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Biochemical characterization of *Biomphalaria arabica*, the molluscan intermediate host for Schistosomes in Saudi Arabia

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Abstract

Schistosomiasis is the most important human helminth infection in terms of morbidity and mortality. The present study provides a comparative analysis on *Biomphalaria arabica*, the intermediate hosts of *Schistosoma mansoni* from two different areas having different prevalence level of Schistosomiasis in Saudi Arabia. *B. arabica* snails were collected from Riyadh and Hofuf district, and the levels of various enzymes like aspartate and alanine aminotransferase (AST, ALT), Lactate dehydrogenate (LDH), acid and alkaline phosphatase (ACP,ALP), α amylase and lipases were measured in the tissue homogenate of snails, to confirm the relationship between the biochemical and endemic of the disease. Also amino acid profile of *B. arabica* was determined using amino acid analyzer. The result showed higher levels of AST and LDH in the sample of *B. arabica* from Hofuf as compared to Riyadh while ALT, ACP, ALK, α amylase and lipase enzymes were found lower in samples from Hofuf as compare to Riyadh. The amino acid profile shows that Riyadh samples have remarkably higher levels of most of the measured amino acids. The present study confirms that biochemical profile of *B. arabica* is critically important for the success of schistosome life cycle and that snail host definitely plays a part in prevalence of the disease.

Keywords: *Biomphalaria arabica, Schistosoma mansoni,* Schistosomiasis, Saudi Arabia, Human helminthes infection.

Introduction

Schistosomiasis is the most important human helminthes infection in terms of morbidity and mortality; a recent meta-analysis assigned 2 to 15% disability weight to the disease (King *et al*., 2005). The prevalence rate of schistosomiasis in Kingdom of Saudi Arabia (KSA) was 2.2/ 100,000; the percentage of urinary schistosomiasis caused by *Schistosoma haematobium* was 33.4% while that of the intestinal schistosomiasis caused by *S. mansoni* was 66.6% (HSB, 2006). In KSA, it has been found that snail of species *Biomphalaria arabica* acts as the intermediate host for *S. mansoni* (Arfaa, 1976). Schistosomiasis or bilharzias is primary tropical parasitic disease that was first described in 1851 by Theodor Bilharz. It is caused by blood-dwelling fluke worms of the genus *schistosoma* that reside in the abdominal veins of their vertebrate definitive hosts (Chitsulo *et al.*, 2000). The distribution of the different species mainly depends on the ecology of the snail hosts (Oomen *et al*., 1990). Snail population, cercarial density and patterns of human water contact have major role in distribution of the infection in different countries (Mott, 1990).World Health Organization (2002) reports estimate that 500-600 million people in 74 tropical and subtropical countries are at risk for schistosomiasis. Over 200 million people in these countries are infected, of these, 120 million are symptomatic and 20 million having severe clinical disease. Human infection with both intestinal and urinary schistosomiasis has a wide distribution in the kingdom of Saudi Arabia (Ashi, 1989).

There are four species of snail vector existing in the kingdom which include *Biomphalaria arabica*, the intermediate host for *S.mansoni*, *Bulinus truncates* the intermediate host for *S.haematobium*, *Bulinus wright* the intermediate host for *S.haematobium* and *Bulinus beccarii* the intermediate host for *S.haematobium* (Arfaa, 1989).The prevalence of schistosomiasis is clustered in the Eastern and Southwestern provinces due to the preferable environmental conditions (Arfaa, 1976). Other factors may contribute to the increase in prevalence of the infection including the large number of expatriates, many from countries with higher prevalence of schistosomiasis, and hence the possibility of parasitic infection among them (Abahussain, 2005). Wallac (1979) studied the distribution, etiology and pathology of clinical aspects of urinary schistosomiasis in Saudi population. He observed that mortality from the disease among Saudi population is not due to parasitic ova passed by the urine and feces but due to the complications caused by fibrotic immunological reactions to the retained ova. He recommended application of hygienic, educational and snail control for the complete eradication of the disease in this country.

