

ASSESSMENT OF SAPONIN RICH FRACTION FROM *BALANITES AEGYPTIACA* (L.) FRUITS AS ANTI SCHISTOMIASIS, ANTI-OXIDANT, ANTI- MUTAGENIC AGENTS AND *IN VITRO* PRODUCTION OF SAPONINS FOR DRUG MANUFACTURE

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Abstract

Saponin rich fraction of *Balanites* fruits (SRF) was administered orally at a dose 250 mg/kg to schistosoma infected mice. Treatment with SRF showed amelioration signs in all biomarkers that confirmed by significant reduction in oogram, ova count and worm burden. Histopathological examination showed extensive reduction in granuloma sizes six weeks post treatment. Our results showed time dependant inhibition in the DNA damage induced in infected mice after treatment. Saponins were also successfully synthesized by callus cultures using Murashige and Skoog media. NMR analysis illustrated the presence of four major saponins of furostanol type from both fruits of natural plant and calli. Thus, SRF of *B.aegyptiaca* fruits possesses antischistomiasis activity. Antioxidant and antimutagenic activities could be considered as a possible mechanisms of action. Additionally, *Balanites aegyptiaca* saponins could be produced in continuous manner using *in vitro* cultures as future vision for drug production to overcome scarce of active metabolites and endangered plant.

Keywords: *Balanites* saponins, Antioxidant, Antimutagenicity, Antischistomiasis, Callus

Introduction

Balanites aegyptiaca (L.) Del. (Balanitaceae) is a semi-arid forest tree. It is widely distributed from Guinea through the Sahara into Egypt and sporadically distributed on sandy soil. The fruits are edible. The species which occur in arid zones grow very slowly and have slow fruits development (Ndoye et al., 2003).

The plant has wide range of biological activities. In ancient Egypt and North Africa as vermifuge and purgative. The preparations include infusion of fresh and dry fruits (Unesco, 1960; Saad, 1998; IUCN, 2005). The materia medica of ancient Egypt includes 134 plants among the plants significance to pharmacy in the past century including *B. aegyptiaca* (Evans, 2007). The fruits are used in Western Sudan traditionally as laxative and antibilharzia. As well as, the root, bark, kernel, fruit and branches have shown to be lethal to snails' miracidia and cercariae of *Schistosoma mansoni* (Koko et al., 2005). In folk medicine, the fruit is known to kill the snails which carry schistosomiasis and bilharzias flukes. Also, the roots are used for abdominal pains and as a purgative. Gum from the wood is mixed with maize meal porridge to treat chest complaints (Tredgold, 1986). Besides, the fruits are used in folk medicine for skin diseases, the bark is used as anti- malarial and, stem and root barks are used in the management of *Candida* infections (Al-Ashaal et al., 2010). Moreover, the fruit extract is used in folk medicine for treatment of hyperglycemia (Gad et al., 2006). Different parts of the plant are used in the ayurvedic and other folk medicine in the treatment of syphilis, jaundice, liver and spleen problems, epilepsy, yellow fever. (Gajalakshmi et al., 2013). Al-Ashaal et al. (2010) reported that fixed oil separated from the fruits has anticancer, anti mutagenic, antioxidant and antiviral activities. The authors also reported that, the oil exhibits anti parasitic activity against *Fasciola gigantica* and *Schistosoma mansoni*, anti candida and antimicrobial properties.

Saponin compounds derived from *B. aegyptiaca* and other plants are reported to be associated with variety of beneficial biological activities, such as larvicidal and molluscicidal effects (Gajakshmi et al., 2013; Treyvaud et al., 2000). The biological activities of saponin have attracted more attention because of their immunomodulatory and antitumor effects (Dzubak et al., 2006). *B. aegyptiaca* steroid saponin glycosides are reported to have potent molluscicidal effect for the control of fresh water snails that represent the intermediate host of *S. mansoni* parasite (Kela et al., 1989).

Hence, for their biological importance, trials of *in vitro* saponin biosynthesis were studied and formation of diosgenin sapogenin was reported (Heble and Staba, 1980; Gour and Kant, 2006).

On the other hand, schistomiasis is considered as prevalent tropical disease affecting millions of people worldwide and constitutes a major health

problem in Egypt that represents one of the most highly infected populations (Bogitish and Cheng 1990). There is evidence indicated that schistosoma infection is involved in the incidence of several cancers and has been suspected as risk factor for various types of cancers e.g., bladder cancer, colorectal cancer and hepatic cancer (Osada et al., 2005). However, the mechanisms of the carcinogenesis are still unclear. The fact that schistosomiasis is found to have a mutagenic effect (Aboul-Ela, 2002) and a comutagenic effect (El-Starkey et al., 2003) may be one of those mechanisms. Urothelial cells collected from patients infected with *S. haematobium* were shown to have an increased frequency of micronuclei suggesting the induction of chromosome injury (Raafat et al., 1984). Schubber and Saleh (1987) presented data indicated that infection with *S. haematobium* elevated the values of sister chromatid exchanges (SCEs) and chromosomal aberrations in blood lymphocytes. Infection of mice with *S. haematobium* resulted in a marked increase in the incidence of chromosomal aberrations in bone marrow cells and sperm head abnormalities (Hamada et al., 1992).

Since the plant has significant multipurpose medicinal properties and increased human pressure for its valuable timber, the plant is endangered because of the high rate of clearance (Ndoye et al., 2003). Besides, it is of great importance to have anthelmintic drugs from natural sources because synthetic ones may lead to drug resistance and decreasing activity against encapsulated larval stages of parasite. Replacing natural compounds by synthetic ones is usually expensive and very difficult.

The present study aims to verify the traditional use of *Balanites aegyptiaca* L. fruits as anti-schistosomiasis and to highlight the role of steroidal saponins rich fraction isolated from the fruit of natural plant as the active principals. Besides, study the antioxidant and antimutagenic effects as possible mechanisms of action of SRF for further promising applications. As well as the possible production of saponins from *in vitro* cultures of *B. aegyptiaca* L. fruits as future vision for drug production just in case of depletion of these active saponins due to climatic conditions or plant diseases and to overcome the problem of endangered plant due to increased human demand.

Materials and methods

Plant material

Balanites aegyptiaca (L.) Del. fruits (Balanitaceae) were obtained from Al Wadi Al Gadeed, Egypt, and identified by prof. Salwa Kawashity, Department of Phytochemistry and Plant Systematic, National Research Centre, Egypt. A voucher specimen is deposited at the NRC herbarium with registration number CAIRC 3665.

Chemicals

All chemicals used in the present study are high analytical grade products from Sigma (USA), Merck (Germany), BDH (England), Riedel de Haen (Germany) and Fluka (Switzerland)

Instruments

¹H-NMR Varian; 300 MHz, ¹³C- NMR;75 MHz, TMS as internal standard, U.S.A.

Light microscope; Olympus, Saitama, Japan, Eye piece:25X,Oil, objective:100X.

Spectrophotometer; LKB, Biochrom, Novaspec, Germany.

