

Effect of the Glycoside Convicine on Female and Male Albino Rats: Pregnancy and Sperm Quality

Mohamed M. Amin and Mahmoud S. Arbid

Department of Pharmacology, Medical Division, National Research Centre, Giza, Egypt

Abstract: Convicine (CV) is a glycoside isolated from Faba beans (*Vicia faba* L.). It is causing hemolytic crisis called favism especially with young males suffers from a deficiency in glucose-6-phosphate dehydrogenase. In the present study these experiments were conducted to investigate the side effect of CV on the pregnant albino rats and on the sperm quality of male albino rats. The first experiment included four groups of pregnant females were injected with CV in three dose levels (80, 100, 150 mg/100g b.wt, ip) at 12th, 13th and 14th days of gestation. The weight of the alive feti decreased significantly while resorbed feti and abortion percent increased significantly in a dose dependent. The second experiment, offspring's of control and treated pregnant rats (100 mg/100g b.wt, ip) on the day 12th, 13th and 14th days of gestation were weighed weekly. The offsprings weights decreased significantly ($p<0.01$) than control group starting from 1st up to 56th date of birth. The third experiment, the male rats were injected for 60 days with CV (100 mg/100g b.wt, ip) and the results revealed a significant decrease in spermatozoa number. Moreover, there were an increase in the abnormality and mortality percent of spermatozoa, while the weight of male genital organs decreased significantly than control group.

Key words: Convicine • Albino rats • Pregnancy • Sperm quality

INTRODUCTION

Convicine is a glycoside that is found primarily in Faba beans (*Vicia faba* L.) which is one of the most important plant. Pulse crops in the world, being consumed in large quantities in the Middle East, Far East and North Africa particularly in Egypt.

Convicine is a compound which is hydrolysed by the intestinal micro flora [1] to highly reactive free radical generating compound isouramil [2] which have been strongly implicated as the causative agent in favism [3] a hemolytic disease in human's particularly young males that have a deficiency of erythrocytic glucose-6-phosphate dehydrogenase (G-6-PD) activity [4].

The free radical generators may also cause often adverse effects including lipid peroxidation [4] and mitochondrial metabolism [4] and possibly diabetes [5].

This study was conducted to investigate the effect of convicine on the pregnant rats and sperm quality of male rats.

MATERIALS AND METHODS

Material: Convicine was prepared according to the procedure of Arbid and Marquardt [6]. The solution was freshly prepared by dissolving convicine in distilled water and adding 2 drops of tween80.

Animals: Pregnant female rats weighing (180-200g) at 12th, 13th and 14th days of gestation and male mature rats weighing (150-180g) were obtained from the animal house colony, National Research Centre, Dokki, Giza. The animals were maintained on commercial balanced diet and tap water. The experiments were performed after approval from the ethics committee of National Research Centre and in accordance with recommendations for proper care and use of laboratory animals (NIH No. 85:23 revised 1985).

Methods Experimental Design: These experiments were conducted, the first one was performed on 32 pregnant females (the 12th day of concept was determined by

vaginal smear) and they were divided into 4 groups (8rats each): the rat was kept as control and injected with saline (0.5 ml/100g b.wt. ip).

The 2nd, 3rd and 4th groups were injected with convicine levels 80, 100 and 150 mg/100g b.wt., ip respectively, each dose was injected for 3 times on the day 12th, 13th and 14th of gestation. The weight of alive feti and percent of resorption and abortion were recorded and statistically analyzed.

The 2nd experiment offsprings of control and CV treated pregnant rats (100 mg/100g b.wt., ip) on the days 12th, 13th and 14th of gestation was weighed weekly.

The 3rd experiment was done on 2 groups of male mature rats (8 rats each). The 1st group was a control and injected with saline (0.5 ml/100g b.wt., ip) and the 2nd group was treated with CV (100 mg/100g b.wt., ip) for 60 successive days of daily treatment. Spermatozoa concentration and percent of mortality and abnormal sperms were determined according to the method of Baloch and Cohen [7].

The male genital organs (testes, epididymis, seminal vesicle, prostate gland and vasa deferentia) were weighed using digital balance.

Statistical Analysis: The differences between groups were tested for significance using t test determined by SPSS software program, version 21. Values are expressed as mean \pm S.E. The level of statistical significance was taken at $P < 0.05$.