El-Ansary and Qurashy (1994) stated that the ability of the parasite to develop within the snail host is correlated to the snail intrinsic biochemical composition rather than any regulatory immune response. Moreover, Thompson *et al.* (1991) reported that free living stages of *schistosomes* are completely dependent on the endogenous reserves acquired from their host in the previous parasitic stage (Nabih *et al*., 1998). Aminotransferases, (AST and ALT) catalyze the interconversion of amino acids and α-keto acids by transferring amino group (Moss *et al*.,1998). The aminotransferases have an important role in the linking of the amino acids and carbohydrate metabolism, being an

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essential group of enzymes in the gluconeogenesis pathway. Beyond this, the aminotransferases are good indicators of tissue lesions (Pinhero *et al*., 2001). Acid and alkaline phosphatase (ACP and ALP) can catalyse the breakdown of ester bonds in the orthophosphate esters under acidic and alkaline conditions respectively. In *B. glabrata,* molluscan snail host to *S. manasoni,* ACP and ALP were detected histochemically among the enzymes that are important for the encapsulation reaction formed around schistosome sporocysts (McKerrow *et al.,* 1985). Biological markers have been defined as xenobioticaly-induced variations in cellular or biochemical components in any biological system (Icen *et al.,* 2005). Carbohydrate metabolizing enzymes (e.g. α-amylase), lipid degrading enzymes (e.g., lipase) were recorded among target enzymes which should be disturbed and may provide more accurate information on the molluscicide induced stress on molluscs (Abdel- Kader *et al.,* 2005). Amino acids are of critical importance in energy metabolism of mollusks since they provide intermediates of Krebs cycle, for example glutamic acid represents the amino nitrogen pool for amino transferases activities (Schnell *et al*., 1985). The concentration of free amino acids in the tissues and extracellular fluid compartments of mollusks varies with the diet, season, temperature, reproductive, developmental states, environmental stress and parasitism. Aspartate, glutamate, glycine, alanine and serine arise from the metabolism of a variety of gluconeogenic compounds and glycogen. Adjustment of these free amino acids level in freshwater species is a complicated process. It is well known that all free amino acids can be derived from the amino acids released during peptide and protein turnover and may accumulate if catabolism is slowed (Stephens, 1972). The present study is a comparative analysis of all the above mentioned biochemical profiles of *B. arabica* snails, the molluscan hosts of *S. mansoni* in Saudi Arabia, from Riyadh and Hofuf cities as these two areas have remarkable different prevalence levels of Schistosomiasis. Our results from the study concluded that biochemical profile of B. Arabica is critically important for the success of schistosome life cycle and that snail host definitely plays some part in prevalence of the disease.

Materials and methods

Sample collection and maintenance

Specimens of *B.arabica* snails were collected from Riyadh and Hofuf cities. They were fed with lettuce leaves *ad lib.* A sample of the snail was randomly chosen and dissected.

Preparation of tissue homogenates

One gram of snail soft tissue was homogenized in 5ml distilled water and then centrifuged at 3000 rpm, the supernatant was used for the biochemical analyses (Nabih I *et al*., 1989).

Enzyme assays

Measurement of transaminases: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured by kinetic diagnostic kits (United Diagnostic Industry, Riyadh). In a coupling reaction, decrease in extinction of NADH was followed at 340nm. The activity was expressed as µmoles/min/gram tissue (Henry, 1974).

Measurement of lactate dehydrogenase (LDH): The quantitative determination of LDH is achieved using lactate to pyruvate kinetic method. Activity was evaluated by measuring the extinction decrease in NADH at 340 nm (Henry, 1979).

Measurement of phosphatase: ACP and ALP were measured according to the method of Bowers and Macomb (Bowers & McComb, 1966) using a kinetic diagnostic kits, a product of United Diagnostic Industry, Riyadh, KSA.

Measurement of lipase: The quantitative determination of lipase is achieved by kinetic diagnostic kit (United Diagnostic Industry, Riyadh, KSA). The method based on the decrease in the turbidity of reaction caused by lipase, which leads to decrease in the absorbance measured at 400nm (Vogel & Zieve, 1963).

Measurement of α-amylase: Amylase was measured according to the method of Wallenfels *et al.*, (1978) using kinetic diagnostic kits, a product of United Diagnostic Industry, Riyadh, KSA.

