Vol-2, Issue-12 PP. 1813-1823 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

POTENTIAL FUTURE APPLICATIONS OF SPERMATHECAL EXTRACT FROM THE LAND SNAIL *ACHATINA FULICA* SPECIALLY IN IMMUNO CONTRACEPTION

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ABSTRACT
Cytrosol fraction of spermatheca and / or ovotestis gland from Giant African Land Snail *Achatina Fulica* was found to have antifertility and immuno modulatory properties. The present experiment was conducted to explore the biochemical nature and pharmacological properties of cytrosol fraction along with its biochemical effects on the Gonadal system of vertebrates invivo. This glandular extract was analyzed and found to cross react with antisperm antibodies developed in rabbit. The anti serum of the spermathecal extract was found to interfere in the protein synthesis in the testis of Albino mice and causes regression of testis. There was a significant reduction in the protein and DNA content in the testis of extract treated and antisera treated mice compared with the control. The crude extract of the spermatheca gland therefore appear to be inherent with the immuno contraceptory properties, which hypothetically could further be employed for many pathological conditions in general, including immunocontraception in particular.

Keywords: Spermatheca, Snail, contraceptive, immunocontraception

1. INTRODUCTION
The invertebrates have been recognized as an important source of bioactive compounds having medicinal potential. The research on invertebrate natural product in the last four decades has led to the discoveries of many chemically and biologically interesting molecules. The major sources of biomedical compounds obtain from invertebrates includes sponges (37%), coelenterates (21%), micro organisms (18%), Algae (9%), Echinoderms (6%), tunicates (6%), molluscs (2%) and bryozoans (1%). Molluscs one of the most abandoned marine and land species yielded some anti cancer compound like dolastine, acharan sulphate, kahalalide and kulolide. Search for suitable male anti fertility agents from invertebrate sources remains a potential area of investigation. Recently scientist are working extensively in search of and to develop drugs immuno modulators or biochemical markers against various diseases as well as a suitable antifertility agents from different bioactive substances of invertebrate sources. The cytrosol fraction of spermatheca and / or ovotestis, a complex organ of the marine moluscs *Telescopium telescopium* exhibited reversible antifertility effect. Thus on the basis of some preliminary studies with the extract obtained from species *Achatina fulica*, which showed encouraging results as an antifertility agent, inspire to undertake detail investigation about its efficacy ,mode of action and effect on different organs for the present study.

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(Scholarly Peer Review Publishing System)

2. MATERIALS AND METHODS

2.1. Animals and chemicals

Achatina fulica was collected from marshy land and washed under tap water and the matured spermatheca was collected. The spermatheca were homogenized in 0.9% saline water and the extract was kept in deep freeze.

Inbred strain of Newzealand male rabbit, reared and maintained in the animal house of the Department of Zoology, Kalyani University, were used for collection of antibody against spermathecal proteins. Rabbit weighing between 800 gm. to 1 kg. were used. Inbred strain of Swiss albino male mice, *Mus musculus*, reared and maintained in the animal house of the Department of Zoology, Kalyani University, served as the materials for different fixation intervals for different experimentation. Mice weighing between 25-30 gms. were used.

The animals were given food and water *ad libitum* and kept in the animal house under proper hygienic condition.

2.2.1. Injection procedure

After acclimatization legs of rabbits were washed with rectified spirit to avoid any contamination before treatment. For treatment, the required amount of extract of spermatheca solution of *Achatina fulica* along with Freund's complete adjuvants were emulsified and then injected intramuscularly on the hip & back region of the rabbits. After 28 days boosting was done by the same kind of extract along with Freund's incomplete adjuvant.

After 7-10 days when the sore appears on the site of injection, blood was collected from ears of rabbits and serum was isolated. Then the serum was titrated against the freezed spermathecal extract used earlier for immunization of rabbits to check the immune response through the Gel Diffusion Precipitation test. Blood was collected from the heart of rabbits after observing the positive band formation in GDP test and the serum was isolated. Then the serum was lyophilized and kept in deep freeze for future use.

2.2.2. Treatment schedule

Four sets of mice, each set forty in number, properly acclimatized were taken for the experiment. Four sets were marked as C, T_1, T_2, T_3 respectively. Set-C mice were used as Control, Set-T_1 mice were treated with complete adjuvant, Set-T_2 mice were treated with crude sparmathecal extract and Set-T_3 mice were treated with rabbit antisera. Body weight of mice was within the range 25 + 1 gms.

