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FENITROTHION INDUCED CHANGES IN AMINO ACIDS PROFILE OF *BIOMPHALARIA ALEXANDRINA* MOLLUSCAN HOST OF *SCHISTOSOMA MANSONI*

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ABSTRACT

Amino acid profiles of control and Fenitrothion treated normal control snails were investigated in a trail to correlate the amino acids profile of treated snails to their previously reported molluscicidal and biological effects. Amino acid profiles of the snails were greatly manipulated with the treatment of Fenitrothion sublethal concentration (LC_{25}), The disturbed amino acid profiles of treated snails, reduction in total protein content and significant increase in protease enzyme activity while significant inhibition in steroid hormones (estriol and progesterone), were discussed in relation to the decrease in snail's egg laying capacity, reduction of their compatibility for the development of the schistosome larvae and cercarial penetration of mammalian skin and snails reproductive efficiency.

Key words: Fenitrothion, *Schistosoma mansoni*, *Biomphalaria alexandrina*, amino acids.

INTRODUCTION

Schistosomaisis, a dreadful disease caused by parasitic trematode worm in both humans as well as in animals is widespread in the world specially in the developing countries [1]. It is considered second only to malaria as a major target disease of the World Health Organization [2]. Schistosomes as digenetic trematods have two hosts a final mammalian hosts and a molluscan intermediate snail hosts. The freshwater snail B*iomphalaria alexandrina* (Mollusca; Gastropoda), is widely distributed in Egypt and acts as intermediate host of

Schistosoma mansoni. Control of snail is regard as one of the best preventative measures in controlling schistosomiasis [3].

El –Ansary and Qurashy [4], stated that the ability of the parasite to develop within snail host is correlated to the snail intrinsic biochemical composition rather than any regulatory immune response. Moreover, Thompson et al. [5] reported that free living stages of schistosomes are completely dependant on the endogenous reserves acquired from their host in the previous parasitic stage. Cercariae for example, live on their endogenous glycogen and fatty acid stores that they build up while they are inside the snail host [6].

It is well known that amino acids are among the Snail Conditioned Water (SCW) signals needed by schistosome miracidiae to identify their snail host species [7]. In fact, amino acids spectra released by fresh water snails varied not only among species, but also within the same species. This may play a critical role in snail–finding mechanisms by the parasite miracidia [8]. Reduction in free amino acid level in haemolymph and tissues of mollusks during schistosome infection have been reported which proved their uptake by the developing parasite [9]. Certain amino acids (Glu, Asp, Gly and Gln), have stimulatory effects on the development of parasitic helminthes [10], because schistosomes have usual nutritional requirement for essential amino acids. Prasitic absorption and utilization likely explain much of the observed decrease.

El-Ansary et al. [11], could induce in vivo attenuation of schistosome cercariae using sublethal concentrations of selected plant molluscicides. The pathogenicity of attenuated *Schistosoma mansoni* cercariae were evaluated [12]. Although the reduced number of attenuated cercariae released from the treated snails showed normal skin penetration rate, worm burden and egg count in the liver and intestine of mice infected with plant molluscicides–attenuated cercariae were remarkably lower compared to those infected with normal cercariae, showing reduced fecundity of worms developed from attenuated cercariae. Number and size of granulomatous reactions showed significant reduction in attenuated cercariae–infected mice. Moreover AST, ALT, glycogen level and lipid peroxides were normalized in these mice when compared to control–infected group [8].

These information initiated our interest to compare the amino acid profiles of control and molluscicides–treated snail in a trail to find out if different amino acids are contributed to the previously reported remarkable reduction in snail compatibility to schistosome parasite [12],

which could easily be correlated to the attenuation of cercariae released from molluscicide–treated snails .

