OXYTOCIC ACTIVITY AND TOXIC EFFECTS OF GLOBULINS OF ABRUS SEEDS (SCARLET VARIETY) IN RABBITS

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SUMMARY: Seeds of Abrus precatorius L (scarlet variety) were investigated for the isolation and for the oxytocic effects of water insoluble protein (globulin) on sequester rabbit uterus. Powdered seeds were macerated with 5% (w/v) aqueous sodium chloride solution and the protein was subsequently precipitated by saturated solution of ammonium sulphate. Oxytocic activity was confirmed by correlating with a standard oxytocic drug. Other pharmacological effects such as toxicity, on the ovarian tissues and its hormones were also explored. Globulin injected to the animals for 24 hours altered the cyclic rhythm of the ovaries. It lowered the plasma level of oestradiol, while the plasma level of progesterone remained unaltered. It was also responsible for the hemorrhage of the ovarian follicles.

It has been concluded that the oxytocic component interferes with the cyclic rhythm of the female rabbit either through modifying the steroidal metabolism of the ovaries or through its toxicity on the ovarian tissues.

Key Words: Abrus seeds, oxytocic activity, globulins.

INTRODUCTION

Abrus seeds are rich in toxic proteins. In addition to extremely toxic protein "abrin" the seeds also contain agglutinins with much lower toxicity (1). Albumin and globulin, two simple proteins isolated earlier, were reported to have an oxytocic effects (2). Abricin and abridin, two steroids were also reported in the seeds; the latter exhibited anti-fertility property (3). Very little work has been published on the oxytocic and anti-fertility property (3). Very little work has been published on the oxytocic and anti-fertility effects of abrus seeds. The present work was carried out with the following objectives: 1. To confirm the oxytocic effect of globulin on isolated rabbit uterus. 2. To study the toxic effects of globulin on ovarian tissues and its hormones, in order to determine its suitability as an oxytocic drug.

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MATERIALS AND METHODS

Chemical

Unless otherwise stated, all the chemicals used in the present work were of analytical grade.

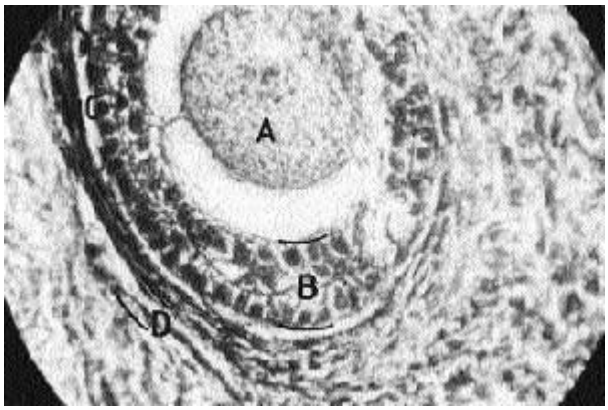
Animals

Healthy female rabbits of subspecies Lapus nigricollis dayanus (Chinchilla), with an average body weight of 2 $\pm$ 0.6 kg were acclimatized for a month in the animal house. During this period, fresh green clover and tap water were provided *ad libitum*.

Test Drug

Mature dried seeds of *A. precatorius* were purchased from the local market. The oxytocic principle (globulin) was extracted with a yield of 10.95% from the powdered seed kernels according to the method o Saha *et al.* (2). 10 g of the powder was macerated with 100 ml of 5% w/v sodium chloride solution for 4 hours with occasional stirring. The macerate was treated with 50 ml of saturated ammonium sulphate solu-

Figure 1: Section of the ovary from an untreated (negative control) rabbit, showing a developing follicle.



A. Oocyte with its surrounding zona pellucida.

B. Cells of granulose layer.

C. Theca interna.

D. Theca externa.

tion and on next day the globulin was precipitated out. It was filtered off, washed with distilled water and finally dried in a desiccator.

Standard Drug

Oxytocin 10 i.u./ml (Geofman) was purchased from a local pharmacy. According to Saha *et al.* (2), oxytocic activity of 0.02 mg of globulin is equivalent to the oxytocic activity of 0.003 i.u. of oxytocin. Maximum dose of oxytocin for an adult female albino rabbit is u to 2.0 i.u. (2); therefore six doses of oxytocin (i.e. 0.225, 0.50, 0.675, 0.900, 1.125 and 1.350 i.u./ml) below the maximum dose and corresponding doses of globulin (i.e. 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 mg/ml) respectively were prepared in isotonic saline solution.