After acclimatization legs of mice were washed with rectified spirit to avoid any contamination before treatment. After recording body weight control mice were treated with 0.1ml 0.9% saline subcutaneously in the peritoneal region. Similarly with Set-T_1, Set-T_2 , and Set-T_3 mice were treated with 0.1ml of Freund's incomplete adjuvant, spermathecal homogenate of 0.5% concentration in 0.9% saline and rabbit antisera (0.1ml in dose) respectively.

On the 7^{th} day i.e. after one week of 1^{st} treatment body weights of the mice of each set recorded. Behavioral changes if any were also recorded during the week after 1^{st} treatment. On the 8^{th} day, 10 mice from each set i.e. Set-C, Set-T_1, Set-T_2 and Set-T_3 , were sacrificed by cervical dislocation for observing the changes, if happened in liver, kidney and testis. Weight of the testis of the treated mice were recorded. After weighing testis, Liver and kidney tissues were collected for Biochemical enzymatic analysis. On the 8^{th} day, rest mice of each set were again treated in the same manner with 0.1ml 9% saline, 0.1ml incomplete adjuvant, 0.1ml 0.5% total spermathecal homogenate with 0.1ml rabbit antisera. This is the 1^{st} boosting dose.

On the 14^{th} day equal no of mice of each set were sacrificed by cervical dislocation and then dissected and testis tissue is collected. Weight of the testis of control and treated mice taken and recorded. On the 15^{th} day 2^{nd} boosting dose administered. Control animals were treated with 0.1ml of 0.9% saline after taking body weights. Rest 20 mice of Set-T_1 were treated with 0.1ml of Freund's incomplete adjuvant, similarly 20 Set-T_2 mice were treated with crude sparmathecal

GLOBAL JOURNAL OF ADVANCED RESEARCH
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homogenate of *Achatina fulica*, and 20 Set-T_3 mice were treated with 0.1ml rabbit antisera after recording the body weights of mice of all sets. This is the 2nd boosting dose. Behavioral changes of the mice after treatment were observed on the subsequent days.

On the 21st day after 1st treatment, equal no of mice of each set were sacrificed by cervical dislocation and then dissected and liver, kidney, testis tissues were collected for observing biochemical changes, if any in those tissues.

The last course of boosting was done on 22nd day after 1st treatment. Control, Set-1, Set-2 and Set-3 mice were treated, after recording body weights, with 0.1ml of0.9ml saline, 0.1ml crude spermathecal homogenate and with 0.1ml rabbit antisera respectively. This is the final boosting dose. Behavioral and other changes were recorded on the next 7 days. On the 28th day rest mice of each set were sacrificed by cervical dislocation and then dissected to collect liver, kidney, testis tissues.

2.2.3. Study schedule

Effects were recorded on mortality, weight of body and vital organs after treatment with specific doses and the animals were sacrificed on specific days. Body weight, organ weight, total protein , and DNA were determined in testis. For these observations four different fixation intervals namely 7 th day,14th day,21th day and 28th day and four different doses such as 0.1m1,0.2m1. and 0.3 ml and 0.4ml were considered.

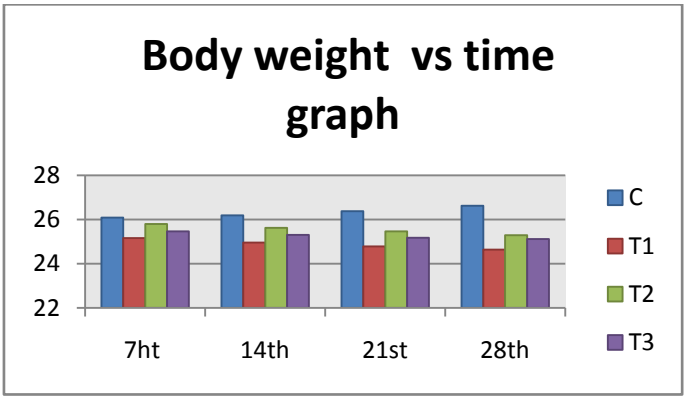
SDS gel electrophoretic analysis of testis tissue was considered in all the cases.

2.3. Determination of the body weight and organ weight

Body weight of individual animal was determined gravimetrically with the help of sensitive weighing balance. Organ weight was taken after taking the total organ on a thin slide or watch glass, as the case may be, and its wet weight was determined by deducting the tare weight.