Organophosphate insecticides Fenitrothion represent one of the most widely used classes of pesticides with high potential for human exposure in both rural and residential environments [13]. Pesticides, including organophosphate (OP), organochlorine (OC), and carbamate (CB) compounds, are widely used in agricultural and indoor purposes. OP and CB act as acetyl cholinesterase (AChE) inhibitors that affect lots of organs such as peripheral and central nervous systems, muscles, liver, pancreas, and brain, whereas OC are neurotoxic involved in alteration of ion channels. There are several reports about metabolic disorders, hyperglycemia, and also oxidative stress in acute and chronic exposures to pesticides that are linked with diabetes and other metabolic disorders. In this respect, there are several *in vitro* and *in vivo* but few clinical studies about mechanism underlying these effects. It was found that, orally administered Fenitrothion in rats led to significant dose-dependent increase in serum glucose and cholesterol levels. Serum total protein, albumin and triglycerides were decreased not significantly in exposed groups when compared with control. In addition histopathological changes were examined in the liver represented by parenchymatous degeneration of hepatocytes with mild necrosis, leucocytic infiltration in the portal area, severe congestion and hemorrhage. Marked tubular dilation, hydropic degenerescence in tubular epithelium, moderate congestion and hemorrhage in the cortical and medulla part of the kidney. Generally the degrees of observed variations were found to be dose dependent [14].

In earlier studies, the effect of Fenitrothion has been seen on the survival of developing eggs and hatchlings, percentage hatchability, frequency of morphological and physiological deformities at different embryonic stages and survival and behavioral response of the fry/fingerlings of *Cyprinus carpio* communis Linn. [15] this study indicated Fenitrothion is one of the very toxic pesticides being used. Thus, the present study deals with the effect of Fenitrothion on the control snails strategy of molluscan host of *Schistosome mansoni.*

MATERIAL AND METHODS

Biological materials were obtained from the Medical Malacology Laboratory, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. A total of 100 normal control snails of an Egyptian strain left for 45 days to ensure that they were free from infection.

Snail maintenance

Snails were maintained in plastic trays, each containing 10 snails and 1L of aerated tap water (26±2˚C) replaced twice a week, and fed fresh lettuce and blue green algae [16]. Aquaria were cleaned weekly for removal of feces and dead snails [17].

Molluscicides-treatment

Biomophalaria alexandrina were exposed to LC_{25} of the Fenitrothion which was obtained from the toxicity lines statistically calculated according to the method of Finney [18]. LC_{25} values were dissolved in dechlorinated water [11]. Amino acid composition: High performance system Epdrof–Germany LC3000 Amino acid analyzer was used for determination of amino acid composition. The analysis was performed in the National Research Center (Unit of central services), Dokki, Cairo, Egypt.

Preparation of samples

The whole snails soft tissue was extracted and homogenized in 5 Ml saline solution. Equal volume of 10% TCA added. Protein was precipitated as while amorphous precipitate, collected by centrifugation, washed with 5%TCA solution then with ether and absolute ethanol and dried in a vacuum desiccators. Twenty five milligram protein was then hydrolyzed with 6 N HCl at 105 $^{\circ}$C for 24 h in a sealed tube [19]. After cooling and filtering, the residue was washed with distilled water and the combined filtrates were completed to 25 ml in a volumetric flask. A portion of the filtrate (5ml) was evaporated to dryness at room temperature in a desiccator under vacuum. The residue was dissolved in 5 ml buffer (0.2N sodium citrate pH 2.2) and the solution was filtered through 0.22 um membrane. Twenty microliters of the final were injected in the instrument capsule for quantitave determination of the amino acids [20]. The cationic exchange resine UL-trope sodium and special programmed buffer system were used (citrate buffer 0.2 N with three different pH at 3.2, 4.25 and 6.6 at flow rate 0.2 ml min^{-1}.). The effluent was met by a stream of ninhydrin reagent. The quanitative estimation of the amino acids depends on the colorimetric determination of blue color. Retention time and peak area were determined using computerized system for standard and sample using computerized system for standard and samples. The relative percentage of each amino acid was calculated.