Amino acid composition: Amino acid composition was determined using an Automatic Amino acid analyzer S 433 that was obtained from Sykam.

Preparation of samples

Whole snail's soft tissue was extracted and homogenized in 5ml saline solution. Equal volume of 10% TCA added. Protein was precipitated as white amorphous precipitate, collected by centrifugation, washed with 5% TCA solution then with ether and absolute ethanol and dried in vacuum desiccators. Twenty five milligram protein was then hydrolyzed with 6N HCL at 105ºC for 24h in a sealed tube (Bailey, 1967). After cooling and filtering, the residue was washed with distilled water and the combined filtrates were completed to 25ml in a volumetric flask. A portion of filtrate (5ml) was evaporated to dryness at room temperature in desiccators under vacuum. The residue was dissolved in 5ml buffer (0.2N sodium citrate pH 2.2) and the solution was filtered through 0.22 µm membrane. Twenty micro liters of final filtrate were injected in the instrument capsule for quantitative determination of the amino acids (Ibrahim, & El-Eraqy, 1996). The quantitative estimation of the amino acid depends on the blue color. Retention time and peak area were determined using computerized system for

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standard and samples. The relative percentage of each amino acid was calculated.

Statistical analysis

The statistical analysis of the data was carried out using one-sample t-test by Statistical Package of Social Science (SPSS) software (version 10.0), $p<0.05$ were considered significant.

Table 1. AST, ALT, and LDH activity in B.arabica tissue homogenates of Riyadh and Hofuf samples

Enzyme	*Activity in µ/g tissue ± S.D		♣P Value	
	Riyadh	Hofuf	Riyadh	Hofuf
AST	0.17±0.04	0.82±0.53	0.00	0.013
ALT	2.45±0.33	2.013±0.92	0.00	0.003
AST/ALT	0.07±0.02	0.86±0.86	0.00	0.139
LDH	1.16±0.29	1.31±1.96	0.00	0.00
LDH/ALT	0.49±0.17	1.2±1.04	0.00	0.014

***** Data are presented as mean ±S.D in all experiments.
♣ Significant level at P<0.05.

Results and discussion

Comparative analysis of enzyme like AST, ALT, LDH activity in *B.arabica* tissue homogenates of Riyadh and Hofuf samples are shown in table 1 and ACP, ALP, α-amylase, and Lipase activity are shown in table 2. The percentage change of AST ALT,AST/ALT, LDH, LDH/ALT, ACP, ALP, α-amylase, and Lipase activities in tissue homogenates of *B.arabica* from Riyadh and Hofuf samples are shown separately from Fig. 1 to Fig. 9 respectively. Enzymatic activity of AST in tissue homogenates of *B.arabica* from Riyadh and Hofuf as shown in table 1 are much higher than those recoded by Nabih *et al.,* (1989) for *B.alexandrina,* a molluscan host for *S.mansoni* in Egypt. This could be easily correlated to the remarkable variation in the prevalence of schistosomsis in both countries.

Lower value of AST in *B.alexandrina* snails proved that they are aerobes but highly adapted to intramolluscan anaerobic conditions usually induced by the developing schistosome parasite. This explanation could find a support through comparing the difference in AST activities of *B.arabica* from Riyadh and Hofuf. Riyadh samples have significantly lower AST value compared to Hofuf which show high prevalence of the disease. As shown in Table 1, it could be easily noticed that *B.arabica* has significant difference in ALT activity in relation to the area from where the snails were obtained. The increase of ALT activity compared to *B.alexandrina* snails could be used as measure of susceptibility to *schistosame* infection, since pyruvate as a product of ALT is usually metabolized to lactate by LDH (Nabih and El-Ansary 1991).

Accumulation of lactate usually reduces the capacity of schistosome parasite to adapt the anaerobic conditions within the snail's tissues. LDH activity in tissue homogenates as shown in Table1 from Riyadh and Hofuf shows more or less similar activities when compared to LDH of *B.alexandrin* from Egypt. On the other hand, difference in LDH activity between Riyadh and Hofuf could be helpful to consider the variation in prevalence level.