Experimental Design

The present work was conducted in three series of experiments. In the first, the oxytocic activity of the test drug on the isolated uterus of non-gravid rabbits was confirmed and bio-assayed against the standard drug. Six doses of each drug prepared above, were used according to the method described in British Pharmacopoeia 1973. Six responses to each dose were recorded. The dose of the oxytocin and that of the globulin which gave nearly the same responses were selected for further experiments.

In the second series of experiments, twelve non-gravid rabbits (in oestrous) were selected. Half of them were treated subcutaneously, each with 0.900 i.u/ml of oxytocin and rest with 6 mg/ml of globulin respectively. Blood samples were collected at zero hour (before the administration of drug), and after 12, 24 and 36 hours, following administration of each drug. These blood samples were sent to a private laboratory for hormone analysis by radio-immunoassay.

In the third series of experiments, 24 rabbits (in oestrous) were divided into 4 groups. Rabbits of first three groups were treated separately with a subcutaneous injection (1 ml) of 0.900 i.u/ml of oxytocin, 6 mg/ml of globulin and isotonic saline solution (positive control) respectively. The fourth group was kept untreated (negative control). After twenty four hours, the rabbits were sacrificed and their ovaries were removed. Each ovary was cut longitudinally into two equal halves, fixed in Bouin's fluid for 24 hours and processed for histological examination using standard methods. All paraffin sections were cut at 6 μ and stained in eosin-hematoxylin.

Statistical Analysis

The mean heights of the uterine muscles contraction curves, after the application of standard and test drug were recorded along with their effective ranges. The student' t test was used for comparing the mean activities of standard and the test drugs (5); while the comparative relationship between the doses, drugs and contraction of uterine muscles were further done by analysis of variance (ANOVA) with two way completely randomized experimental design, along with the

Table 1: The average heights of contraction of uterine muscles, following the application of standard and test drug, in rabbits.

DOSE		HEIGHT OF CONTRACTION (mm)		
Standard Drug (i.u./ml)	Test Drug (mg/ml)	With Standard Drug	With Test Drug	p-values (Student's t test)
0.225	1.5	1.23±0.45	1.71±0.31	p>0.05
0.450	3	2.0±0.00	2.25±0.25	p>0.05
0.675	4.5	3.8±1.04	3.5±1.12	p>0.05
0.900	6	4.5±0.29	4.37±0.37	p>0.05
1.125	7.5	9.0±0.91	6.91±1.15	p>0.05
1.350	9	13.0±0.81	8.5±0.29	p>0.05

F= 512.227.... p= .000 (***) ---Between dose levels.
F= 77.692.... p= .000 (***) ---Between drugs.
F= 43.037.... p= .000 (***) ---Between interaction (dose X drug)

Table 2: Duncan's multiple range test, with factors. Dose and drug, with the mean square= 0.32168 and degree of freedom=60 at significant level= 0.05.

DOSE (L.S.D.0.05 = 0.4631)						DRUG (L.S.D. 0.05=0.26740)					
Rank	Treat	Mean	n	Non Sig.	R.	Rank	Treat	Mean	n	Non Sig.	R.
1	6	11.0416	12	a		1	1	5.6472	36	a	
2	5	7.9916	12	b		2	2	4.5068	36	b	
3	4	.3416	12	c							
4	3	.6500	12	d							
5	2	.0833	12	e							
6	1	.4650	12	f							

Table 3: Amount of ovarian hormones, estimated after the injection of standard drug (2 ml of 900 i.u.) in controlled rabbits and test drug (2 ml of 6 mg) in treated rabbits.

Sampling Time (Hours)	CONTROL *		TREATED *	
	Oestradiol (pg/ml)	Progenesterone (ng/ml)	Oestradiol (pg/ml)	Progenesterone (ng/ml)
0	96±4.615	4±1.32	100±6.719	3±1.872
12	97±3.107	4±1.30	70±5.137	3±1.50
24	90±3.019	4±0.19	28±3.041	3±0.573
36	89±2.17	4±0.50	18±2.153	3±0.384

* Mean readings of hormones in plasma levels of six animals.

Duncan's multiple range tests, with least significant differences (LSD) at p=0.05 (5, 6).