Determination of total protein content

Total protein content was estimated in tissue homogenates of snail according to the method of

Bradford [21], whereas protease enzyme activity was determined in tissue homogenates of *Biomphalaria alexandrina* snail according to the method described by Church et al. [22].

Determination of estriol and progesterone

Fractions of unconjugated steroids and mono-steroid were isolated from cord haemolyph of molluscan host of Biomphalaria alexandrina snail, and the concentrations of estriol, and progesterone were determined by gas-liquid chromatography according the method of La atikainen et al. [23].

RESULTS AND DISCUSSION

The mean of different amino acids in tissue of *Biomphalaria alexandrina* snails treated with Fenitrothion are shown in table 1 and Fig 1 (a-d). It can easily be noticed that amino acid profiles of the snails were greatly manipulated with molluscicides treatment. Aspartic, glutamic, tyrosine, lysine, arginine and proline are the most affected amino acids. Amino acids are of critical importance in energy metabolism of mollusks, such as, glutamic acid represents the amino acid nitrogen pool for amino transferases to provide Krebs cycle intermediates. Moreover, amino acids are necessary for trematode-infected Biomphalaria species [9,24]. The amino acid profile of control *Biomphalaria alexandrina* snail reported in the present study is more or less similar to that reported in the Digestive Gland Gonad complex (DGG) of *Biomphalaria glabrata* [25]. Qualitative analysis revealed the presence of histidine, lysine, serine, alanine, valine and isoleucine or leucine in both samples with non significant decrease in Lysine and valine in *Echinostoma caproni* infected *Biomphalaria glabrata*. Altered amino acid spectra recorded in the present study may lead to abnormal SCW signal which in turn could disturb the snail –finding mechanisms by schistosome miracidiae [26].

Boehmler et al. [27], proved that poly L-lysine affecting behavior of hemocytes of *Biomphalaria glabrata* on the parasite. The cells showed minimal spreading, moved significantly faster and formed aggregations. In the present study, induced lysine levels is of critical importance in inducing parasite killing by hemocytes of molluscicides–treated snails. The effective Fenitrothion colud has immunostimulatory effect through lysine. This could be used to explain the decrease in snail compatibility previously recorded by El Ansary et al. [11] as reduction in the mean total number of cercariae shed/each *Biomphalaria alexandrina* snail treated with selected plant mollscicides. This could be easily correlated to the induced

levels of aspartic acid which was recorded as effective phago-stimulant in *Biomphalaria glabrata* [28].

Infection by larval trematodes, often causes a cessation of egg production of molluscan intermediate host and this is referred to as parasitic castration. Manage et al. [29] and Bai et al. [30] attributed this to the depression of biogenic monoamines (Dopamine) and the inhibition of phenol oxidase activity as an enzyme playing a major role in egg laying capacity of snails.

El Ansary et al. [12], showed that, phenyl alanine as precursor for dopamine was remarkably higher in Fenitrothion treated snail which confirmed the impairment of dopamine synthesis and correlated positively with reducing the fecundity of treated snails. Moreover, in the present study, increase of L-tyrosine (penol oxidase substrate) levels in tissues of Fenitrothion treated snails could be functionally linked to the significant reduction in fecundity of treated snails [12]. This in turn provides strong support for the crucial role of phenol oxidase in normal egg production. This suggestion could find support in the study of Bai et al [30], which assessed the effects of larval infection on L-tyrosine (PO substrate) levels in the albumin gland and ovotestis and revealed a significant increase in the level of L-tyrosine in both organs in castrated infected snails. .

Serine and alanine were significantly reduced in Fenitrothion treated snails. This could be explained on the basis of the molluscicide reduce the concentration of pyruvate through inhibition the glycolytic flux. Pyruvate is the precursor of alanine through alanine amintranferase enzyme (ALT). On the other hand, glutamate as a second product of ALT activity was not elevated in Fenitrothion treated snails. This could be easily attributed to the fact that glutamate is an amino group donor in many other transaminases reactions.