Isolation of saponin rich fraction from natural plant and from in vitro culture

Fruits from intact natural plants were sliced, dried at 45⁰c and extracted with water. Water extract was dried under reduced pressure and temperature using rotary evaporator. The dried residue was successively extracted with hexane, chloroform and finally ethanol. The ethanolic extract was concentrated under reduced pressure and temperature. The ethanolic residue was chromatographed on silica TLC plates with eluting system CHCl₃: MeOH: H₂O (60: 30: 5 v/v) and sprayed with 20% H₂SO₄acid as colorogenic reagent and gave 4 major greenish spots. The saponins were isolated on preparative high performance thin layer plates HPTLC using the same eluting solvent, and the 4 bands corresponding to the 4 major spots were separated and extracted with ethanol. The extract for each band was evaporated under reduced pressure and temperature not exceeding 50°C. The residue for each band was analyzed and the NMR analysis (DMSO) for each compound was recorded.

In parallel *B. aegyptiaca* L. fruits were cleaned by tap water, then immersed for seconds in 10% sodium hypochlorite solution and then disinfected with 70% ethanol. The seeds were obtained from the fruits and inoculated in air laminar flow cabinet in Murashige and Skoog media (MS) (Murashige & Skoog, 1962) supplemented with auxins and cytokinins including, NAA, 2, 4 - D, IAA and Kinetin. The cultures were incubated at 24 ±2⁰c and 16/8h photo period and transferred to fresh media every 4 weeks for 3 sub cultures. The produced calli were dried at a 45⁰c. The dried materials were successively extracted with hexane chloroform and finally ethanol. The ethanolic residue was subjected to the same chromatographic analysis procedures described above from fruits of natural plant and the data were compared.

Saponin elutes from both natural fruit extract and calli extract were further subjected to acid hydrolysis. Elute was evaporated and 2 mg of the residue was dissolved in 3 ml solution of 2 N hydrochloric acid and methanol (1: 1 v/v), then heated under reflux for 2 hours. The methanol was then evaporated and 5 ml distilled water was added to the reaction mixture. The

aqueous phase was extracted three times with chloroform. The combined chloroform extract was washed with distilled water till became acid free. The chloroform was evaporated and the saponin aglycones were identified by co-chromatography against standards on silica gel (HPTLC) (chloroform, methanol, and 9:1 v/v) and visualized by vanillin sulfuric acid reagent.

Biological Study

Animals

Male Swiss albino mice of CDI strain (20-25 g) were obtained from Theodor Bilharz Research Institute, Cairo, Egypt and maintained on water and stock commercial pellet diet ad libitum (El-Kahira Company for Oil and Soap). Handling, anesthetic and sacrifice procedures followed ethical guidelines and adhered to internationally accepted principles for laboratory animals use and care found in European Community Guidelines (EEC, Strasbourg, 18.III. 1986, Text amended according to the provisions of the protocol; ETSNo.170 in 2005).

Doses and route of administration

Plant extract of saponin rich fraction derived from fruits of intact natural *B. aegyptiaca* L. plant was given orally at a dose 250mg/kg body weight. Which equal to 1/20 LD50 (Ya'u, et al., 2011) that represents submaximal dose and given for three and six consecutive weeks after two months of *S. mansoni* infection.

***S. mansoni* infection**

Mice were infected with 80 cercariae of the *S. mansoni* Egyptian strain via the tail-immersion technique (Olivier and Stirewalt, 1952) and let stand for two months.

Experimental design

Mice were divided into six groups of six mice each. Group 1: normal healthy control mice. Groups 2, 3: normal healthy mice orally treated with plant extract daily for three and six consecutive weeks. Group 4: *S. mansoni*-infected mice; Groups 5, 6: *S. mansoni*-infected mice for two months then treated with plant extract for three and six weeks respectively. After treatment mice subjected to diethyl ether anesthesia, then serum were collected by cutting sub-lingual vein in sterile centrifuge tubes and centrifuge at 3000 rpm for 15 minutes. Serum was storage at -80 OC for analysis of liver function enzymes; aspartate and alanine aminotransferases (AST & ALT), as well as alkaline phosphatase (ALP).

Tissue homogenate

Liver tissue was homogenized in 0.9 N NaCl (1:10 w/v) for the estimation of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphatase (G-6-Pase), acid phosphatase (AP), 5'-nucleotidase, malondialdehyde (MDA), glutathione (GSH), vitamin C and E.

Extraction of the tissue for urea cycle enzyme activities

The hepatic tissue of albino mice was removed, blotted, weighed and homogenized in 4-5 volume of 0.1% hexadecyl trimethyl ammonium bromide (CTB). The CTB solution was added at room temperature but subsequent homogenization was carried out around 0 °C. The crude homogenate was centrifuged at 4500 rpm. For 15 min at 2 °C. The supernatant was kept at 0 °C and the residue re-extracted by homogenization as before. The combined supernatants were used as the enzyme source for assay of different enzyme activities. All assays, were carried out in a total volume of 1-2 ml at 38°C. The reactions were started by addition of either the specific substrate or the enzyme source, and were stopped after the incubation time by addition of 5 ml 0.5 M HClO₄ and the precipitated protein was removed. The supernatant fluid served for the analytical procedures.

Biochemical assays

Cell organelles marker enzymes and protein content

SDH is measured when the reduction of flavin adenine dinucleotide is coupled with a reduction of tetrazolium salt as 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT); the produced formazan of INT is measured colorimetrically at 490 nm (Rice and Shelton, 1957).

LDH is measured when the reduction of nucleoside derived amino acids (NAD) is coupled with the reduction of tetrazolium salt with phenazine methosulfate serving as an intermediate electron carrier; the produced formazan of INT was measured colorimetrically at 503 nm (Babson and Babson, 1973).

The three enzymes, G-6-Pase, acid phosphatase and 5'-nucleotidase, were measured colorimetrically at 660 nm by measuring the inorganic phosphorus release following the methods of Swanson (1955), De Duve and Wattiaux (1956) and Bodansky and Schwartz (1963), respectively.

Biomarkers for liver functions tests

Aspartate and alanine aminotransferases were measured by the method of Gella et al. (1985), where the transfer of amino group from aspartate or alanine formed oxalacetate or pyruvate, respectively and the developed colour was measured at 520 nm. Alkaline phosphatase catalyzed in alkaline medium the transfer of phosphate group from 4-nitrophosphatase to 2- amino-2-methyl-1-propanol (AMP) and liberated 4- nitrophenol. The developed color was measured at 510nm (Rosalki et al., 1993).

Oxidative stress biomarkers

Malondialdehyde as a product of polyunsaturated fatty acids oxidation was determined by the method of Buege and Aust (1978). Its concentration was calculated using the extinction coefficient value $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and read at 535 nm.

Glutathione was estimated by the colorimetric assay according to the method of Moron et al. (1979) using pithiobis-2-nitrobenzoic acid (DTNB) in phosphate buffer. The developed color was read at 412nm.

Vitamin C was estimated by the method of Jagata and Dani (1982) using Folin reagent and the developed color was read at 560 nm.

