

BIOCHEMICAL STUDIES ON THE EFFECT OF SAPONINS
EXTRACTED FROM CUCURBITA FOETIDISSMA
PLANT ON BIOMPHALARIA SNAILS

(With 3 Tables)

By

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**دراسات بيوكيميائية على تأثير الصابونينات
المستخلصة من نبات كوكوربيتا فوتيديسما
على قواقع البيومفالاريا**

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قام الباحثون بدراسة تأثير الصابونينات المستخلصة من جذور وثمار نبات كوكوربيتا فوتيديسما (المعروف باسم جاموس الأرض) وذلك على مستويات الترايجليسرايدات ، الكوليسترول ، اليوريا ، حمض البولييك والكرياتنين فى قواقع بيومفالاريا الكسندرينا وبيومفالاريا جلابراتا . كما تم تعيين مستوى الانزيمات اسبارتايت امينوترانسفيريز والالانين امينوترانسفيريز فى هذه القواقع بعد معاملتها بالتركيز القاتل لخمسين فى المائة من هذه الصابونينات . وقد لوحظ وجود ارتفاع ذو دلالة احصائية فى مستويات الترايجليسرايدات والكوليسترول فى قواقع بيومفالاريا الكسندرينا المعاملة بالصابونينات المستخلصة من جذور النباتات بينما كانت الزيادة فى الكوليسترول فقط فى نفس القواقع عند معاملتها بصابونينات الثمار . كما لوحظ زيادة فى مستوى حامض البولييك فى القواقع المعرضة لصابونينات الجذر والثمار . كذلك لوحظ ارتفاع فى مستوى اليوريا فى قواقع بيومفالاريا جلابراتا المعاملة بصابونينات الثمار فقط ، أما الكرياتينين فلم يتأثر مستواه فى نوعى القواقع ويرى الباحثون ان هذه النتائج قد تكون ذات تأثير هام فى المقاومة البيولوجية للقواقع الناقلة للبلهارسيا .

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SUMMARY

The effect of saponins obtained from root and fruit of *Cucurbita foetidissima* plant (commonly called buffalo ground) at their LC50 on triglycerides, cholesterol, urea, uric acid and creatinine levels in *Biomphalaria alexandrina* and *Biomphalaria glabrata* snails was studied. Moreover, the levels of aspartate aminotransferase (AST) and alanine amino transferase (ALT) enzymes were determined in both *Biomphalaria* snails species treated with the LC50 of saponins obtained from the fruit and root of "buffalo ground" plant. It was observed that there is a highly significant increase in the levels of triglycerides and cholesterol in *B. alexandrina* snails treated with saponin extracted from the root of the plant and in cholesterol content only in *B. alexandrina* snails treated with saponin extracted from the fruit of the plant. Uric acid level increased in *B. alexandrina* snails treated with saponins obtained from the root and fruit of the plant. Saponin obtained from the fruit exhibited a slight increase in the level of urea in treated *B. glabrata*. Creatinine level was unchanged in all treated groups. It is hoped that these results might help in biological control of *Schistosoma* transmitting snails.

Keywords: Biochemical effect, saponin, *Cucurbita foetidissima* plant
Biomphalaria, snails

INTRODUCTION

While the prevalence of schistosomiasis has been reduced in a few countries such as Japan and Venezuela, there is abundant evidence from Africa, the Middle East, South America and South East Asia that the disease is spreading and increasing in intensity; largely as a result of man-made water resources development related to increase power and food requirements (WEBBE and JAMES *et al.*, 1977). Such evidence lends emphasis and justification to the search for naturally occurring plants to combat *Biomphalaria* snails which are the intermediate host for bilharziasis and to avoid more pollution of the environment as chemical molluscicides affect fish and pollute the water (WHO, 1965). Naturally, plant molluscicides must be effective at lower concentrations, easily applicable

and cheap (HILAL et al., 1988). Recently, FADDAH (1994) screened some water soluble saponins from seeds of *Luffa cylindrica* L. and *Momordica charantia* L. for their molluscicidal activity against *B. alexandrina* snails. It was found that they have a potent molluscicidal activity and exhibited a significant suppressive effect not only on the number of egg masses but also on the rate of the hatchability of these eggs and on the growth rate of the newly hatched *B. alexandrina* snails.