Some amino acids are anaplerotic and contribute to gluconeogensis because all or part of their carbon skeletons enter the citric acid cycle after deamination or transamination such as alanine, cysteine, glycine, serine, threonine for pyruvate, arginine, histidine, glutamine and proline are convertible to α- ketoglutarate *via* glutamine; isoleucine and valine are converted to succinyle COA, in addition to tyrosine and phenyl alanine which form fumarate. The significant elevation of glutamate, aspartic, glycine, valine, proline, isoleucine, histidine and arginine as glucogenic amino acids in Fenitrothion treated snails proved the impairment of gluconeogensis .

It has previously been postulated that L- arginine emitted by penetrating cercaiae serves as an intra-specific signal guiding other cercariae to the penetration site [31]. Moreover, Hass et al. [32] recorded that, attachment of *Schistosoma mansoni* cercariae to mammalian skin is specifically stimulated by L-arginine which acts as pheromone which could guide cercariae towards common penetration sites. In addition, schistosomula (transformed cercariae) could use L-arginine to produce nitric oxide for blood vessel dilation during their migration in the host. Based on these information, the remarkable decrease of arginine in the treated snail's tissues, could prevent the development of intramolluscan parasitic stages and abolish the release of cercariae with reduced level of arginine and hence prevent the penetration to the mammalian host skin.

The present results clearly demonstrate that, significance decrease in estradiol (E2) treated snails. This may be explained on the basis of both androgenic and estrogenic steroids are growth promoters because they significantly enhance feed efficiency, rate of gain and muscle growth. However, despite relatively little is known about the biological mechanism by which androgenic and estrogenic steroids enhance rate and efficiency of muscle growth [33]. The low concentration of estradiol in treated snails may be related to decrease rates of proliferation and protein synthesis, in addition to increase rates of protein degradation that in turn leads to decrease in total protein content as documented in the present results. The effects of estradiol is mediated at least in part through the classical receptor, estrogen receptor-α (ESR1), the IGF-1 receptor (IGFR1), and the G protein- coupled estrogen receptor (GPER)-1, formerly known as G protein-coupled receptor (GPR)-30. Based on current research results, it is becoming clear that significant decrease of anabolic steroid-suppressed snails muscle growth involves a complex interaction of numerous pathways and receptors. Consequently, additional *in vivo* and *in vitro* studies are necessary to understand the mechanisms involved in this complex process [33]. Furthermore, the significant low level of progesterone in Fenitrothion treated snails leads to poor reproductive efficiency thus, treatment option declared preventive management for control snails strategy [34].

Moreover, the current results show, significant increase in protease enzyme activity in treated snails as compared to the normal control one which again confirmed the present significant decrease of total protein content in Fenitrothion treated snails. In this context, Kraut [35] reported that, ATP-dependent proteases are responsible for the most energy-dependent protein degradation across all species. Proteases initially bind an unstructured region on a

substrate, and then translocate along the polypeptide chain, unfolding and degrading protein domains as they are encountered. Although this process is normally processive, resulting in the complete degradation of substrate proteins to small peptides, some substrates are released prematurely. Regions of low sequence complexity within the substrate, such as the glycine-rich region (GRR) from p105 or glycine-alanine repeats (GAr) from the Epstein-Barr virus nuclear antigen-1 (EBNA1) protein, can trigger partial degradation and fragment release. Loss of processivity could be due to inability to hold on to the substrate (faster release) or inability to unfold and degrade a substrate domain (slower unfolding). Previously Kraut et al. [36] showed that the GRR slows domain unfolding by the proteasome. In contrast, a recently published study by Kraut [35] concluded that GArs increase the rate of substrate release from ClpXP, a bacterial ATP-dependent protease. Here, we show that these apparently contradictory results can be reconciled through a reanalysis of the ClpXP GAr data. This reanalysis shows that, as with the proteasome, low-complexity sequences in substrates slow their unfolding and degradation by ClpXP, with little effect on release rates. Thus, despite their evolutionary distance and limited sequence identity, both ClpXP and the proteasome share a common mechanism by which substrate sequences regulate the processivity of degradation.