Table 2. ACP, ALP, α-amylase, and Lipase activity in B.arabica tissue homogenates of Riyadh and Hofuf samples

Enzyme	*Activity in µ /g tissue ± S.D		♣P Value	
	Riyadh	Hofuf	Riyadh	Hofuf
ACP	0.98±0.28	0.74±0.15	0.00	0.00
ALP	2.1±1.47	1.24±0.22	0.009	0.00
α-amylase	10.08±2.29	5.29±1.27	0.00	0.00
Lipase	53.5±17.5	26±13.9	0.009	0.033

***** Data are presented as mean ±S.D in all experiments.
♣ Significant level at P<0.05.

Goromosova and Tamozhnya (1984) proved that the correlation of AST and ALT served as index of the metabolic "aerobity" degree or relative role of the aerobic and anaerobic parts in the energetic metabolism in the invertebrates. The comparison of AST/ALT activities ratio with the viewpoint of ecology showed that the invertebrates were divided into three major groups. The first group classified as aerobic, but highly adapted to anaerobic condition with AST/ALT value fluctuated within 0.3-1. The second group with higher AST/ALT ratio (1-1.7) lived in the surface zone, at the same depth where a sufficiently intensive oxygenation of the surrounding water being found. In this group, the AST was higher than that of the facultative anaerobes.

The third group unites oxyphilic and mobile mollusks and crustacean in which the AST/ALT was 2.0 and higher. In the present study AST/ALT ratio show values of 0.07±0.02 and0.86±0.86 for Riyadh and Hofuf respectively. So both samples could be classified as facultative anaerobes, but with those of Riyadh are less adapted to anaerobic conditions as showing a value lower than 0.3 for AST/ALT ratio.

Considering the LDH/ALT ratio, Goromosova and Tamozhnya recorded that a ratio of 0.04-0.1 was observed in sponges and hydroids. It was somewhat higher than 0.2 in a number of the little mobile gastropods and attached bivalve mollusks. In the present study, LDH/ALT ratio in *B.arabica* snails recoded values of 0.49±0.17 and 1.2±1.04 from Riyadh and Hofuf respectively (Table1).

According to Goromosova and Tamozhnya (1984) and Nabih *et al.* (1989), snails from Riyadh show lower adaptive capabilities to anaerobic respiration which could be related to the difference of disease prevalence between the two cities with Hofuf showing higher prevalence. Thus, the biochemical profile of the snail host could easily affect the developing parasite survival within the snail's tissues and confirmed the importance of metabolic integration in the success of snail-schistosome relationship previously reported by El-Ansary and Qurashy(1994).

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Fig.1. The percentage change of AST activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

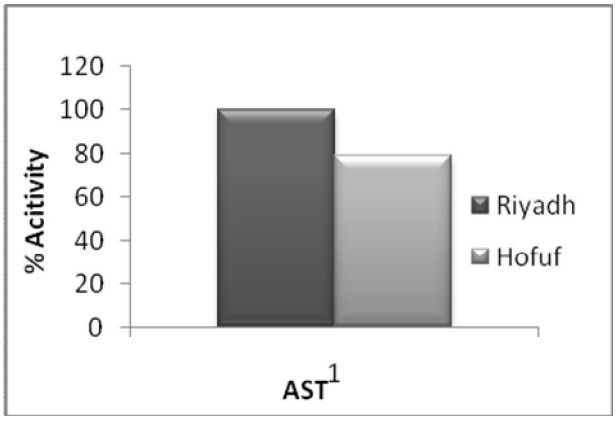

Fig.2. The percentage change of ALT activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

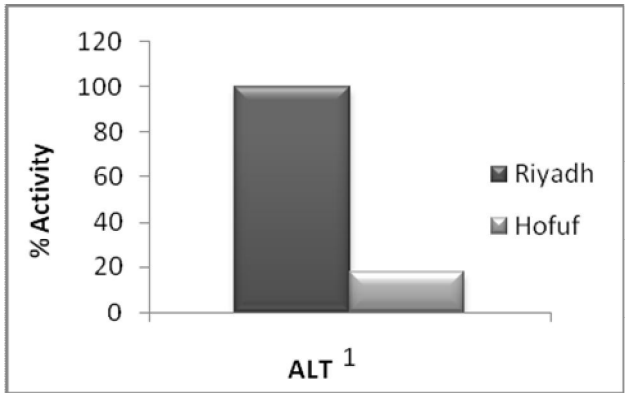

Fig.3. The percentage change of AST/ALT activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