RESULTS

Experiment 1, resorption and abortion percent were increased significantly ($P < 0.01$), while the weight of alive feti was decreased significantly in pregnant injected females with the higher doses of CV (100 and 150 mg/100g b.wt., ip). No detectable resorption or abortion was seen in the lowest dose of CV injected pregnant rats (Table 1).

Offsprings weight in the experiment 2, decreased significantly ($P < 0.01$) than control group starting from the 1st to 56th date of birth. In table 2, the percent of decrease in the body weight was ranging from 10.34% to 18.76% (at 1st to 24th date of birth). This decrease was cleared by time as the percent of decrease reached to 33.67% at the 56th date of birth (Table 2, Figure 1).

Concerning the effect of CV on the sperm quality in male (experiment 3), it was observed that spermatozoa number and mortality percent were significantly decreased ($P < 0.01$) (Table 3) as well as the percent of abnormal spermatocytes was increased significantly when compared to control group. The weights of male genitals organs were decreased significantly ($P < 0.01$) when compared with control group (Table 4). The percent of decrease was in testes 41.78%, epididymis 52%, prostate 42.85%, seminal vesicle 60.97% and vasa deferentia 51.25% (Figure 2).

Table 1: Effect Of Different Doses Of Convicine On Albino Rats

Groups	Resorption (%)	Abortion (%)	Alive feti weight (g)
Control (0.5 ml saline/100g b.wt., ip)	0	0	4.52 \pm 0.032
1 (80 mg CV/100g b.wt., ip)	0	0	3.92 \pm 0.6
2 (100 mg CV/100g b.wt., ip)	29.8 \pm 2.5*	18.5 \pm 1.4*	2.62 \pm 0.26*
3 (150 mg CV/100g b.wt., ip)	35.6 \pm 1.6*	20 \pm 1.3*	2.12 \pm 0.024*

Values are means \pm S.E of 8 animals. As compared with normal control (*) group (t test) at $P < 0.01$.

Table 2: Effect Of Convicine (150 Mg/100g B.Wt. Ip On 12th, 13th And 14th Days Of Gestation) In Pregnant Rats On Body Weight Of Offsprings

Days of birth	Offsprings from control mothers (g)	Offsprings from treated mothers (g)
1	5.8 \pm 0.24	5.2 \pm 0.02*
8	12.2 \pm 0.25	11.22 \pm 0.24**
16	17.2 \pm 0.28	16.23 \pm 0.18**
24	23.24 \pm 0.32	18.88 \pm 0.26**
32	32.24 \pm 0.33	23.64 \pm 0.36**
40	41.23 \pm 1.63	26.42 \pm 0.34**
48	47.6 \pm 1.22	32.1 \pm 2.26**
56	58.2 \pm 2.8	38.6 \pm 3.34**

Values are means \pm S.E of 8 animals. As compared with normal control group (*) (t test) at $P < 0.05$, (**) (t test) at $P < 0.01$.

Table 3: Effect Of Convicine (150 Mg/100g B.Wt. Ip) On Epididymal Spermatozoa Characters Of Male Albino Rats After 60 Days Of Daily Administration

Groups	Spermatozoa concentration (Million/ml)	Mortality (%)	Abnormality (%)
Control	690±2.8	86±2.6	5.3±0.44
Convicine	355±2.24**	13.2±1.38**	25.6±1.8**

Values are means ± S.E of 8 animals. As compared with normal control (**) group (t test) at $P<0.01$.

Table 4: Effect Of Convicine (150 Mg/100g B.Wt. Ip) On Weight Of Male Albino Rats Genital Organs After 60 Days Of Daily Administration

Groups	Testes	Epididymis	Seminal Vesicle	Prostate	Vasa deferentia
Control	2.13±0.141	1.25±0.05	0.41±0.022	0.35±0.012	0.8±0.005
Convicine	1.24±0.13**	0.6±0.045**	0.16±0.012**	0.2±0.016**	0.39±0.003**

Values are means ± S.E of 8 animals. As compared with normal control (**) group (t test) at $P<0.01$.