Saponins obtained from the root of "buffalo ground" plant was firstly isolated by MARIE et al. (1988). In the present study, it was the first time to isolate saponin obtained from the fruit of the plant which is recently cultivated in Egypt. In a previous work, (FADDAH et al., 1994) declared that saponins obtained from root and fruit of "buffalo ground" plant have a potent molluscicidal activity against *B. alexandrina* and *B. glabrata* and also studied the effect of these saponins on the levels of some catecholamines and glucose in the same snails. Continuing the previous work and in order to attain more information, the effect of the newly extracted "Buffalo ground" saponin, some metabolic parameters were studied in these snails. This is considered as a trial to elucidate the changes that may be induced within the snails's body leading to alteration in their normal patterns, hence influence the vitality of *Biomphalaria* snails. So, it was of interest to measure the levels of cholesterol, triglycerides and some enzymes such as AST and ALT. Moreover, the effect of these saponins on metabolic end products (urea, uric acid and creatinine) in *Biomphalaria* snails were detected which may have a harmful effect on these snails hoping to gain more understanding correlation between these parameters.

MATERIAL AND METHODS

I. Sources:

Biomphalaria alexandrina snails (shell diameter 6-8 mm) were obtained from water collections of Abu Rawash (Giza Governorate). *Biomphalaria glabrata* snails were laboratory bred snails obtained from strains that occur naturally in Brazil and Venezuela (shell diameter about 20 mm). Snails were maintained in the laboratory under standard conditions of feeding and aeration. They were fed on fresh lettuce leaves, aeriated with air pumps and kept at 25-30 °C for 5-6 weeks.

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II. Materials:

Saponins used in this study were extracted from the root and fruit of *Cucurbita foetidissima* plant which is cultivated in the farm of National Research Centre (Cairo).

- All chemicals were obtained from Sigma (USA), Merck (Germany) and El Nasr pharmaceutical companies.
- Cholesterol was measured using diagnostic kits obtained from Boehringer Mannheim Germany.
- Triglycerides kits was obtained from Chimica.
- AST and ALT kits were obtained from Egyptian American Co. for lab. services.
- Urea and creatinine kits were obtained from Boehringer Mannheim Germany.
- All reagents used for uric acid analysis were of analytical grade. Sodium tungstate, sulfuric acid and Sodium carbonate were obtained from Sigma, BDH chemical companies

III. Methods:

Preparation of Plant Saponins:

Saponins were extracted using the method of *MARIE et al.* (1988). Defatted powdered fruits and roots of buffalo ground plant were extracted with chloroform and then with methanol (80%). The methanolic extract was evaporated to a syrupy brown liquid which was dissolved in water and extracted with n. butanol saturated with water. The butanolic extract was evaporated under reduced pressure. The residue was dissolved in hot 90% alcohol and saponins were precipitated by addition of large volume of acetone. The obtained saponins were purified again by mixing with magnesium oxide aqueous paste to form saponin magnesium complex. The paste was then dried on boiling water bath and the saponins were released from the complex by boiling with alcohol and filtered. The filtrate was evaporated to dryness, the residue dissolved in the least amount of alcohol and precipitated again by large volume of acetone, then filtered and kept away from light, in vacuum desiccator. The saponin is yellowish white in colour and give positive Liebermann-Burchard and Molish's test. It also gives voluminous froth when shaken with water and haemolyzed red blood cells.

Treatment:

Six groups of the snails (100 snails each) (exposure time is 24 hours). The LC50 of the saponin from the root of the plant was (150, 160 ppm) while for that obtained from the fruit was (140, 150 ppm) for *B. alexandrina* and *B. glabrata* respectively (*FADDAH et al.*, 1994)

1. **First group:** was composed of *B. alexandrina* snails treated with the LC50 of saponin extracted from the fruit of the plant.
2. **Second Group:** Consisted of *B. alexandrina* snails treated with LC50 of the saponin extracted from the root of the plant.
3. **Third Group:** Consisted of *B. glabrata* snails treated with LC50 of the saponin isolated from the fruit.
4. **Fourth group:** Consisted of *B. glabrata* snail treated with LC50 of saponin isolated from the root of the plant.
5. **Fifth group:** Control untreated *B. alexandrina* snails.
6. **Sixth group:** Control untreated *B. glabrata* snails.

Preparation of tissue homogenate:

The tissue homogenate of all the previous groups was prepared (1 g/5 ml bidistilled water) centrifuged and the supernatant liquid was subjected to the following analysis:

Estimation of Cholesterol:

Cholesterol was assayed using the method of *KATTERMANN et al.* (1984).

Determination of Triglycerides:

The measurement of triglycerides was performed according to *YOUNG* (1975).