Vitamin E was determined by the colorimetric assay using the method of Angustin et al. (1985).

Urea cycle Enzyme assays

All the assay systems used were employed according to Linton and Campbell (1962) which is the modified method of Brown and Cohen (1959). Heat inactivated (10 min, 100 °C) tissue homogenate was used as control, in addition to the blank sample. Specific activity is defined as q moles of product formed or substrate disappeared / mg protein / hr at 38°C.

Measurement of ornithine aminotransferase (OAT): The activity of the enzyme was determined through measurement of citrulline which was determined by the method of Linton and Campbell (1962).

Measurement of argininosuccinate synthetase activity (ASS) : This assay was carried out according to the modified system of Linton and Campbell (1962). The enzyme was determined through measuring the unreacted citrulline.

Measurement of argininosuccinate lyase (ASL): The assay system used was that of Linton and Campbell (1962). Incubation was carried out in a final volume of 1 ml and in this step arginase splits arginine to urea before addition of liver enzyme.

Measurement of arginase: The assay system used was that of Linton and Campbell (1962) urease was added so that urea produced could be determined as ammonia. 100% activation of enzyme was reached by preheating or 30 min. at 50 °C, with $50 \text{ } \mu\text{M}$ Mn^{2+} (manganous ion).The enzymatic activity was determined by measuring the liberated ammonia.

Histopathological examination

Representative slices from liver tissue were taken from the eviscerated animals and fixed in 10% buffer formalin. Paraffin-embedded sections (4µm thick) were taken after fixation and slides were stained using haematoxylin and eosin by the method of Hirsch et al. (1997).

Statistical analysis

Results were evaluated with independent student t-test (SPSS-Statistical Package for Social Sciences, version 10.0) for determination the significant values between groups.

Results were expressed as mean \pm SD of six mice in each group. Significant level was $p < 0.05$.

Mutagenicity Study

Animals were sacrificed 24h after the last treatment. For somatic and germ cells preparations, animals from the different groups were injected i.p. with colchicines, 2-3h before sacrifice.

Chromosome aberrations in somatic cells

Chromosome preparations from bone - marrow and spleen (somatic cells) carried out according to the method of Yosida and Amano (1965). 100 well spread metaphases were analyzed per mouse. Metaphases with gaps, chromosome or chromatid breakage, fragments, deletions, Robertsonian translocation as well as numerical aberrations (polyploidy) under 100X magnification with a light microscope (Olympus, Saitama, Japan) were recorded.

Chromosome abnormalities in spermatocytes cells

Chromosome preparations from spermatocytes (germ cells) were made according to the technique of Evans et al. (1964). 80 well spread diakinase-metaphase I cells were analyzed per animal for chromosomal aberrations. Metaphases with univalents, and chromosome translocations (chain IV) were recorded.

Sperm-shape abnormalities

Sperms were prepared according to Wyrobek and Bruce (1978). At least 1000 sperm were examined /animal.

Statistical analysis

The significance of the results was between the negative control data and infected mice with schistosomiasis worm. Also, between infected mice with schistosomiasis worm plus SRF and infected mice alone was calculated using t-test.

Results and discussion

Saponins characterization from fruits of natural plant and from in vitro cultures

Examination of the defatted ethanolic extract of fruits derived from

intact natural *B. aegyptiaca* L. and from calli from cultures revealed the presence of 4 major saponins. Hydrolysis of saponins extract and co chromatography of the hydrolysate against standard revealed the presence of diosgenin aglycone. Saponins isolate and their acidic hydrolyzed aglycones gave positive tests characteristics for saponins (Al-Ashaal et al., 2012). In the present study, calli were successfully initiated from seeds of *B. aegyptiaca* on Murashige and Skoog media supplemented with 2 mg/L 2, 4-D and 2 mg/l kinetin. The obtained calli were friable with yellowish green to brown color that may be due to accumulation of secondary metabolites (Fig.1).This compatible with reports that found kinetin to support callus induction (Bidawat et al., 2011). While other reports showed that kinetin combination enhanced vegetative growth and shoot initiation (El- Mekawy et al., 2012). The results coordinate with other reports illustrated the formation of diosgenin sapogenin from cultures of *B. aegyptiaca* by the use of 2, 4, D and NAA (Gour and Kant, 2006). In vitro production of the diosgenin was also reported from *Dioscorea deltoidea* cultures (Heble and Staba, 1980). The success of *in vitro* biosynthesis of saponins could open the door for mass production and stable supply of these active metabolities for drug manufacture.

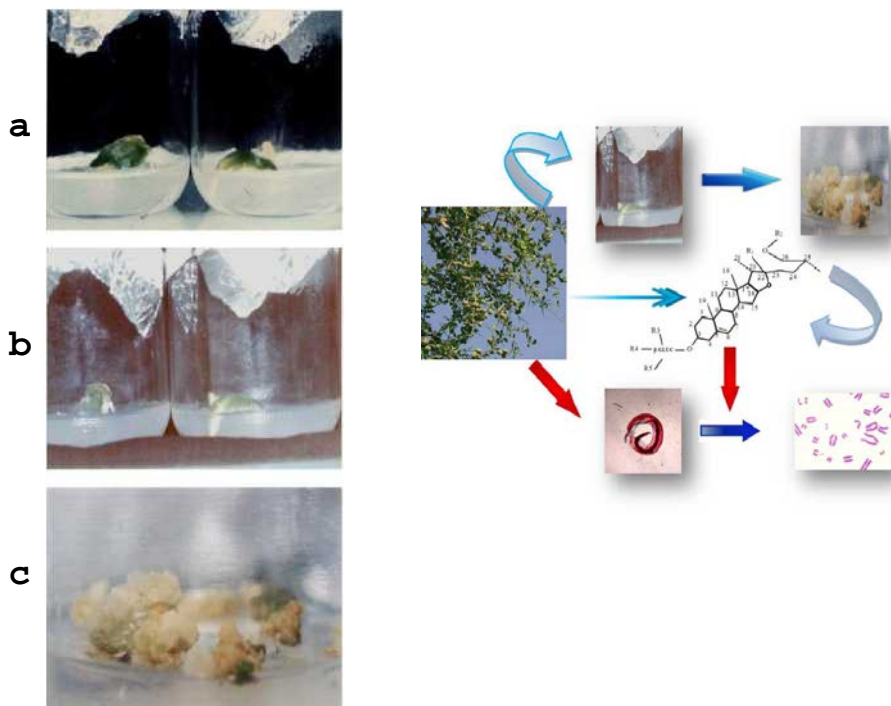


Fig. 1. Callus induction from *B.aegyptiaca* (L.) Del seeds
a Seed inoculation on MS media containing growth regulators
b Germinated seeds
c Callus culture

NMR analysis of the saponins from the fruits of natural mother plant and fruits derived calli cultures revealed the presence of 4 saponins glycosides of the furostanol type (Fig.2). The results are in a good agreements with ¹H-NMR and ¹³C- NMR analysis of the separated saponins from *B. aegyptiaca* reported ones (Kamel, 1991). Production of the saponin glycosides active constituents from *in vitro* cultures, helps to overcome the problem of slow development of the plant and fruits. Besides, saving the plant from being endangered (Ndoye et al., 2003).