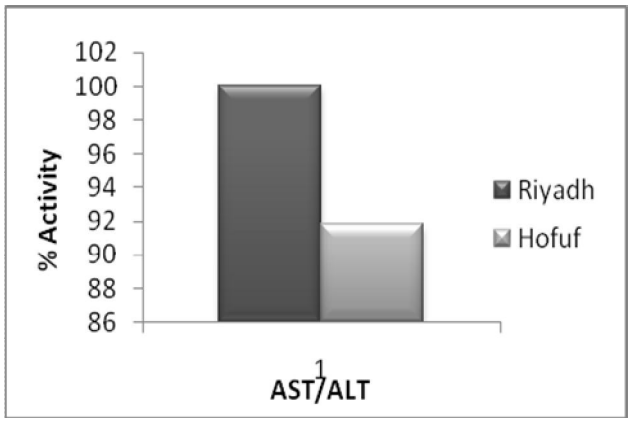

Fig.4. The percentage change of LDH activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

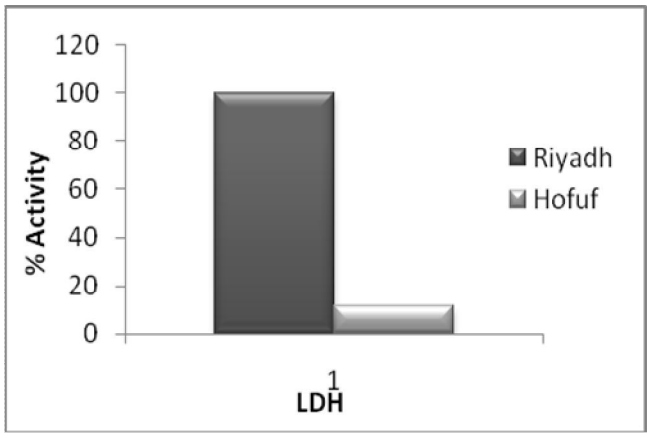

Fig.5.The percentage change of LDH/ALT activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

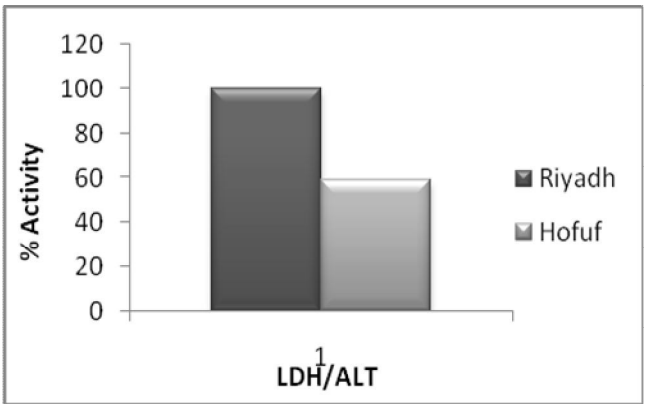

Fig.6. The percentage change of Acid Phosphatase activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

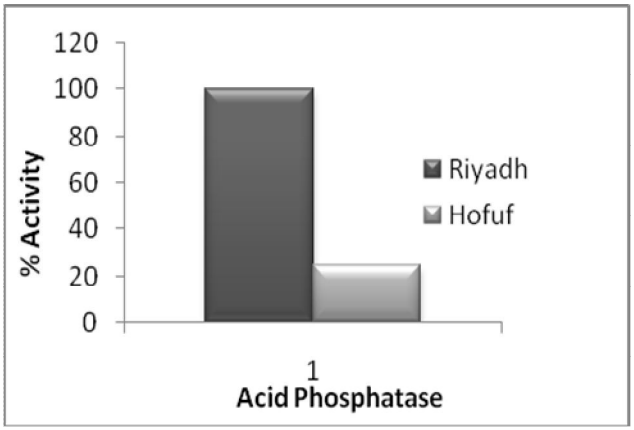

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Fig.7. The percentage change of Alkaline Phosphatase activities in tissue homogenates from B.arabica of Riyadh and Hofuf samples.