Thus , it could be conclude that, treatment of *Biomphlaria alexandrina* snails with sublethal concentration of Fenitrothion was effective in altering the amino acid profile of this snail species which could be contributed to the impairment of snail egg laying capacity, snail–schistosome miracidiae finding mechanisms and immune response of the molluscan hosts and abolish mammalian skin penetration rate by schistosome cercariae.

Table 1: **Amino acids concentration ug mL^{-1} changes in *Biomphalaria alexandrina* snails treated with Fenitrothion using amino acid analyzer.**

Groups		Mean ± SD	P ≤ value
Aspartic	Control	1.46 ±0.22	0.05 significant
	Treated	3.38 ±0.52*	
Glutamic	Control	21.37± 3.72	0.01 significant
	Treated	29.20±0.98**	
Serine	Control	3.74±0 .84	0.05 significant
	Treated	2.03±0 .69*	
Glycine	Control	21.46±2 .74	0.006 significant
	Treated	33.01±2.5***	
Histidine	Control	4.39±0 .71	0.01 significant
	Treated	6.04±1 .59**	

Arginine	Control Treated	2.88±0.54 1.51±0.20*	0.024 significant
Threonine	Control Treated	5.76±0.90 5.92±0.54	0.14 insignificant
Alanine	Control Treated	1.29±0.21 0.67±0.10**	0.01 significant
Proline	Control Treated	37.65±3.22 52.43±5.47**	0.01 significant
Tyrosine	Control Treated	0.32±0.54 1.23±0.02*	0.05 significant
Valine	Control Treated	0.86±0.10 1.93±0.14*	0.05 significant
Methionine	Control Treated	2.49±0.39 2.39±0.57	0.8 insignificant
Cysteine	Control Treated	2.32±0.11 2.24±0.34	0.7 insignificant
Isoleucine	Control Treated	0.54±0. 04 0.98±0.15*	0.05 significant
Phenyl-alanine	Control Treated	1.65±0.72 2.76±0.46**	0.01 significant
Lysine	Control Treated	1.17±0.14 2.12±0.72***	0.005 significant

- Statistical analysis is carried out using student T-test , where * is slightly significant at $p \leq 0.05$, ** is significance at $p \leq 0.01$ and *** is high significance at $p \leq 0.001$

Table 2: Total protein content and protease enzyme activity levels in tissue homogenate of *Biomphalaria alexandrina* snails treated with Fenitrothion.

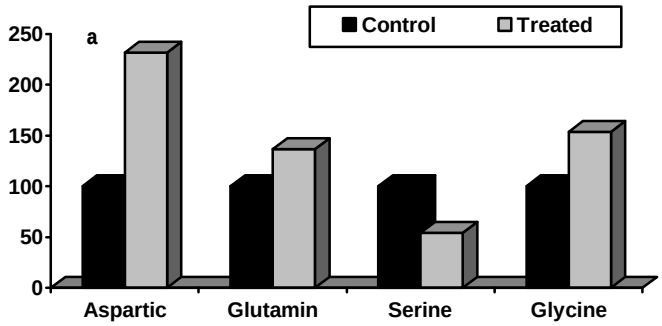
	Groups	Mean±SD	P ≤ value
Total protein	Control	5.29±0.70	0.05 significant
	Treated	2.03±0.43*	
Protease	Control	47.72±7.93	0.000 significant
	Treated	76.59±10.43***	