Measurement of Urea:

urea was estimated in the tissue homogenate of the six groups as described by *FAWCETT and SCOTT* (1960).

Estimation of Uric Acid:

This was performed using phosphotungstic acid (*CARAWAY*, 1963).

Determination of Creatinine:

Creatinine was determined according to *POPPER et al.* (1937).

Measurement of Transaminases:

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the method of *TEITZ* (1970).

RESULTS

Data listed in Table (1) demonstrated the effect of saponin extracted from the root of "buffalo ground" plant on the levels of cholesterol, triglycerides, urea, uric acid and creatinine in *B. alexandrina* and *B. glabrata* snails.

It was observed from Table (1) that treatment of the snails with saponin obtained from "buffalo ground" root exhibited a marked increase in cholesterol and triglycerides level in *Biomphalaria* snails. Uric acid level increased in *B. alexandrina* snails treated with root saponin. Urea and creatinine levels were unchanged in treated snails

table (2) illustrates the effect of saponin obtained from the fruit of "buffalo ground" plant on the levels of cholesterol, triglycerides, urea, uric acid and creatinine in *B. alexandrina* and *B. glabrata* snails.

It was observed from data listed in Table (2) that saponins obtained from "buffalo ground" fruit induced a highly significant increase in the level of triglyceride and uric acid in *B. alexandrina* snails and a significant increase in urea content in *B. glabrata* snails.

Measurement of the level of ALT and AST in *Biomphalaria* treated snails:

Data listed in table (3) illustrate the effect of "buffalo ground" saponins on the levels of AST and ALT enzymes.

It is clear from Table (3) that saponions obtained from fruit and root of the plant moderately affect the levels of AST and ALT in *Biomphalaria* snails.

DISCUSSION

The use of naturally occurring molluscicides is considered an effective tool in prevention of bilharziasis (HILAL et al., 1988). Saponins are of the pharmacodynamic groups which have biological activities. Some of them are haemolytic and others are not. They are used as expectorant and antimicrobial agents (TSCHESCHE and WULFF, 1973). A few plants were screened for the molluscicidal activity of the contained saponins e.g. *Canna indica* (MAHRAN, et al., 1979). Recently, FADDAH (1994) found that saponins from *L. cylindrica* L. and *M. charanita* L. have a significant suppressive effect not only on the number of egg masses but also on the number of the hatched eggs with a decrease in their growth rate. FERGUSON (1955) found that alcoholic extract obtained from the root of "buffalo ground"

plant exhibited some oxytocic action, spasmolytic effect on the isolated intestine and constriction of coronary vessels of rat's heart. FADDAH *et al.* (1994) found that saponins obtained from the root or fruit of "buffalo ground" plant was considered as potent molluscicides and induced marked decline effect on the egg laying and on the hatchability rate of these eggs in *B. alexandrina* and *B. glabrata* snails. Moreover, they found that saponins obtained from the fruit are more effective than the obtained from the root in increasing the level of nor-epinephrine in both snail species, on the level of epinephrine in *B. alexandrina* snails and on the dopamine level in *B. glabrata*. Glucose and lactate dehydrogenase levels were markedly increased especially in *B. glabrata* snails treated with saponin obtained from the root of the plant. The increase in the glucose level may lead to enhance the rate of glycolysis and hence elevate the level of pyruvate, the latter by oxidative decarboxylation gives rise to acetyl con which is a building block of cholesterol (HARPERS, 1993).

In the present study, the level of cholesterol was increased in *Biomphalaria* snails treated with the saponins of "buffalo ground" plant. This agrees with the results of ISHAK *et al.* (1975) who found that glycogen break down increases in infected snails which may lead to increase fat deposition; and also with results of FADDAH *et al.* (1992) who declared that the level of nor-epinephrine increased in the same snails treated with "buffalo ground" saponins and hence increase glycogenolysis.

The level of triglycerides was increased in the present study which in turn increase the level of glycerol and fatty acids, the latter undergoes β -oxidation to give acetate. Active acetate involved in the biosynthesis of fatty acids which is in turn combined with the great amount of alpha-glycerol phosphate which resulted from glycolysis to give out triglycerides (HARPERS, 1993). Previous studies by BARLOV (1966) showed that cholesterol content increased with the increase in triglycerides level in snails which agrees with the present results. Cholesterol and uric acid levels were increased in the present study. This finding is in accordance with that of SCHOENFELD and GOLDBERGER (1963) who found that there was a positive correlation between cholesterol and uric acid content in infected *B. alexandrina* snails. FADDAH *et al.* (1992) measured the levels of urea, creatinine, uric acid in *B. alexandrina* snails subjected to UV and gamma radiations and also in infected snails. It was observed that there was a marked increase in their levels. Since the levels of urea, uric acid and creatinine are ranging within certain limits in the

snail's body, any disturbance in these metabolic end products may be harmful or toxic to both snails and cercarial liability for infection. Uric acid level increased in *B. alexandrina* snails treated with saponins of the plant.