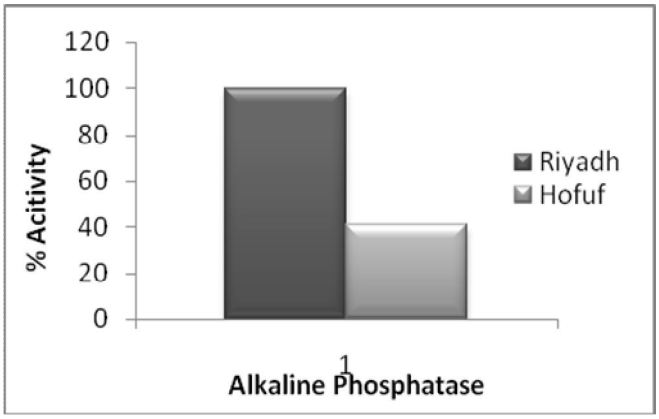


Fig.8. The percentage change of Amylase activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples.

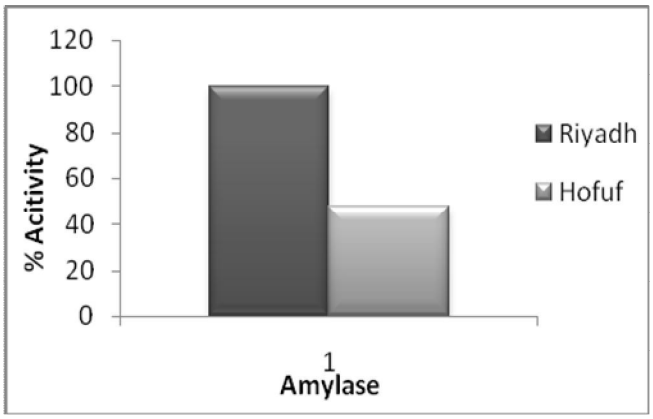


Fig.9. The percentage change of Lipase activities in tissue homogenates of B.arabica from Riyadh and Hofuf samples

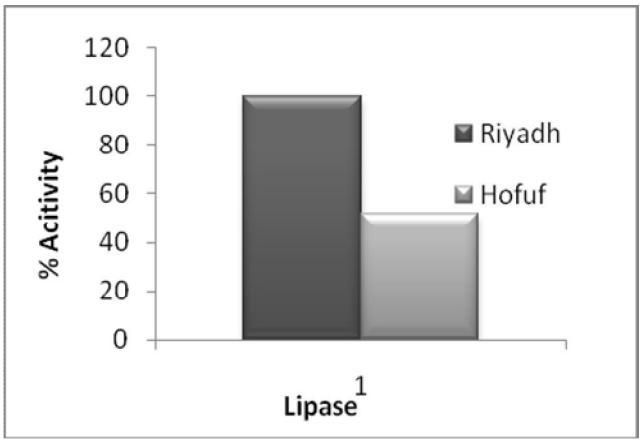


Fig.10. The percentage change of glucogenic amino acid in snail tissue homogenates of B.arabica from Riyadh and Hofuf samples

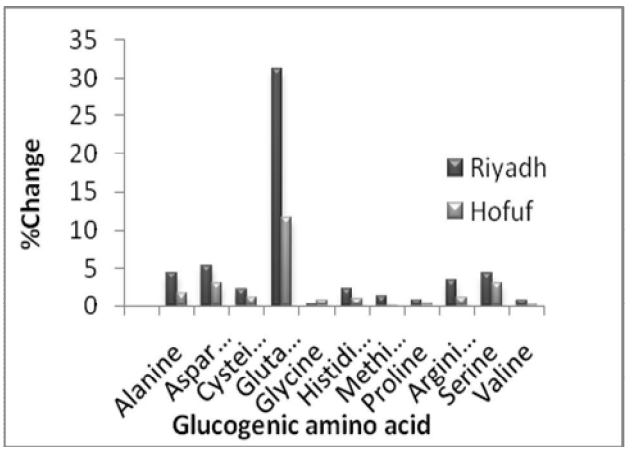


Fig.11. The percentage change of ketogenic amino acid in snail tissue homogenates of B.arabica from Riyadh

