation period, respectively.

In the same time, weight of fetuses was reduced to 2.80, 3.50 and 4.0g by the treatment with 1/10 LD₅₀, and to 1.40, 2.0 and 2.55g the treatment with 1/4 LD₅₀ during 1st, 2nd and 3rd week of gestation period, respectively.

The tested plant extract elongated the gestation period as compared with control (21 days).

From the previously mentioned results, it could be concluded that the Datura plant extract have potential harmful effects on the fetuses in addition to fetal retardation of embryonic formation and development

inside the uterus of the pregnant females especially in the 1st week followed by the 2nd and 3rd weeks. Also, the tested plant extract reduced the number of newly born individuals and the weight of fetuses.

These findings are in agree with those obtained by Hussein (1991), Hiremath *et al.* (1996), Desheesh *et al.* (1997) and Tannis *et al.* (2008).

In summarizing, it should be emphasized that chemosterilants for regulating rodent populations are in the initial stages of development, and only after much intensified research will they fulfill our awaiting needs. Greater insight into rodent chemosterilants and their efficacy in the environment must be attained before we can

employ them in rodent control practices. Most encouraging, however, is the current development of information on all aspects of rodent chemosterilants, especially practical application. This relatively new approach to a safer and more effective rodent control, while no panacea, will greatly broaden our present technology. Perhaps the greatest importance of chemosterilants is that they will reduce the need for lethal rodenticides, an especially worthy goal now that concern over environmental contamination is at an all-time high. Chemosterilants will change the emphasis from increased mortality to reduced natality for regulating population densities. This is supported by sound biological principles.

Table (1): Toxic effect of Datura Leaves ethanolic extract against albino rat, *Rattus norvegicus*.

Dose (mg/kg b.w.)	Mortality %	LD ₅₀ (mg/kg b.w.)	LD ₉₀ (mg/kg b.w.)	Slope of regression line
40	10	116.70	315.29	2.67
60	20			
80	30			
100	40			
150	70			

Table (2): Effect of Datura leaves ethanolic extract on the fertility of male albino rat, *Rattus norvegicus*.

Dose	Ratio of male : female	No. of pregnant females	Pregnancy %	Av. Wt. of testes (g)
$\frac{1}{10}$ LD ₅₀	1 : 3	8	53.33	0.94
$\frac{1}{4}$ LD ₅₀	1 : 3	3	20.0	0.77
Control	1 : 3	15	100.0	1.21

(5Males: 15 Females used for each treatment)

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Table (3): Effect of Datura leaves ethanolic extract on pregnant female albino rat, *Rattus norvegicus* during gestation period.

Dose	Gestation period	Stages of pregnancy					
		Delivered		Resorbed		Aborted	
		No.	%	No.	%	No.	%
1/10 LD ₅₀	1 st week	7	70	3	30	0	0
	2 nd week	8	80	2	20	0	0
	3 rd week	8	80	2	20	0	0
1/4 LD ₅₀	1 st week	2	20	7	70	1	10
	2 nd week	3	30	7	70	0	0
	3 rd week	4	40	6	60	0	0
Control	1 st week	10	100	0	0	0	0
	2 nd week	10	100	0	0	0	0
	3 rd week	10	100	0	0	0	0

Table (4): Effect of Datura leaves ethanolic extract on the embryos development during the gestation period.

Dose	Gestation period	Av. of gestation period (day)	Av. No. of newly born individuals	Av. Wt. of Fetuses (g)
1/10 LD ₅₀	1 st week	23.0	3.60	2.80
	2 nd week	23.0	4.20	3.50
	3 rd week	22.0	6.40	4.00
1/4 LD ₅₀	1 st week	24.0	2.00	1.40
	2 nd week	23.0	3.20	2.00
	3 rd week	22.0	4.40	2.55
Control	1 st week	21.0	8.0	5.75
	2 nd week	21.0	8.0	5.75
	3 rd week	21.0	8.0	5.75

REFERENCES

Abou- Hashem, A.A.M. (2012). Evaluation of the rodenticidal effects of some plant extracts under laboratory and field conditions. *The Journal of Basic & Applied zoology*, 65: 282- 288.

Afifi, F.A., A.A. Gomaa, Z.H. Zidan, E.Z. Fam and M.S. Salwa Ahmed (1992). Preliminary evaluation of chemical

constituents in certain bioactive plant extracts. *Arab Univ. J. Agric. Sci., Ain-Shams Univ., Cairo*, 1 (1):97- 111.

Chaturvedi, M., P. C. Mali and A. S. Ansari (2003). Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* schard fruits in male rats. *Pharmacology*, 88 (1): 38-48.