¹H-NMR analysis for compound 1; 0.832 ppm (3H₂S, 18-CH₃), 0.876 ppm (3H, d, J= 6.8 Hz, 27-CH₃), 1.073 ppm (3H, S, 19-CH₃), 1.263 ppm (3H, d, J= 6.8Hz, 21-CH₃), 1.784 ppm (6H, d, J= 6.1Hz, Rha-CH₃), 4.64 ppm (1 H, bs, H-1 Rha), 5.01 ppm (1 H, d, J= 7.7 Hz, H-1 Glc).

The results revealed that compound 1 is identical with 26 - O - P- glucopyranosyl - 25 - furost - 5. ene, -3P, 22, 26 - triol 3 - O[a-rhamnopyranosyl - (1[^]2)]- [a-rhamnopyranosyl - (1[^]4)] P- glucoryranoside.

¹H-NMR for compound 2; 0.73 ppm (3-Hs, 18-CH₃), 0.92 ppm (3H, d, J=6.8H2, 27-CH₃), 0.99 ppm (3H, S, 19-CH₃), 1.15 ppm (3H, d, J=6.8 Hz, 21-CH₃), 1.50 ppm (6H, d, J=6.1 Hz, Rha-CH₃), 3.22 ppm (3H, S, OCH₃), 4.4690

ppm (1H, bs, H-1 Rha), 4.449 ppm (1H, d, J=7.7 Hz, H-1 Glc), 5.00 (1H, d, J=7.7, Glc-H1)

5.30 ppm (1H, bs, H-1 Glc). The compound 2 is identical with 22 methoxy - 26 - O - P-glucopyranosyl - 25 - furost - 5 - ene, -3P, 26 - diol 3 O[α -rhamnopyranosyl - (1²)]-[α -rhamnopyranosyl - (1⁴)] P- glucoryranoside.

¹H-NMR for compound 3; 0.730 ppm (3H, S, 18-CH₃), 0.887 ppm (3H, d, J= 7.0Hz, 27-CH₃), 0.957 ppm (3H, S, 19-CH₃), 1.290 ppm (3H, d, J=6.6 Hz, 21-CH₃), 1.780 ppm (6H, d, J=6.0 Hz, 2 Rha-CH₃), 4.460 ppm (1H, d, J=6.8 Hz, H-1Glc), 4.90 ppm (1H, bs, H-1 Rha), 4.673 ppm (1H, bs, H-1 Rha), 5.058ppm (1H, d, J=6.4 Hz, H-1 Glc), 5.3 (1H, bs, H-1 xyl). Thus compound 3 is identical with 26 - O - P - glucopyranosyl - 25 - furost - 5 - ene - 3P, 22, 26 - triol 3 - O [α - rhamnsosy - (1²)] - [P - xylopyranosyl - (1³)] - [α - rhamnopyranosyl - (1⁴)] - P - glucopyranoside.

¹HNMR analysis for compound 4; 0.710 ppm (3H, S, 18 - CH₃), 0.823 ppm (3H, d, J=6.8Hz, 27-CH₃), 0.929 - ppm (3H, S, 19 - CH₃), 1.202 ppm (3H, d, J=6.5 Hz, 21 - CH₃), 3.22 ppm (3H - S OCH₃), 1.75 ppm (6H, d, J=6.2 Hz, 2Rha - CH₃), 4.90 ppm (1H, bs, H-1 Rha), 4.460 ppm (1H, d, J=7.1Hz, H-1 Glc), 4.690 ppm (1H, bs, H-1 Rha), 5.049 ppm (1H, d, G=7.1Hz, H-1 Glc), 5.30 ppm (1H, bs, H-1 xyl).

The compound 4 is identical with 22 methoxy - 26 - O - P - glucopyranosyl - 25 - furost - 5 - ene - 3P, 26 - diol 3 - O [α - rhamnsosy - (1²)] - [P - xylopyranosyl - (1³)] - [α - rhamnopyranosyl - (1⁴)] - P - glucopyranoside.

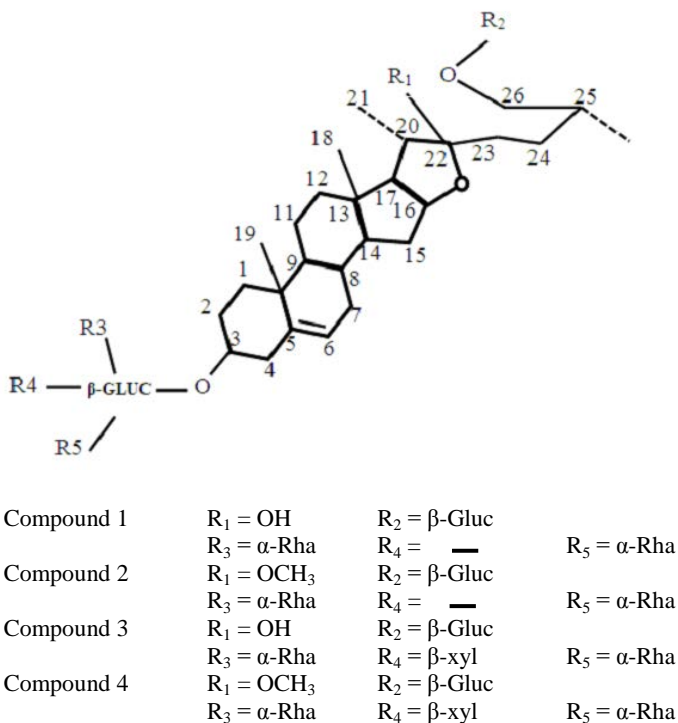


Fig. 2. Furostanole saponins from *B. aegyptiaca*(L.)

Biological study

Biochemical assays

Concerning marker enzymes, Table 1 shows the effect of *B. aegyptiaca* SRF treatment on liver function enzymes. Normal mice treated with SRF recorded insignificant changes in AST, ALT and ALP enzyme activities, while infected mice showed significant increase in AST, ALT and ALP in serum of infected mice by 27.33, 25.91 and 47.32%, respectively.

Table 1 - Effect of *B. aegyptiaca* SRF treatment on liver function enzymes in *S. mansoni* infected and treated mice.

Parameters	Control	Control-treated (3weeks)	Control-treated (6weeks)	Infected	Infected-treated weeks)	Infected-treated (6 weeks)
AST	66.93±1.54 ---	65.53±1.80 (-2.09)	65.85±0.59 (-1.61)	48.64**±1.92 (-27.33)	56.35*±0.84 (15.80)	60.82*±0.70 (-9.12)
ALT	35.66±1.38 ---	34.81±1.58 (-2.38)	34.81±0.21 (+2.38)	26.42**±1.66 (-25.91)	30.39*±1.05 (-14.77)	33.02*±0.43 (-7.40)
ALP	3.55±0.54 ---	3.28±0.38 (-7.60)	3.51±0.03 (-1.12)	1.87**±0.21 (-47.32)	2.27**±0.28 (-36.05)	2.78**±0.22 (21.69)

Values are mean ±SD of six mice in each group.