3. RESULTS

	BODY WEIGHT				WEIGHT OF TESTIS			
	7TH	14TH	21ST	28TH	7TH	14TH	21ST	28TH
AVER.	*26.69*	*24.71*	*25.11*	*25.14*	*0.45*	*0.37*	*0.46*	*0.38*
AVER	*26.62*	*24.71*	*25.08*	*25.17*	*0.44*	*0.37*	*0.46*	*0.37*
AVER	*26.63*	*24.72*	*25.07*	*25.19*	*0.45*	*0.37*	*0.44*	*0.38*
AVER	*26.62*	*24.70*	*24.99*	*25.18*	*0.45*	*0.38*	*0.45*	*0.38*

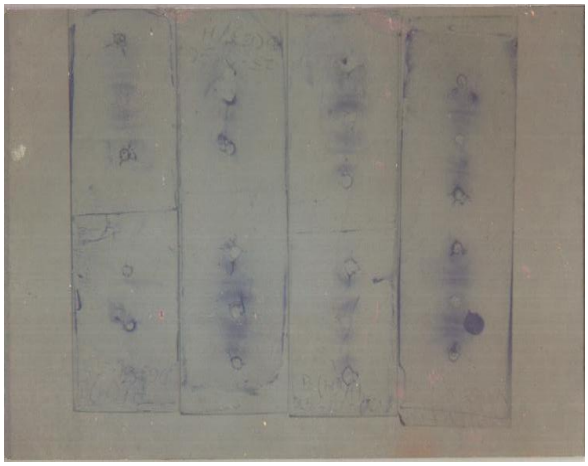


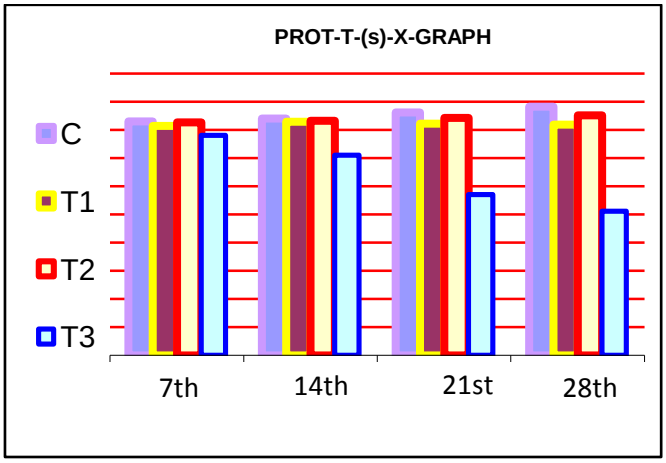
Vol-2, Issue-12 PP. 1813-1823 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Total protein content was also measured in testis after 7th, 14th, 21st and 28th day of treatment interval. No significant change in the level of protein was observed upto 20th day in case of C, T_1 and T_2 set mice. A slight increase of total protein content level was observed at 21st day & 28th day in case of control & T_2 set. On the contrary an insignificant change of protein level observed in case of T_1 set on 21st & also further on 28th day. But T_3 mice showed a gradual decline in protein level in a dose dependant manner. Maximum decline was observed on 28th day.

3.1 Precipitin Band





PROTEIN-T-(Sum)-x,sd-graph

Fixation Intervals	C	T1	T2	T3
7th	166.04	162.82	165.27	156.10
14th	168.03	165.68	166.49	142.08
21st	172.31	164.39	168.33	114.06
28th	176.42	163.76	170.23	102.35

3.2 DNA Testis :

DNA content in the testis of control mice increased regularly with time but with short increment. That of the T_2 mice also increased slightly after each treatment except after 2nd treatment, when the value diminished very slightly. In case of T_1 mice, the DNA content decreased very shortly after 1st treatment, but measurably decreased after 2nd as also after 3rd treatment. DNA content of antisera treated mice (T_3) declines regularly after each treatment. After 4th treatment the level of DNA reaches the minimum value at least in compare to that in liver & kidney.