- Cheeke, P.R. and L.R. Shull (1985). Natural toxicants in feeds and poisonous plants. AVI Publishing Company, Inc., Westport, Conn., USA 429 PP.
- Desheesh, M.A., A.H. Mansee and F.A. Kassem (1997). Identification of cardenolide uscharin from *Calotropis procera* plant and their rodenticidal activities. Alex. J. Agric. Res., 42 (3): 227- 238.
- El- Gengaihi, S.E., N.Z. Dimetry, S.M. Mohamed (1997). Chemical and biological investigation of harmful plant. 2- Alkaloidal investigation. J. Appl. Entomol. 12 (3): 165- 167.
- Fellows, L.E. (1979). Plant secondary compounds as a chemical defense. In: Wright, E.N. (Ed.), Bird Problems in Agriculture. pp. 188- 195.
- Freedman, B., L.J. Nowak, W.F. Ewolek, E.C. Berry and W.D. Guthrie (1979). A bioassay for plant- derived pest control agents using the European corn borer. J. Econ. Entomol., 72 (4): 541- 545.
- Ghanem, N. F., S. I. Attia, A. M. Rizk and M. H. Shwaireb (1995). Effect on antithyroid drug carbimazole on the fertility and testicular structure of BALB/ C mice. J. Egypt. Ger. Soc. Zool., 18 (c): 1-12.
- Hiremath, S.P., H.K.S. Swamy, S.M. Bandami, S.B. Pati and R.L. Londonkar (1996). Post coital antifertility activity of the plant *Sriga densiflora* (Scrophulariaceae) on female albino rats. In. J. pharm., 34 (1): 84- 92.
- Horn, H.J. (1956). Simplified LC₅₀ (or ED₅₀) calculations Biometreics. 12: 311- 322.
- Hussein, H.I. (1991). Rodenticidal properties of cardenolides crude extracts isolated from Sodom Apple *Calotropis procera*. ph. D. Thesis, Fac., Agric. Alexandria Univ., 160 pp.
- Kim, J. C., H. I. Yun, H. C. Shin, S. S Han and M. K. Chung (2000). Embryo Lethality and teratogenicity of a new fluoroquinolone antibacterial DW-116 in rats. Arch. Toxicol., 74 (2): 120-124.
- Lohiya, N. K., R. B. Goyal, D. Jayaprakash, A. S. Ansari and S. Sharma (1994). Antifertility effect of aqueous extract of *Carcia papaya* seeds in male rats. Planta. Med., 60: 400-404.
- Loomis, T.A. (1976). Essentials of toxicology. 2nd ed., Lea of Febiger philadelphia.
- Matic, S., S. Snezana, B. Desanka, G. Nevena, V. Melita and M. Mirjana (2011). Genotoxic potential of *Cotinus coggygria* Scop. (Anacardiaceae) stem extract in vivo. Gen. Mol. Biol., 34 (2): 298- 303.
- Mourad, A.A. (2010). Toxicity effect of ethanolic *Eucalyptus* leaves extract on white Norway rat, (*Rattus norvegicus* Var. ALBUS). J. plant prot. and path., Mansoura Univ., 1 (11): 877- 884.
- Mourad, A.A. and F.K. Khidr (2011). Chemosterilant effect of *Citrullus colocynthis* fruits ethanol extract on the male albino rat, *Rattus norvegicus*. J. Egypt. Ger. Soc. Zool., (63c): 23- 31.
- Muley, B. P., S. S. Khadabadi and N. B. Banarase (2009). Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteracea): a review. Trop. J. Pharm. Res., 8 (5): 455-465.
- Oji, O., F. N. Madubuik, P. C. Ojimekwe and C. M. Ibeh (1994). Rodenticide potential of *Thevetia peruviana*. J. Hebs, species and Medical plants, 2: 3, 3-10.
- Prakash, A. O., V. Saxena, S. Shukla, R. K. Tewari,, S. Mathur, A. Gupta, S. Sharma and R. Mathur (1985). Anti-implantation activity of some indigenous plants in rats. Acta Eur. Fertil., 16: 441-448.
- Preissel, U. and P. Hans - Georg (2002). Brugmansia and Datura Angel's Trumpets and Thorn Apples. Buffalo, New York. Firefly Books. PP 124 - 125.
- Schmutterer, H. (1981). Some properties of components of the neem tree (*Azadirachta indica*) and their use in pest control in developing countries. Mededelingen Van de Facultet

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- Landbouwetent, Schappen, coggygia and gallic acid on *Drosophila melanogaster*. Arch. Biol. Sci., 61: 261-266.
- Rijksuniversiteit Gent., 46 (1): 39-47.
- Sherif, R. M., M M. I. Amir, K. A. Gouhar, H. E. Lokma and A. M. Hegab (1989). Teratogenic activity of warfarin on pregnant females of the white rat. Egypt J. Appl. Sci., 4 (1): 219 - 231.
- Tannis, H. W., S. S. Eric and Y. K. Laurence (2008). Angel's Trumpet (*Datura stramonium*) poisoning and delirium in adolescents in Winnipeg, Manitoba: Summer 2006. Paediatr Child Health, 13 (3): 193-196.
- Stanic, S., S. Matic, S. Solujic and T. Milosevic (2009). Genotoxicity testing of the methanol extract of the plant *Cotinus*

تأثير مستخلص أوراق الداتورة الإيثانولي على تعقيم الفأر النرويجي الأبيض

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تم دراسة تأثير مستخلص أوراق الداتورة الإيثانولي على خصوبة ذكور الفئران البيضاء والتأثير التشويهي على الأجنة والإناث الحوامل.

أوضحت النتائج أن التركيز النصفى المميت LD_{50} كان 116.70 مجم/كجم من وزن الجسم. كما أظهرت النتائج أن تأثير معاملة ذكور الفئران البيضاء بمستخلص الداتورة بالجرعتين ($1/10$ و $1/4$ النصف مميتة) أدى إلى انخفاض نسبة معدل الحمل ونقص في وزن خصية الذكور. وأيضا تم دراسة تأثير نفس الجرعتين النصف مميتة من مستخلص أوراق الداتورة الإيثانولي على التأثير التشويهي على الأجنة والإناث الحوامل وقد أكدت كل النتائج التي سجلت نتيجة المعاملة حدوث أضرار بالغة وواضحة على الأجنة حيث أنها سببت إيقاف نمو الأجنة داخل رحم الإناث الحوامل خصوصاً في الأسبوع الأول. كذلك أدى المستخلص النباتي المختبر إلى امتصاص الأجنة داخل رحم الإناث وخفض في نسبة التناسل ووزن الصغار كما أثرت سلبياً على عدد الأفراد الناتجة المولودة وزادت من نسبة الأفراد الميئة.