Data are expressed as µmol/min/ mg protein

Values between brackets are percentage change over control group

Statistical analysis is carried out by independent t- test

Table 5 illustrated that treatment with plant extract for three weeks recorded enhancement by 11.52, 11.13 and 11.26%, respectively, while treatment for six weeks showed improvement by 18.19, 18.50 and 25.63%. Table 2 demonstrates the effect of SRF treatment on cell organelles marker enzymes in *S. mansoni* infected mice. Insignificant change in cell organelles markers enzyme in control mice treated with plant extract for three and six weeks. Infected mice recorded significant decrease in SDH, LDH and G-6-Pase by 45.76, 59.14 and 28.42%, respectively. AP and 5'-nucleotidase showed significant increase in *S. mansoni* mice by 49.03 and 124.42%, respectively. Treatment of *S. mansoni* infected mice with SRF for three weeks showed improvement by 16.94, 19.29, 22.02, 23.87 and 56.89% for SDA, LDH, G-6-Pase, AP and 5'-nucleotidase, respectively. Table 5 showed that treatment of *S. mansoni* infected mice with SRF for six weeks recorded improvement by 41.52, 20.85, 27.20, 35.48 and 75.03% for SDA, LDH, G-6-Pase, AP and 5'-nucleotidase, respectively.

Table 2 - Effect of *B. aegyptiaca* SRF treatment on cell organelles marker enzymes in *S. mansoni* infected and treated mice.

Parameters	Control	Control-treated (3weeks)	Control-treated (6weeks)	Infected	Infected-treated (3 weeks)	Infected-treated (6 weeks)
SDH	1.18±0.14 ---	1.09±0.05 (-7.62)	1.14±0.03 (-3.38)	0.64**±0.05 (-45.76)	0.84**±0.04 (-28.81)	1.13±0.02 (-4.23)
LDH	330.63±16.52 ---	322.52±12.66 (-245.00)	326.16±3.67 (-1.35)	235.08**±23.54 (-59.14)	298.87**±19.62 (-9.60)	304.02*±4.00 (-8.04)
G-6-Pase	99.55±3.15 ---	96.85±3.63 (-2.71)	96.92±2.60 (-2.64)	71.25**±6.24 (-28.42)	93.18*±8.18 (-6.39)	98.33±0.36 (-1.22)
AP	1.55±0.14 ---	1.58±0.15 (+1.93)	1.60±0.03 (+3.22)	2.31**±0.26 (+49.03)	1.94±0.02 (+25.16)	1.76*±0.09 (+13.54)
5`-nucleotidas	7.37±0.57 ---	7.50±0.06 (+1.76)	7.59±0.07 (+2.98)	16.54**±0.79 (+124.42)	12.34**±1.27 (+67.43)	11.01**±0.32 (+49.38)

Values are mean ±SD of six mice in each group. Data are expressed as μmol//min/ mg protein.

Values between brackets are percentage change over control group.

Statistical analysis is carried out by independent t- test.

*is the level of significance at p<0.05 and ** is at p<0.001.

Regarding to oxidative stress markers, Table 3 demonstrates that normal mice treated with *B. aegyptiaca* SRF showed insignificant changes in MDA, GSH, and Vits. C and E. *S. mansoni* infected mice showed significant increase in MDA by 343.18%, while GSH, Vits. C and E recorded significant decrease by 32.72, 37.03 and 61.30%, respectively. Table 5 showed that treatment of infected mice with SRF for three weeks showed improvement by 131.81, 16.05, 24.60 and 34.58% for MDA, GSH, and Vits. C and E, respectively, while treatment for six weeks showed amelioration by 138.63, 22.33, 33.89 and 39.72%.

With respect to urea cycle enzymes, Table 4 illustrates that normal control mice treated with SRF exhibited insignificant change as compared to normal untreated one. *S. mansoni* infected mice showed significant increase in OAT by 93.77%, while significant decrease in ASS, ASL and arginase enzyme activities by 76.82, 28.48 and 74.22%, respectively.

Table 3 - Effect of *B. aegyptiaca* SRF treatment on oxidative stress markers in *S. mansoni* infected and treated mice.

Parameter	Control	Control-treated (3weeks)	Control-treated (6weeks)	Infected	Infected-treated (3 weeks)	Infected-treated (6 weeks)
MDA	0.44±0.22 ---	0.42±0.02 (-4.54)	0.40±0.04 (-9.09)	1.95***±0.04 (+343.18)	1.37***±0.04 (+211.36)	1.34***±0.04 (+204.54)
GSH	44.77±2.63 ---	47.48±2.16 (+6.05)	45.16±1.50 (+0.87)	30.12**±1.06 (-32.72)	37.31**±0.69 (-16.66)	40.12*±2.45 (-10.38)
Vit. C	8.29±0.41 ---	8.98±0.23 (+8.32)	8.44±0.13 (+1.80)	5.22**±0.52 (-37.03)	7.26*±0.20 (-12.42)	8.03**±0.10 (-39.32)
Vit. E	2.92±0.10 ---	2.97±0.08 (+1.71)	3.00±0.10 (+2.73)	1.13**±0.08 (-61.30)	2.14**±0.11 (-26.71)	2.29**±0.03 (-21.57)

Values are mean ±SD of six mice in each group.

Data are expressed as µg & µmol /mg protein for GSH, Vit. C, Vit. E and MDA.

Values between brackets are percentage change over control group.

Statistical analysis is carried out by independent t- test.

*, ** and *** are the levels of significance at p<0.05, p<0.001 and p<0.0001, respectively.

Table 4 - Effect of *B. aegyptiaca* SRF treatment on urea cycle enzymes in liver of *S. mansoni* infected mice

Parameters	Control	Control-treated (3weeks)	Control-treated (6weeks)	Infected	Infected-treated (3 weeks)	Infected-treated (6 weeks)
OAT	4.18±0.15 ---	4.19±0.45 (+0.23)	4.23±0.34 (+1.19)	8.10±1.16*** (+93.77)	5.12±1.50** (+22.48)	4.67±1.25 (+11.72)
ASS	1.23±0.60 ---	1.29±0.40 (+4.87)	1.35±0.20 (+9.75)	0.65±0.03** (-76.82)	1.00±0.26* (-18.69)	1.12±0.22* (-8.94)
ASL	23.87±3.15 ---	22.99±4.16 (-3.68)	23.87±5.35 (0.00)	6.80±6.10*** (-28.48)	16.22±3.34** (-32.04)	19.56±4.35* (-18.05)
Arginase	47.88±5.14 ---	46.34±6.67 (-3.22)	47.00±7.10 (-1.84)	12.34±1.67*** (-74.22)	30.42±3.56** (-36.46)	39.96*±3.29* (-16.54)

Values are mean ±SD of six mice in each group. Data are expressed as µmol/min/ mg protein.