AST and ALT levels were normal. So these saponins have no effect on gluconeogenesis. This result coincides with that obtained by EL-ANSARY and FADDAH (1993) who declared that AST and ALT were not affected by treatment of *B. alexandrina* snails with harmful substances such as organophosphorus insecticides.

In conclusion, any biochemical disturbance in the normal metabolic pattern of *Biomphalaria* snails may lead to break the life cycle which is highly specific and depend mainly on the species of *Biomphalaria* intermediate host. This may help in decreasing the incidence of schistosomiasis transmitting snails.

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Table (1): Effect of "buffalo ground" saponin obtained from the root of "buffalo ground" plant on the levels of cholesterol, triglycerides, urea, uric acid and creatinine in *B.alexandrina* and *B.glabrata* snails (mg/100 m).

	<i>B.alexandrina</i>		<i>B.glabrata</i>	
	Control	Treated snails	Control	Treated snails
Triglycerides X+ S.D.	70.47±4.3	100.63±8.1	200.65±1.3	320.87±2.4
P < % Change		0.001±29.9		0.001±59.9
Cholesterol X+ S.D.	15.155±2.1	37.27±3.4	15.75±1.5	24.7±5.1
P < % Change		0.001±145.9		0.001±56.8
Urea X+ S.D.	20.55±3.9	21.96±4.5	28.5±4.3	27.9±5
P < % Change		N.S. + 6.8		N.S. - 2.1
Uric Acid X+ S.D.	2.7±0.21	4.21±0.25	2.76±0.34	2.94±0.48
P < % Change		0.001±55.9		N.S. + 6.5
Creatinine X + S.D.	0.99±0.02	0.955±0.034	1.53±0.043	1.505±0.021
P < % Change		N.S. + 3.5		N.S. + 1.6

Data are mean of 10 determinations
P < 0.001 highly significant.

Table (2): Effect of saponin obtained from the fruit "buffalo ground" plant on the level of cholesterol, triglycerides, urea, uric acid and creatinine in *Biomphalaria* (mg/100 ml).

	<i>B.alexandrina</i>		<i>B.glabrata</i>	
	Control	Treated snails	Control	Treated snails
Triglycerides X+ S.D.	70.47±5.1	69.4±6.2	200.65±15.3	198.4±13.1
P < % Change		N.S.+1.51		N.S.1.12
Cholesterol X+ S.D.	15.155±2.1	42.1±3.4	15.75±2.4	16.975±4.1
P < % Change		0.001±177.7		N.S. 7.7
Urea X+ S.D.	20.55±2.2	19.8±3.4	28.5±2.4	33.18±5.3
P < % Change		N.S. + 3.6		0.01 16.4
Uric Acid X+ S.D.	2.7±0.21	3.606±0.03	2.76±0.04	2.58±0.09
P < % Change		0.001±33.5		N.S. + 6.5
Creatinine X + S.D.	0.99±0.02	1.08±0.09	0.53±0.023	1.47±0.0432
P < % Change		N.S. + 9		N.S. + 3.9

Data are mean of 10 determinations
P < 0.001 Highly significant
P < 0.01 Significant

Table (3): Effect of saponins of "buffalo ground" plant on the level of AST and ALT in *Biomphalaria* U/gm tissue.

	<i>B.alexandrina</i>			<i>B.glabrata</i>		
	Control	Treated with root saponin	Treated with fruit saponin	Control	Treated with root saponin	Treated with fruit saponin
AST+S.D.	0.43±0.01	0.447±0.032	0.460±0.61	0.49±0.02	0.489±0.02	0.49±0.03
P < % Change		N.S. +3.9	N.S. + 8.3		N.S	N.S.
ALT+ S.D.	0.96±0.06	1.04±0.03	0.0978±0.02	1.03±0.04	1.06±0.02	1.05±0.05
P < % Change		N.S. 8.33	N.S. 1.8		N.S. 2.9	N.S. 1.91