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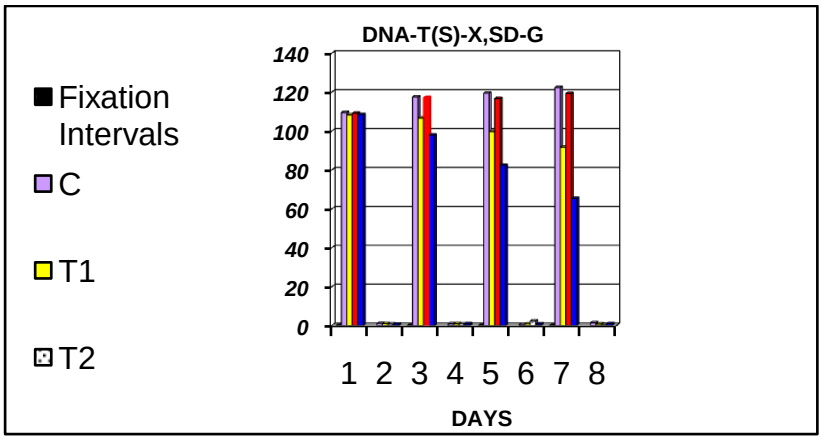
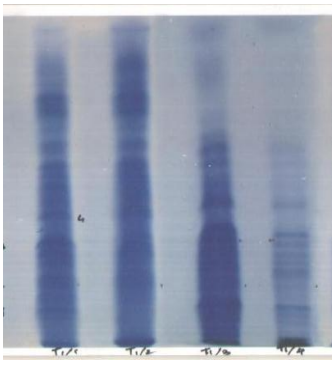
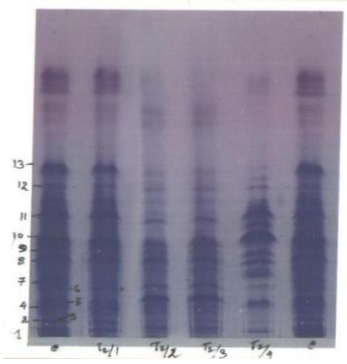
Table - . Total DNA in (mg/g) estimated in Testis of mice

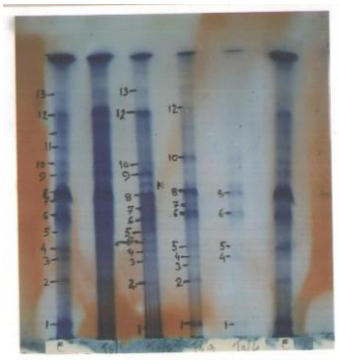
Fixation Intervals	C	T1	T2	T3
7th	109.48	108.22	109.09	108.43
	1.10	0.96	0.75	0.71
14th	117.45	106.67	117.13	97.84
	0.89	0.90	0.82	0.89
21st	119.42	99.71	116.66	82.34
	0.58	0.84	2.29	0.81
28th	122.34	91.66	119.25	65.36
	1.37	0.75	0.67	0.94



3.3 SDS Gel Band







4. DISCUSSION

Antibody against the sperm(homogenate) of Achatina fulica raised in the rabbit and found positive on the fifth week after administration and the titre value of the antisperm antibody found noticeably high after 8th week as evidenced by the

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formation of distinct precipitin band. Several faint bands besides the distinct one also formed probably due to the presence of a number sperm protein/antigen in the spermathecal extract against each of which specific antibodies formed in the immunized rabbit.

In our present investigation we made a study of comparative effectiveness of aspermotogenic action of spermatheca / ovotestis gland of *Achatina fulica* (inverbebrate source) in mammals through different type of administration. Viz, i) Isotonic total homogenate of matured spermatheca gland (T_2)

ii) Emulsion of total homogenate with FCA (T_1)

iii) Antisera collected from rabbit immunised with spermatheca homogenate emulsified with FCA (T_3)

Testicular regression observed in all the cases though in different degrees and different rate (ie, at different time interval) indicating plausible existence of phylogenetic relationship between mollusc and mammals. and thus plausibly supports that relatively similar molecules and their basic physiological functions in molluscan system may remain the same in vertebrates regardless structural changes of these ancestral bio molecules, (Ottiviani; Halvey, 1990) as also found by Kemeness (2005) that brain molecules in humans and pond snails actually share important characters unchanged by evolution.

This apparent loss of body weight and organ weight and loss in total protein content would point to the failure of protein synthesing machinery of the cells. The mechanism of protein synthesis in both lower and higher forms of organisms is under genetic control and is well documented.(coopes, 1997) Leusin 1997)

The changes observed in the activities of enzymes are also very significant specially in the testis. There was a steady decrease in the DNA, and protein contents in the testis of all the treated mice (T, T_2 & T_3) at all the fixation time interval. The extent of decrease is less in case of T_2 set group of animals but it is pronounced in case of T_1 and T_3 mice.

As it is known protein is synthesized basically through an elaborate mechanism by transcription of specific parts of DNA to form various types of RNA (mRNA, tRNA , rRNA) which interact with specific amino acids. The amino acids are attached with one another in a definite sequence to produce a certain type of protein (poly peptide). Therefore any degradation or denaturation of protein would be reflected in gel electrophoretic band profiles and likely to be reflected in the DNA, RNA contents as well.

Our findings on the protein band from SDS PAGE also indicates that some of the Genes involved in the synthesis were switched off resulting in the disappearance of some of the proteins. The appearance of some new protein bands may be due to the switching on of some genes.

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