Values between brackets are percentage change over control group.

Statistical analysis is carried out by independent t- test.

*is the level of significance at p<0.05 and ** is at p<0.001

Table 5 demonstrates that treatment of infected mice with plant extract for three weeks showed improvement by 71.29, 28.45 , 39.46 and 26 .62 % , respectively for OAT,ASS,ASL and arginase enzyme activities, while treatment for six weeks showed amelioration percentages reached to 82.06, 38.21, 53.46 and 57.68%.

Table 6 illustrates oogram (dead, immature and mature eggs), worm burden and ova count in both liver and intestine of infected- treated mice with *B. aegyptiaca* for 3 and 6 weeks and showed significant decrease in these parameters in infected -treated mice with *B. aegyptiaca*.

Table 5- Improvement levels of cell organelles markers, liver function enzymes, oxidative stress biomarkers and urea cycle enzymes after SRF treatment.

Parameters	% of improvement after 3 weeks of treatment	% of improvement after 6 weeks of treatment
SDH	16.94	41.52
LDH	19.29	20.85
G-6-Pase	22.02	27.20
AP	23.87	35.48
5`-nucleotidase	56.89	75.03
AST	11.52	18.19
ALT	11.13	18.50
ALP	11.26	25.63
MDA	131.81	138.63
GSH	16.05	22.33
Vit. C	24.60	33.89
Vit.E	34.58	39.72
OAT	71.29	82.06
ASS	28.45	38.21
ASL	39.46	53.46
Arginase	26.62	57.68

% of improvement= ((mean treated- mean infected)/ mean control) x 100.

Table 6 - Oogram, worm and ova count in Infected and infected -treated mice with SRF

Parameters	Infected			Infected-treated (3 weeks)			Infected-treated (6 weeks)		
	Dead	Immature	Mature	Dead	Immature	Mature	Dead	Immature	Mature
Oogram	4.00±0.89	45.2±14.98	48.2±14.06	32.33±8.52***	31.20±8.52 **	35.05±9.30**	40.66±9.80***	33.00±10.84*	32.77±8.90*
Worm count	Female	Male	Couple	Female	Male	Couple	Female	Male	Couple
	4.25±1.92	8.50±2.20	8.00±2.10	3.00±1.24*	3.66±0.94**	5.66±1.49**	2.00±0.82***	1.66±0.47***	4.4±0.93***
Ova count	Liver		Intestine	Liver		Intestine	Liver		Intestine
	19853.00±6543.73		13411.80±1566.71	14935.66±2069.35**		11716.00±3290.22**	7587.5±2085.4***		6846.06±1937.8***

Values are mean ±SD of six mice in each group. Statistical analysis is carried out by independent t- test.

is the level of significance at $p < 0.01$, * is at $p < 0.001$

Regarding the present data, it is known that host's response to *S. mansoni* infection involved the production of reactive oxygen species where the antioxidant enzymes represented a target for immune elimination of adult worms (Loverde, 1998).

The present data revealed significant increase in malondialdehyde, while there was a significant decrease in the other parameters. These results indicated that infection with *S. mansoni* impairs the antioxidant system since the level of glutathione depletion is used as an index of oxidative stress and is a sign that hepatic cells utilizing more antioxidant defenses (Ip et al., 2000). This is in accordance with Hamed (2006) who found that the glutathione level was decreased after parasitic infection. Pascal et al. (2000) reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxide, since the complex mechanism of lipid peroxidation is known to require the participation of highly reactive oxygen and other reactive oxygen metabolites in the chain of biochemical reactions; thus whenever these free radicals are involved, lipid peroxides are in turn increased. Hence lipid peroxide serves as a marker of cellular oxidative stress and has long been recognized as a major consecutive factor of oxidative damage in chronic diseases (Son et al., 2007).

With regard to vitamin C and in coinciding with the present results, Frei et al. (1988) reported that peroxy radicals are trapped by ascorbate and thus the level of the enzyme and vitamin decreased during the free radical scavenging process. Also, the reduction of vitamin E after schistosomal infection occurs since the vitamin acts as a soluble antioxidant to protect biological membranes against oxidative stress which leads to maintenance of cell function. Moreover, Sokal et al. (1998) reported that vitamin E protects hepatocytes against lipid peroxidation and toxic injury.

Regarding to, AST, ALT and ALP enzyme activities and in accordance with our results, Hamed and Hetta (2005) revealed a significant increase in AST, ALT and ALP after *S. mansoni* infection. They attributed these changes to the elaboration of free radicals due to schistosomal infection, which may cause damage to the mitochondrial membrane as well as an increase in cell membrane permeability that may lead to the discharge of its enzyme content.

Concerning SDH enzyme activity, the present results revealed a significant decrease in its activity two months post infection. Hamed and Hetta (2005) attributed this failure to *Schistosoma* toxins which accumulated within mitochondrial fraction and reflected on enzyme activity.

In agreement with the present results, Maghraby et al. (2010) mentioned that the level of LDH was decreased in liver tissue and increased in serum of infected mice with *S. mansoni*. They attributed the decrease of enzyme activity to tissue damage caused by larvae in the infection period, led to the release of enzyme from the necrotic tissue to blood stream or due to the increased cell

anoxia and irritation by toxic or metabolic products of the worm.

Concerning G-6-Pase enzyme activity, the present results revealed significant decrease after infection with *S. mansoni*. This decrease was attributed to the disturbance of either synthesis or degradation of glycogen (Hara et al., 1981).

Regarding AP enzyme activity, the present results showed a significant increase in enzyme activity after infection. This was in parallel with the results of Hamed et al. (2010). This elevation in AP activity may be due to increased tissue catabolism resulting from increased worm and egg toxins by infection, since all the lysosomal enzymes are activated in conditions characterized by increased tissue catabolism led to enhancement of phagocytic phenomenon and /or due to aberration of the lysosomes.

As concerning the 5'-nucleotidase enzyme activity, the present results recorded significant increase in its level post infection. This increase was the same finding of Hamed (2011) who attributed the increase in the 5'-nucleotidase enzyme activity to enhancement of the active transport process through the plasma membrane where the enzyme appeared in accurate localization at bile canalicular and sinusoidal plasma membrane of liver parenchymal cells and/or due to accelerated nucleic acid metabolism where the catabolism of purine and pyrimidine nucleotides starts with the action of 5'-nucleotidase.

As concerning the effect of *S. mansoni* infection on urea cycle enzymes, the results revealed that, OAT exhibited highly significant increase at two-month post infection. ASS showed decrease at 2nd month post infection. In addition, ASL and arginase exhibited a more intensive reduction in their activities at 2nd month post infection as compared to normal control mice. These results could easily be correlated to those of Rizk et al. (2000), who reported that, bilharzial infection resulted in defect in protein metabolism through a defect in absorption of amino acids or defects in enzyme synthesis and, hence derangement of many metabolic pathways which may involve detoxification mechanism in urea cycle regulation. The same authors added that, during *S. mansoni* infection hepatocyte mitochondria may become swollen and disrupted. OAT is compartmentalized within mitochondria of liver cells and the lack of coordination between this enzyme and cytoplasmic arginase represents a pathological condition rather than adaptive response during infection.