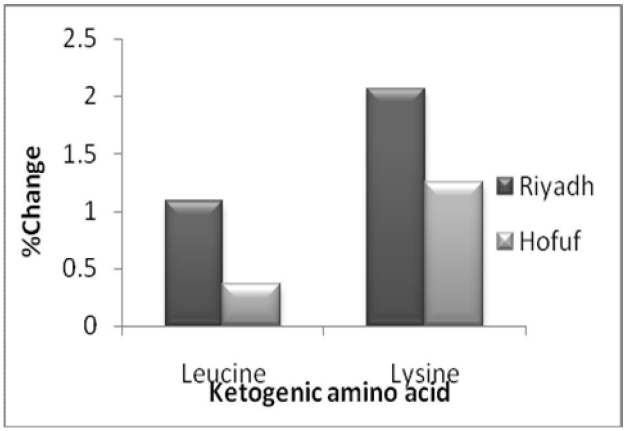
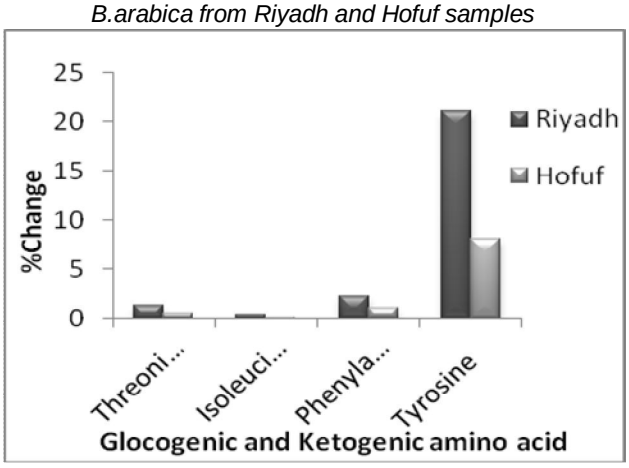


Fig. 12. The percentage change of glucogenic and ketogenic amino acid in snail tissue homogenates of B.arabica from Riyadh and Hofuf samples



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A significant difference in the distribution and abundance of acid phosphatase as lysosomal enzyme marker in blood cells (hemocytes) of schistosome-susceptible (PR albino M-line) and a resistant (10-R2) strain of *B. glabrata* during the course of infection with *S. manasoni* was recorded by Granath and Yoshino (1983) and Sasaki *et al.*(2005). They reported that, a significant increase of ACP after two weeks post infection, indicating a possible response to tissue damage resulting from migrating daughter sporocysts. On the other hand, alkaline phosphatase does not show any significant difference in activity between normal and infected snails (Dardenne *et al* 1979). In the present study, ACP and ALP level measured in tissue homogenates of *B.arabica* snails (Table 2) shows that snails from Hofuf has significantly lower ACP activity as an enzyme involved in the cellular immune response of the host resulting in the killing of larger number of intramolluscan schistosome sporocysts which in turn will lead to shedding of fewer number of cercariae. Lower activity of this enzyme could be easily related to the success of the host-parasite relationship and helps the development of schistosome parasite within the snail host. This could support the previous reports of Mubila and Rollinson, (2002) that the variation in prevalence of schistosomasis was partly related to the snail host.

Table 3. The glucogenic amino acid concentration (µgml^{-1}) changes in B.arabica snail tissue homogenates of Riyadh and Hofuf samples

Glucogenic amino acid	Riyadh (µgml^{-1})	Hofuf (µgml^{-1})
Alanine	4.41	1.641
Aspartic acid	5.19	3.028
Cysteine	2.25	1.15
Glutamic acid	31.2	11.65
Glycine	0.25	0.68
Histidine	2.33	1.03
Methionine	1.25	0.09
Proline	0.82	0.32
Arginine	3.41	1.07
Serine	4.25	3.09
Valine	0.83	0.11