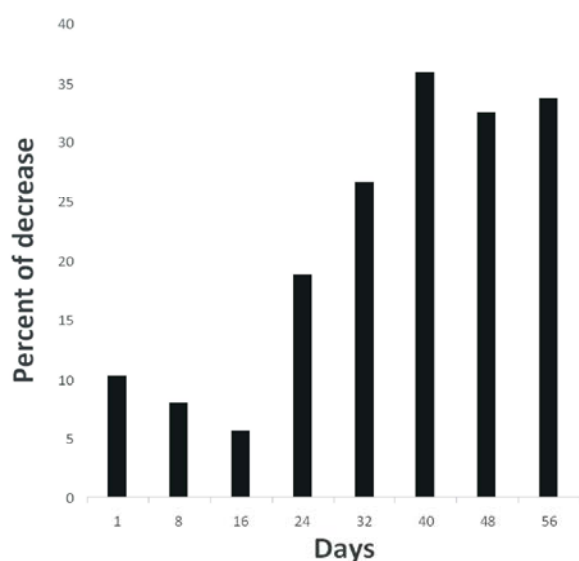


Fig. 1: Effect Of Convicine (150 Mg/100g B.Wt. Ip On 12th, 13th And 14th Days Of Gestation) In Pregnant Rats On The Percentage Of Body Weight Decrease Of Offsprings.

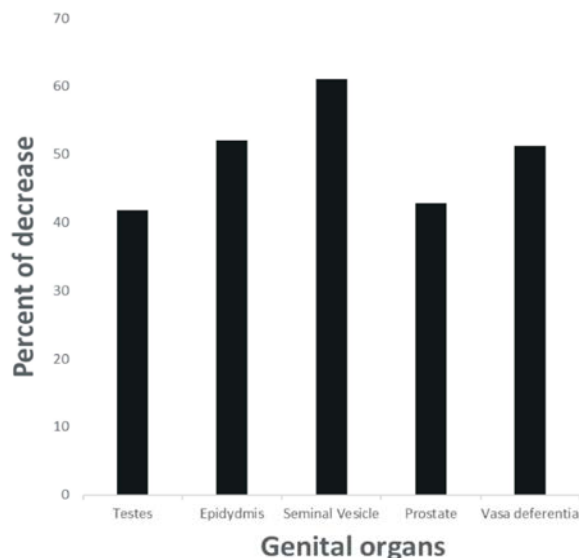


Fig. 2: Effect Of Convicine (150 Mg/100g B.Wt. Ip) On The Percentage Of Weight Decrease Of Male Albino Rats Genital Organs After 60 Days Of Daily Administration.

DISCUSSION

Favism is a genetic disease characterized by hemolytic anemia which occurs in sensitive people shortly after consumption of broad beans (faba beans, *Vicia faba* L.), this beans contain relatively high levels of vicin and convicine [8].

It has been proposed that the main immediate causative agents associated with favism are divicine and isouramil [2], which are cleaved from their respective glycosides vicine and convicine by intestinal microbial α -glycosidase [9]. The favic crisis usually begins several hours after ingestion of raw or cooked beans and can persist for several days [10]. The clinical condition is characterized by a variety of symptoms including fever, weakness, free hemoglobin in blood and urine, icterus, enlarged spleen,

elevated reticulocyte counts and renal failure [11]. The other side effects of convicine on the pregnant rats and on the sperm quality of male rats investigated in our study.

The results of experiment 1 revealed that CV treated pregnant rats has increased percent of resorbed and dead feti and abortion also occurred. These results may be due to the effect of CV on haemoglobin, in which hemoglobin produced ferryl species can react with its aglycon isouramil that accelerating its oxidation and methemoglobin is performed [12], also reduced the partial pressure of oxygen in the blood and the percent of oxygen saturation [13].

In addition, the RBCs have a markedly reduced oxygen carrying capacity which presumably greatly limits aerobic metabolism, of severe enough, will lead to death due to asphyxiation [13].

CV reduced the body weight gain of offsprings from injected pregnant rats in experiment 2. This findings due to the effect of isouramil which (the aglycon of convicine) possesses a thermo stable ant nutritional factor found in faba beans or due to the other depressing factors on the animals during the feeding with the faba beans [14].

The obtained results were in agreement with Robblee *et al.* [15] who demonstrated that when the faba beans content of the poultry diet exceeded 30% there was an increase in mortality rate, decreased egg production and feed convicine efficiency.

The resultant effect of CV on sperm quality in the experiment 3, revealed a significant reduction in the sperm number and motility percent. This findings runs parallel with that obtained by Arbid and Marquardt [13] who reported that vicine (the other glycoside in fava beans) caused arrest of spermatogermes at spermatid level, decreased the number and motility of sperms in male rats.

However, the genital organs weights were also decreased in CV injected male rats. Thus CV depressed the fertility, hatchability of eggs, damaged or denaturated protiens and,or altered the structure of the lipid components in the yolk membrane [16].