Our data confirmed the observation of Aly (2004) who recorded that infection causes changes in arginase and OAT due to changes in absolute enzyme levels. Furthermore, adaptive changes in arginase levels appear to be attributable to a shift in the balance of synthetic versus degradation rates.

Under certain induced pathological conditions, hepatic arginase level can be regulated independently from other urea cycle enzymes. The same authors reported that serum concentration of certain urea cycle intermediates were at abnormal levels. They declared that at 19th week post infection, total liver arginase increased by 61% while OAT decreased by 31%.

A significant decrease in both total and specific activities of serum carbamoyl phosphate synthetase (CPS), OAT and also in the urea level around 10th week post infection were reported by Aly and Maghraby (2012), who suggested that, the granuloma or inflammatory cells induced by *S. mansoni* eggs produce some factors which may be responsible for reduction of these enzymatic activities, or due to liver enlargement and/or a decrease in the number of hepatocytes containing urea cycle enzymes. In addition, CPS requiring N-acetyl-glutamate as a stimulator activator appears to catalyze one of the rate-limiting steps of the urea cycle, there is a possibility that, suppression of this enzyme results in the decline of urea cycle enzyme activities.

The present results illustrate the significant improvement of all liver biomarker enzymes and antioxidant status of infected mice that supported by histopathological improvement, post treatment with *B. aegyptiaca* SRF. The ameliorations signs noticed in *S. mansoni* treated mice with *B. aegyptiaca* SRF in the all investigated biochemical parameters in the present results may be revealed to the antioxidant activities of the isolated saponins (Karou et al., 2011). *B. aegyptiaca* steroid saponin glycosides were proven to be potent molluscicides for the control of fresh water snails that represent the intermediate host of *S. mansoni* parasite (Kela et al., 1989). Moreover, the efficacy of *B. aegyptiaca* fruit mesocarp in infected mice with a Sudanese strain of *Schistosoma mansoni* was proven (Koko et al., 2005). Diosgenyl saponins from *B. aegyptiaca* kernels demonstrated appreciable anticancer effect in human cancer cell lines *in vitro*. The anticancer activity mainly results from a depletion of ATP leading in turn to a major disorganization of actin cytoskeleton (Gnoula et al., 2008).

The results of the present study are confirmed by significant reduction in oogram (dead , immature and mature eggs), worm count (male , female and coupling) as well as ova count in both liver and intestine in *S. mansoni* infected mice treated with *B. aegyptiaca* SRF. In concomitant with our findings, Koko et al. (2005) found that, a significant reduction in EPG (egg count per gram of faeces), eggs burden in tissues as well as the recovery of adult worms upon using *B. aegyptiaca* fruit mesocarp. In parallel results, Al Ashaal et al. (2010) found that, the oil of *B. aegyptiaca* exhibited *in vitro* anthelmintic activity against hepatic worms (*Schistosoma mansoni* and *Fasciola gigantica*). It was

found that aqueous extracts of different parts of *B. aegyptiaca* exhibited reasonable molluscicidal activity against Ethiopian *Biomphalaria pfeifferi* (*B. pfeifferi*), *Lymnaea natalensis* (*L. natalensis*) *Schistosoma mansoni* (*S. mansoni*) cercariae, besides cercariacidal activity against *S. mansoni* cercariae (Molla et al., 2013).

In the same line with our results, *B. aegyptiaca* extract as well as, saponins were found to have antiparasitic activities against other parasites. In a good connection with the present results, Kusch et al. (2011) found that phenolic compounds of *B. aegyptiaca* extract may contribute to the *in vitro* antimalarial activity that in turn may be having antischistosomal effect due to their antioxidative properties. Methanolic extract of *B. aegyptiaca* fruits was found to have high effectiveness against parenteral stages of *Trichinella spiralis* and inhibitory effects on adult *Toxocara vitulorum* and egg development that were dose dependent (Shalaby et al., 2010 and Hatem et al., 2012) Bishnu et al. (2008) revealed that, saponins from *in vitro* cultures of the root explant of *B. aegyptiaca* can be used as a larvicidal agent against *Aedes aegypti* larvae.

Histopathological examination

Histological examination of normal control liver tissue under light microscope revealed the presence of hexagonadal or pentagonadal lobules with central veins, liver sections of control group (A) H&E (A*) Masson's trichome stain showed the normal liver cells as arrows show. The histology was within normal limits in normal mice treated with SRF for three weeks (B) H&E (B*) Masson's trichome stain. Sections (C) & (C*) showed no changes appearing of hepatic cells a result of treatment for six weeks H&E and Masson's trichome stains respectively (Fig.3).

Histopathological examination of H&E and Masson's trichome stained liver sections showed positive histological changes in the areas of hepatocellular and formation of granulomas around degenerated eggs of *S. mansoni* and localized fibrosis around it, arrows show multiple granulomatous lesions and focal areas of necrosis as shown in (D) and (D*) liver sections whereas the histopathological examination revealed reduction in granuloma size after administration SRF for three weeks. More reduction in granuloma size detected in liver sections, (F, G) H&E stain and (F*, G*) Masson's trichome stain in SRF treated groups for six weeks (Fig.4).

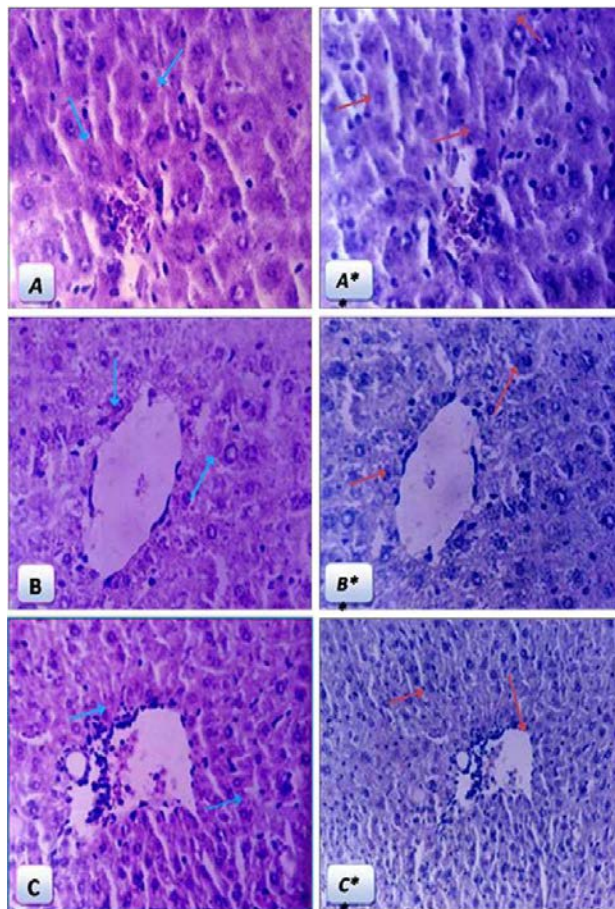


Fig. 3. Liver Sections of control group (A) H&E- 400x (A*) Mason's trichome - 400 x stain showed the normal structure of hepatic cells. Healthy groups treated with *Balanites aegyptiaca* extract for three weeks (B) H&E- 200x, (B*) Mason's trichome - 200 x. showing no change of hepatic cells. Also, sections (C) and (C*) revealed no change in cells as a result of treated the extract for six weeks H&E 200x and Mason's trichome 100x stains respectively.