RESULTS AND DISCUSSION

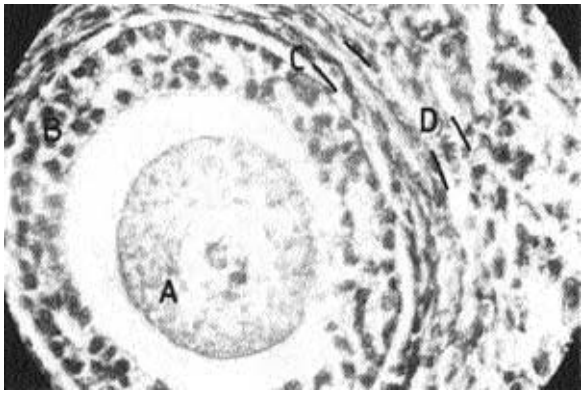
Abrus seeds are rich in both water soluble (albumin) and insoluble (globulin) proteins. Previously, very little experimental work has been done to determine the oxytocic property of *A. precatorius.* Only Saha *et al.* (2) have reported the oxytocic activity and toxic effects of *Abrus* seeds on the uterine muscles of non-gravid female guinea pigs. They reported that globulin appeared to be more active than albumin as far as its oxytocic influence was concerned. It was also observed that 0.02 mg of globulin and 3 mg of albumin gave almost equal responses, to that given by 0.003 i.u. of oxytocin (pituitary extract) (2). Therefore, only globulin was studied further in this present work.

Globulin was extracted from *A. precatorius* (scarlet variety) seeds with a yield of 10.5% (A single *Abrus* seed weighed approximately 113.75 mg, therefore it yielded about 12.4 mg of globulin).

Siegmund (4) investigated that the maximum dose of the standard drug (oxytocin) for an adult female albino rabbit was up to 2 i.u.. Therefore, six doses of the standard drug (oxytocin), below this maximum dose, were used to confirm and standardize the oxytocic activity of the test drug in order to get a suitable dose which elicit almost the same response as that of the standard drug (Table 1).

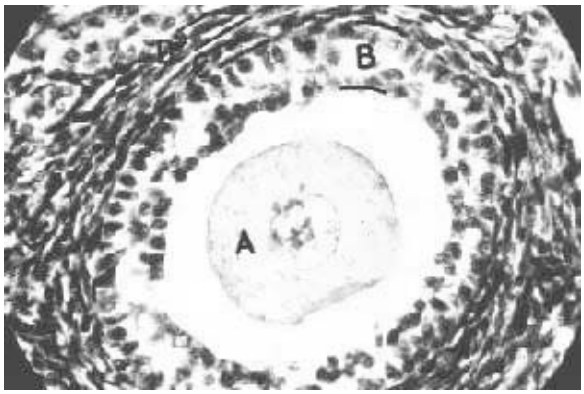
The analysis of variance (ANOVA) revealed that all the six doses and two drug's parameters (standard and test drugs), significantly differ from each other at p=0.05 (Table 1). The least significant differences (LSD) and significant ranges among various parameters in relation to the contraction of uterine muscles of rabbits were further indicated by Duncan's multiple range test (Table 2). Moreover, the student's test further indicated that the responses given by 0.675 and 0.900 i.u. doses of the standard drug and 4.5 and 6.0 mg doses of the test drug respectively is statistically insignificant (p>0.05 and p>0.01); while the differences between the responses given by 1.350 i.u. dose of the

Figure 2: Section of the ovary from a normal saline treated (positive control) rabbit, showing a developing follicle.



A. Oocyte with its surrounding zona pellucida.
Nucleus is also visible in the middle.
B. Cells of granulose layer.
C. Cells of theca interna.
D. Cells of Theca externa.

Figure 3: Section of the ovary from a standard drug treated rabbit showing a developing follicle.

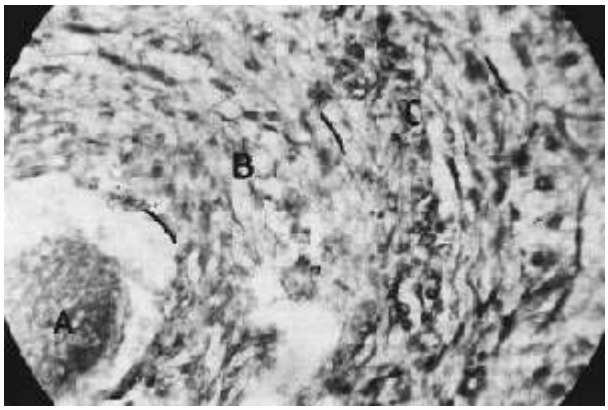


A. Oocyte with is surrounding zona pellucida. Large egg cell nucleus and its nucleus and its nucleolus is also prominent in the middle.
B. Cells of granulose layer.
C. Cells of theca interna.
D. Cells of theca externa.

standard drug and 9 mg dose of the test drug is statistically significant ($p<0.05$) (Table 2). The gradual decrease in responses produced by the test drug was possibly due to the reason that either it occupied the uterine receptors strongly and released them partially when compared with standard drug or it exerted some of its toxic effects on the uterine muscles, which had also been reported earlier (2). Thus the maximum acceptable dose of test drug was 6 mg and it was administered to the rabbits for further experiments.