Due to the formation of free radicals from CV *in vivo* [13] and subsequently reduced blood glutathione (Scott, 1978) and the cell membrane became fragile [17]as well as any cells in the body including the ova in females and sperms in male were affected.

CONCLUSION

It can be concluded that Convicine when hydrolyzed to its aglycan isouramil, in addition to its effect on RBCs membrane and metabolism and on the immunological system, it has adverse effect on pregnant rats as well as on spermatogenesis in male rats.

Funding: The authors didn't received any financial support for research or its publication.

Competing Interests: The authors have declared that no competing interests exist.

REFERENCES

1. Frohlich, A.A. and R.R. Marquardt, 1983. Turnover and hydrolysis of vicine and convicine in avian tissues and digesta. Journal of the Science of Food and Agriculture, 34(2): 153-163.

2. Albano, E., A. Tomasi, L. Mannuzzu and P. Arese, 1984. Detection of free radical intermediate from divicine of *Vicia faba*. Biochemical Pharmacology, 33: 1701-1704.
3. Lin, J.Y. and K.H. Ling, 1962. Studies on favism. I. Isolation of an active principle from faba beans (*Vicia faba*). Journal of the Formosan Medical Association, 61: 484-489.
4. D'Aquino, M., S. Gaetani and M.A. Spadoni, 1983. Effect of factors of favism on the protein and lipid components of rat erythrocyte membrane. Biochimica ET Biophysica Acta, 731(2): 161-167.
5. Dominis, M., S. Rocic, S.J. Ashcroft, B. Rocic and M. Poje, 1984. Diabetogenic action of alloxan-like compounds: cytotoxic effects of 5-hydroxy-pseudouric acid and dehydrouramil hydrate hydrochloride on rat pancreatic beta cells. Diabetologia, 27(3): 403-6.
6. Arbid, M.S. and R.R. Marquardt, 1986. Effect of intraperitoneally injected vicine and convicine on the rats: induction of favismlike sign. Journal of the Science of Food and Agriculture, 37(6): 539-547.
7. Baloch, K. and R.B. Cohen, 1964. A cytochemical technique for studying oxidative enzyme system of mammalian spermatozoa in semen smears. Fert. Steril, 15: 39-55.
8. Gardiner, E.E., R.R. Marquardt and G. Kemp, 1982. Variation in vicine and convicine concentration of faba bean genotypes. Canadian Journal of Plant Science, 62(3): 589-592.
9. Hegazy, M.I. and R.R. Marquardt, 1984. Metabolism of vicine and convicine in rat tissues: absorption and excretion patterns and sites of hydrolysis. Journal of the Science of Food and Agriculture, 35(2): 139-146.
10. McMillan, D.C. and D.J. Jollow, 1999. Favism: divicine hemotoxicity in the rat. Toxicological Sciences, 51(2): 310-316.
11. Arese, P., L. Mannuzzu and F. Turrini, 1989. Pathophysiology of favism. Folia Haematologica. 116: 745-52.
12. Winterbourn, C.C., U. Benatti and A. De Flora, 1986. Contributions of superoxide, hydrogen peroxide and transition metal ions to auto-oxidation of the favism-inducing pyrimidine aglycone, divicine and its reactions with haemoglobin. Biochem Pharmacol., 15: 35(12): 2009-2015.

13. Arbid, M.S. and R.R. Marquardt, 1988. Favism-like effects of divicine and isouramil in the rat: Acute and chronic effects on animal health, mortalities, blood parameters and ability to exchange respiratory gases. *Journal of the Science of Food and Agriculture*. 43(1): 75-90.
14. Olabro, G., L.D. Campbell and R.R. Marquardt, 1981. Influence of faba beans fractions on egg weight among laying hens fed test diet for short time period. *Canadian Journal of Animal science*, 61: 751-755.
15. Robblee, A.R., D.R. Clandinin, R.T. Hardin, G.R. Milne and K. Darlington, 1977. Studies on the use of faba beans in rations of laying hens. *Can. J. Anim. Sci.*, 57: 421-425.
16. Bellairs, R., M. Harkness and R.D. Harkness, 1963. The vitelline membrane of the hen's egg: a chemical and electron microscopical study. *Ultrastruct. Res.*, 8: 339-359.
17. Arbid, M.S., R.R. Marquardt and S.A. Nada, 1996. Effect of vicine and divicine on glutathione haemolysis and mortalities in different sex and age in albino rats. *Toxicology Letters*, 95: 96-96(1).