Recently, Al Daihan (2008) proved that α-amylase and lipase were significantly reduced in *S.nigrem* treated snails and that inhibition of these two enzymes could affect the development of schistosome parasite within snail's tissues. In the present study, surprisingly both enzymes were found to be lower in Hofuf compared to Riyadh which is not supporting the phenomenon of prevalence related to biochemical profile of the snail host

Table 4.The ketogenic amino acid concentration (µgml^{-1}) changes in B.arabica snail tissue homogenates of Riyadh and Hofuf samples

Ketogenic amino acid	Riyadh (µgml^{-1})	Hofuf (µgml^{-1})
Leucine	1.08	0.36
Lysine	2.07	1.25

since both α-amylase and lipase are important for the provident of glucose and free fatty acids respectively for the developing parasite.

Table 5. The glucogenic & ketogenic amino acid concentration (µgml^{-1}) changes in B.arabica snail tissue homogenates of Riyadh and Hofuf samples

Glucogenic and Ketogenic amino acid	Riyadh (µgml^{-1})	Hofuf (µgml^{-1})
Threonine	1.33	0.53
Isoleucine	0.37	0.13
Phenylalanine	2.21	1.07
Tyrosine	21.03	8.13

However, since these two enzymes are involved in the feeding and reproductive competence at the molluscan population level, so lower activity in *B.arabica* from Hofuf could spare more enough energy to support the development of intramolluscan stages of schistosome parasite as a parasite highly sensitive to any change in the ATP level (El- Ansary 1999).

Table 3, 4, and 5 show (glucogenic, ketogenic and glucogenic & ketogenic respectively) amino acid profiles of tissue homogenates of *B.arabica* from Riyadh and Hofuf. The percentage change of amino acid in snail tissue homogenates of *B.arabica* from Riyadh and Hofuf samples are shown in Fig 10, 11, and 12. It could be easily noticed that Riyadh samples have remarkably higher levels of most of the measured amino acids. Regarding glycogenic amino acids they reported higher level in Riyadh that could easily reflects the impairment of gluconeogenesis pathway. This could affect parasitic development since schistosome infected snails usually uptake their glucose requirements from snails tissues.

Reduction in free amino acid levels in haemolymph and tissues of mollusks during schistosome infection have been reported which proved their uptake by the developing parasite (Schnell *et al.,* 1985). Certain amino acids (Glu, Asp, Gly and Gln) have stimulatory effects on the development of parasitic helminthes (Hata, 1994) because schistosomes have usual nutritional requirement for essential amino acids. Parasitic absorption and utilization likely explain much observed decrease.

Boehmler *et al.* (1996) proved that poly L-lysine affecting behavior of hemocytes of *Biomphalaria globrata* on the parasite. The cell showed minimal spreading, moved significantly faster and formed aggregates. In the present study, significantly higher level of lysine in samples obtained from Riyadh could be helpful in inducing parasite killing by hemocytes of Riyadh *B.arabica* snails. On the other hand snails from Hofuf could have weak phagocytic activity of hemocyte, and hence poor parasite killing due to the reduced lysine concentration. Higher lysine level together with the previously observed higher ACP activity could clarify that *B. arabica* snails from Riyadh may have higher cellular reactions and hence be less compatible for the development of schistosome parasite. This could explain

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the lower prevalence of schistosomiasis in Riyadh compared to Hofuf.

Infection by larval trematodes often causes a cessation of egg production of molluscan intermediate host and this is referred to as parasitic castration. Manager *et al.* (1996)and Bai *et al.* (1997)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* (2003) showed that sublethal concentration of selected plant molluscicides was effective in reducing the fecundity of the treated snails. This could find support in the present study, since phenyl alanine as a precursor for dopamine was remarkably higher in Riyadh snails compared to Hofuf, which could be related to the less efficient dopamine synthetic pathway. Moreover, in the present study, increase of L-tyrosine (phenol oxidase substrate) levels in tissues of Riyadh snails could be functionally linked to the significant reduction in fecundity and hence prevalence of the disease in Riyadh compared to Hofuf.

Conclusion

The present study confirms that snail's biochemical profile is critically important for the success of schistosome life cycle and that snail's host definitely plays role in the prevalence of the disease.

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