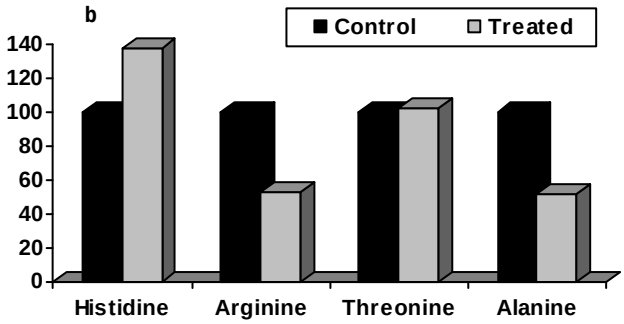
- Total protein content is expressed in mg /g tissue and protease enzyme activity is expressed in umole /min /mg protein.
- Statistical analysis is carried out using student T-test , where * is slightly significant at $p \leq 0.05$, ** is significance at $p \leq 0.01$ and *** is high significance at $p \leq 0.001$

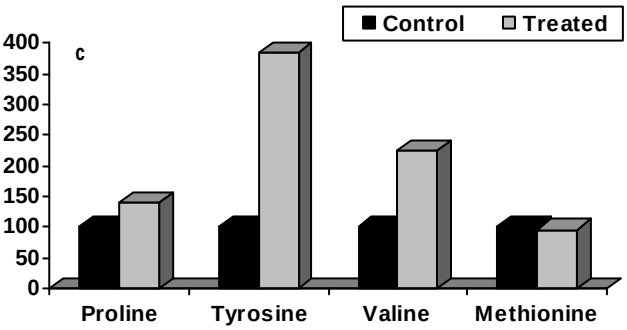
Table 3: Estriol and progesterone levels in tissue homogenate of *Biomphalaria alexandrina* snails treated with Fenitrothion (µg/100 ml of free steroid ±standard deviation)

Groups		Mean ± SD	P ≤ value
Estradiol	Control	0.56± 0.10	0.005 significant
	Treated	0.12± 0.03***	
Progesterone	Control	15.89 ±2.62	0.008 significant
	Treated	7.08±1.70***	

- Statistical analysis is carried out using student T-test , where * is slightly significant at p≤0.05 , ** is significance at p≤0.01 and *** is high significance at p≤0.001







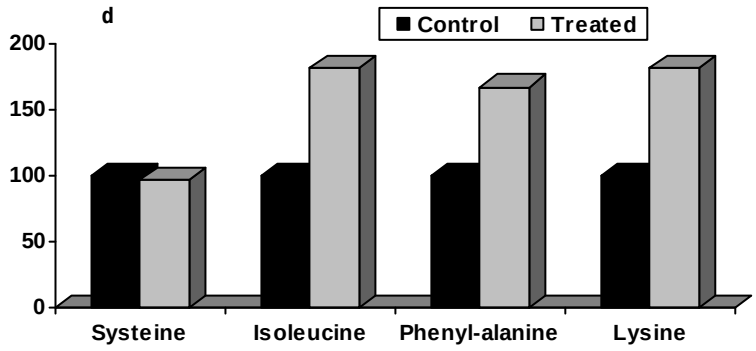


Fig. (1): The percentage change of different amino acids in *Biomphalaria Alexandria* snails treated with Fenitrothione relative to untreated snails (control).

REFERENCES

1. Engles D, Chitsulo L, Montresor A and Savioli L. The global epidemiological situations of schistosomiasis and new approach to control and research. Acta Trop, 2002; 82: 139-146.
2. Xiao S, Tanner M, N'Goran EK, Utzinger J, Chollet J, Bergquist R, Minggang C. and Fiang Z. Recent vestigation of artmether, a novel agent for the prevention of Schistosoma japonicum, S. mansoni and S. haematobium. Acta Trop, 2002; 82: 175-181.
3. Lardans V. and Dissous C. Snail control strategies for reduction in transmission (Review). Parasitol. Today, 1998; 14: 413-417.
4. El-Ansary A and Qurashy A. Factors affecting natural selection between helminthes parasites and their molluscan hosta with special reference to schistosoma. Comp. Biochem. Physiol, 1994; 108: 397-415.
5. Thompson N, Mejia-Scales V and Borchardt DB. Physiologic studies of snail-schistosome interactions and potential for improvement of in vitro culture of scliistosomes. In vitro Cell Dev. Biol, 1991; 27: 497-504.
6. Nabih I, El-Ansary A, Abdel Galil F and Zayed N. On the factors controlling metabolic integration between Schistosoma parasites and their molluscan hosts. J. Egypt Ger. Soc. Zool, 1998; 26: 87-102.
7. Haberl B, Korner M, Spengler Y, Hartel J, Kalbe M and Hass W. Host finding in *Echinostoma caproni*: Miracidia and cercariae use different signals to identify the same snail species. Parasitology, 2000; 120: 479-486.