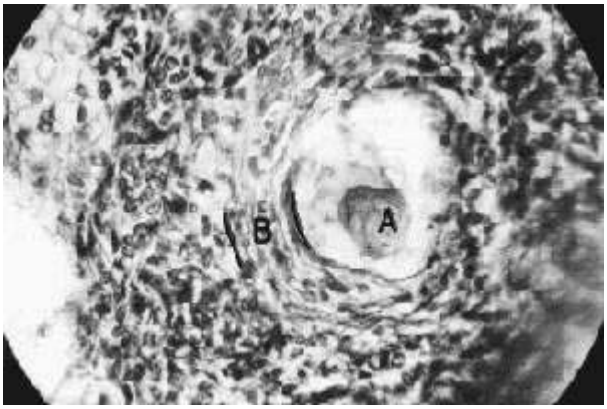
Rabbits are mono-estrous animals and during their breeding season, if they are kept separated from their males for about one month, they automatically acquire their oestrous phase. The oestrous phase was con-

Figure 4: Section of the ovary from a test drug treated rabbit showing an artretic follicle.



A. Oocyte.
B. Disorganized cells of granulose layer.
C. Indistinctive cells of the theca layer.

Figure 5: Section of the ovary from a test drug treated rabbit showing an artretic follicle with hemorrhagic egg cell.



A. Hemorrhagic Oocyte.
B. Cells of granulose layer, theca interna and theca externa, all are indistinctive.

firmed by the examination of the vaginal smears. The administration of the test drug seemed to disturb the oestrous cycle of the female rabbits (in oestrous), while the administration of standard drug did not show such effect. The plasma level of progesterone remained unaltered in both the cases, since it is mainly secreted by the corpus luteum, within the ovaries which is often not formed in rabbits without copulation (Table 3).

Histological examination of the ovaries of test drug treated animals revealed almost all the follicles in different stages of atresia. Granulose cells of these follicles become disorganized and in many cases they are indistinctive. Cells of theca interna and externa of those follicles could not be distinguished (Figures 4 and 5). Moreover, hemorrhagic spots are also seen in some of the follicles of these ovaries (Figure 5). On the other hand, histological examination of the ovaries of standard drug treated animals, along with both their

positive and negative controlled animals, did not show any of the above mentioned abnormalities. In the atretic follicles, granulose cells and the cells of theca interna and externa could easily be distinguished in these ovaries (Figures 1, 2 and 3). During oestrous, oestradiol is mainly secreted by theca cells of the follicles. As the granulose cells and the theca cells of the ovarian follicles were disturbed by the test drug, the plasma level of oestradiol also decreased considerably. The active principle may interfere with the cyclic rhythm directly by altering the steroidal metabolism of the ovaries, or by altering the plasma level of gonadotrophin indirectly by acting on the pituitary glands. Hemorrhagic spots within the ovaries and destruction of the follicles reveal that it is a very toxic drug. However, in the absence of information on the plasma level of gonadotrophin and other related phenomena, it is quite difficult to speculate the nature of exact mechanism involved.

In India and Pakistan, the western or allopathic system of medicine coexists with the traditional system. The seeds of *A. precatorius* have long been used for their ecbolic and anti-fertility properties. The seeds are ground into a paste and made into needles, which are inserted under the skin to produce criminal abortion (7). Present study reveals that large quantity of seeds is required to produce the oxytocic effects. As an oral contraceptive, one decorticated seed is claimed to be effective for one year, two seeds for two years and three seeds for the whole life. According to Siddiqui *et al.* (3) the reputed contraceptive activity would require very large quantity of the seeds for adult women. Therefore, besides abridin (a reported anti-fertility component of *A. precatorius seeds*), the anti-fertility activity of the seeds might be due to the combined effect of the constituents which also include the toxic principles. Globulin, along with its oxytocic activity, also disturbed the oestrous cycle; hence possibly it could be one of the other constituents that triggered the anti-fertility activity of the Abrus seeds. Therefore, our results confirmed the conclusion of Siddiqui *et al.* (3).

Treatment of certain cytotoxic proteins (e.g. abrin, abricin) with B-mercaptoethanol strongly increased their ability to inhibit protein synthesis and concomitantly decreased their toxic effects (8). Similar treatments can also be tried with globulin in order to decrease its toxic effects.

From the present study it may be concluded that globulin from the Abrus seeds can not be used as a useful oxytocic drug due to its minor activity and severe toxicity. Some intermolecular modifications or treating with some suitable agents, to decrease its toxicity, are required for its use as an oxytocic agent. Nevertheless, due to its destructive action on some of the ovarian follicles, it may be used in low doses as an anti-fertility agent like other contraceptives.

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