8. Soliman MS. and El-Ansary A. Induced Changes in the Amino Acid Profile of *Biomphalaria alexandrina* Molluscan Host to *Schistosoma mansoni* Using Sublethal Concentrations of Selected Plant Molluscicides. J. Applied Sci, 2007; 7 (19): 2881-2885.
9. Schnell CA, Becker W. and Winkler A. Amino acid metabolism in the fresh water pulmonate Biomphalaria glabrata infected with the trematode Schistosoma mansoni. Comp. Biochem. Physiol, 1985; 81: 1001-1008.
10. Hata H. Essential amino acids and other essential mponents of Angiostronglus costaricensis from third-stage larvae of young adults. J. Parasitol, 1994; 80: 518-520.
11. El- Ansary A, Sammour EM, Soliman MS and Gawish FA. In vivo, attenuation of schistosome cercarial development and disturbance of egg laying capacity in Biomphalaria alexandrine using sublethal concentrations of plant molluscicides. J. Egypt Soc. Parasitol, 2001; 31: 657-569.
12. El-Ansary A, Mohamed AM, Mahmoud SS and El-Bardicy. On the pathogenicity of attenuated Schistosoma mansoni cercariae released from metabolically disturbed Biomphalaria alexandrina snails. J. Egypt Soc. Parasitol, 2003; 33: 777-794.
13. Tamura H, Maness SC, Reischmann K, Dorman DC, Gray LE and Gaido KW. Androgen receptor antagonism by the organophosphate insecticide fenitrothion. Toxicol. Sci.2001; 60**,** 56–62.
14. Jeremy M S, Kathryn EH, Darshan SA. Rose Brannon, JoeS. Parker, Matthew Weiser, Thai H. Ho, Pei-Fen Kuan, Eric Jonasch, Terrence S. Furey, Jan F. Prins, Jason D. Lieb, W. Kimryn Rathmell, and Ian J. Davis (2013).Variation in chromatin accessibility in human kidney cancer links H3K36 methyltransferase loss with widespread RNA processing defects. Published in Advance October 24, 2013, doi: 10.1101/gr.158253.113 *Genome* Res. 2013.
15. Kapur K, Kamaldeep K and Toor HS. The effect of Fenllronthion on reproduction of a Teleost fish, *Cyprinus carpio communis* Linn: A biochemical study Bull Enivirnm.Toxicol, 1978; 20: 438-442.
16. Becker W. and Lamprecht IB. The freshwater *snail Biomphalaria glabrata* is an intermediate host of the *Schistosoma mansoni*. J.Parasitol, 1977; 53: 297-305.
17. Schneck, JL, and Fried B. Growth of *Biomphalaria glabrata* (NMRI strain) and *Helisoma trivolvis* (Colorado strain) under Laboratory conditions. American Malacological Bulletin, 2005; 20: 71-73.
18. Finney DJ. Prolit Analysis: A Statistical Treatment the Sigmoid Response Curve. Cambridge University Press, London 1952.

19. Bailey JL. Techniques in Protein Chemistry. Elsevier Publishing Co., London 1967.
20. Ibrahim N. EL-Eraqy W. Protein content and amino acid composition of *Nelumbo nucifera* seeds and its evaluation as hypoglycemic agent. Egypt. Pram. Sci, 1996; 37: 635-641.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 7: 248-25.
22. Church FC, Swaisgood DP, Catignani GL. Spectrophotometric Assay Using *o*-Phthaldialdehyde for Determination of Proteolysis in Milk and Isolated Milk Proteins, 1983; 66 (6): 1219–1227.
23. La atikainen T, Peltonen J. and Nylander P. Determination of estriol, estriol sulfate, progesterone and neutral steroid mono- and disulfates in umbilical cord blood plasma. J. Clin Invest, 1973; 21(3), 347–359
24. Nabih I, El-Dardiri Z, El-Ansary A. and Rizk M. Measurement of some selected enzymatic activities in infected Biomphalaria alexandrina snails. Cell Mol. Biol, 1990; 36: 637-642.
25. Ponder EL, Fried B. and Shermal J. Free-pool amino acids in Biomphalaria glabrata infected with Echinostoma caproni as determined by thin-layer chromatography. J. Parasitol, 2004; 90: 665-666.
26. Korner M. and Hass W. Chemo-orientation of echinostome cercariae towards their snail hosts: Amino acids signals a low host specificity. Int. J. Parasitol, 1998; 28: 511-516.
27. Boehmler AM, Fryer SE and Bayne CJ. Killing of Schistosoma mansoni sporocysts by Biomphalaria glabrata hemolymph in vitro: Alteration of hemocyte behavior after poly-L-lysine treatment of plastic and the kinetics of killing by different host strains. J. Parasitol, 1996; 82: 332-335.
28. Thomas D, Kowalczyk C. and Somasundaram B. The biochemical ecology of Biomphalaria glabrata, a snail host of Schistosoma mansoni: Short chain carboxylic and amino acids as phagostimulants. Comp. Biochem. Physiol, 1989; 93: 899-911.
29. Manage P, Jianyong L, Christensen BM. and Yoshino TP. Biogenic monoamines in the fresh water snail, *Biomphalaria glabrata:* Influence of infection by the human blood fluke, *Schistosoma mansoni*. Comp. Biochem. Physiol, 1996; 114: 227-234.
30. Bai G, Johnston LA, Watson CO and Yoshino TP. Phenoloxidase activity in the reproductive system of Biomphalaria glabrata: Role in egg production and effect of schistosome infection. J. Parasitol, 1997; 83: 852-858.

31. Ingram RJ, Bartlett A, Brown MB, Marriott C. and Whitfield. Penetration of human skin by the cercariae of Schistosoma mansoni: An investigation of the effect of multiple cercarial applications. J. Helminthol, 2003; 77: 27-31.
32. Hass W, Grabe Pash T, Stoll K, Fuchs M, Haberl B, Loy DC. Recognition and invasion of human skin by Schistosoma mansoni cercariae: The key role of L-arginine. Parasitology, 2002; 124: 153-167.
33. Dayton WR. and White ME. Role of satellite cells in anabolic steroid-induced muscle growth in feedlot steers. J Anim Sci, 2014; 92(1): 30-8.
34. Katagiri S. and Moriyoshi M. Alteration of the Endometrial EGF Profile as a Potential Mechanism Connecting the Alterations in the Ovarian Steroid Hormone Profile to Embryonic Loss in Repeat Breeders and High-producing Cows. J. Reprod Dev, 2013; 59(5): 415-20.
35. Kraut DA. ATP-dependent protease, Enzyme kinetics, Pre-steady-state kinetics, Proteasome, Protein degradation, Protein denaturation, Protein evolution, Protein translocation, protein unfolding. J. Biol.Chem, 2013; 34, 22-40.
36. Kraut DA, Israeli E, Schrader EK, Patil A, Nakai K, Nanavati D, Inobe T, Matouschek A. Sequence- and species-dependence of proteasomal processivity. ACS Chem Biol, 2012; 17(8): 1444-53.