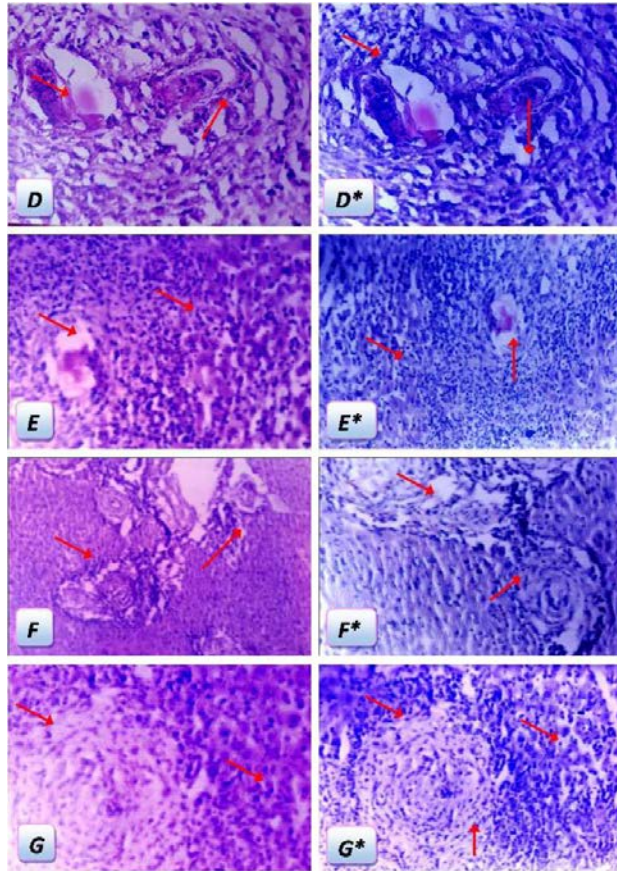


Fig. 4. Liver sections of infected group (D) H&E- 400x (D*) Mason's trichrome - 400 x stain showing abnormal morphological hepatic cells. Sections (E) H&E- 200x (E*) Masson's trichrome 200x revealed a reduction in granuloma size after *B. aegyptiaca* SRF treated for three weeks. Disintegrated of egg in granuloma of liver sections.(F, G) H&E 200x stain and (F*, G*) Masson's trichrome 200x stain in group treated *Balanites egyptica* extract for six weeks.

With respect to, histopathological examination, the present study reveals that hepatic cells, central vein and portal triad were normal in Healthy groups. Fibrous and hepatic parenchyma showed severe fibrosis and severe diffuse inflammatory infiltration, causing the distortion of hepatic lobules. This is in agreement with Ali and Hamed (2006). Granuloma formation results from a delayed hypersensitivity response generated by the host against antigens secreted by the eggs of parasite (EL-Banhawy et al., 2007). The current results showed that *S. mansoni* egg granuloma sizes were reduced after administration of *Balanites aegyptiaca* SRF for three weeks and a significant reduction in granuloma size was shown in liver sections in groups treated for six weeks. Thus, histopathological study confirmed the biochemical observations.

At our laboratories, in previous studies, praziquantel(2-cyclohexylcarbonyl-1,2,3,6,7,11 -hexahydropyrazinol [2,1 -a] isoquinolin-4-one) as positive drug control at a dose of 500mg/kg body weight of *schistosoma mansoni* infected mice was administered orally for two successive days. The drug induced decrease in number of ova count and worm burden that in turn, caused amelioration of liver function enzymes activities including, ALP, AST, ALT and acid phosphatase. Besides, decrease in number, size, cellularity of granuloma and significant improvement of preportal fibrosis (EL-Banhawy et al., 2007). Abouel Ela et al. (1996) reported that praziquantel at the same dose and onset of action induced significant chromosomal aberrations.

Mutagenicity Study

Tables 7 and 8 show the number and percentage of the chromosomal aberrations induced in control and infected animals alone and after treatment with SRF. The percentage of aberrant cells in animals treated with SRF was statistically not significant in comparing to the control group. SRF reduced the number of the chromosomal aberrations when administered with the infected mice. This reduction of chromosomal abnormalities excluding gaps was highly significant ($p < 0.01$) in comparison with the infected mice alone.

Regarding Table 9, it is observed that no significant differences between the animals treated with SRF and the control group. The mean percentage of diakinesis metaphase I cells was 13.75 ± 1.21 ($p < 0.01$) in the mouse infected with schistosomiasis. This percentage was decreased after treatment with SRF at 250 mg/kg b.wt. for 6 weeks to 11.0 ± 1.61 .

Table 7 - Percentage of chromosomal abnormalities induced in infected mice bone marrow cells with *S. mansoni* and after treatment with SRF

Treatments mg/kg b.wt.	Time of Treatment (weeks)	Abnormal metaphases			No. of different types of metaphases				
		No.	Mean % ± (without gaps)	Gaps	Frag.and/or break	Deletion	R.T.	M.A.	polyploidy
I- Control	-----	11	2.20±0.93	12	9	2	----	----	----
	3	15	3.0±0.55	9	12	3	----	----	----
II- SRF									
	6	17	3.40±0.67	11	13	4	----	----	----
III- Mice infected with schistosomiasis	8	48	9.60±0.83**	22	31	7	4	4	2
IV- Mice infected with schistosomiasis	3	35	7.0±0.60**	17	27	4	2	2	----
+ SRF	6	32	6.40±0.93**	16	23	5	1	1	1

Total number of examined metaphases= 500(100 metaphases/ animal,5 animals/group).

R.T. =Robertsonian translocation, Frag. = Fragment , M.A. =Metaphases with more than one aberration.

** Highly significant $p < 0.01$ level (t-test).

◆◆ Highly significant compared to infected mice $p < 0.01$ level (t-test).

Table 8 - Percentage of chromosomal abnormalities induced in infected mice spleen cells with *S. mansoni* and after treatment with SRF

Treatments mg/kg b.wt.	Time of Treatment (weeks)	Abnormal metaphases			No. of different types of metaphases				
		No.	Mean % ± SE (without gaps)	Gaps	Frag. and/or break	Deletion	R.T.	M.A.	polyploidy
I- Control	-----	14	2.80±0.50	11	11	3	----	----	----
II- SRF	3	13	2.60±0.55	10	11	2	----	----	----
	6	15	3.0±0.93	12	12	3	----	----	----
III- Mice infected with schistosomiasis	8	53	10.60±0.93**	21	33	5	3	8	4
IV- Mice infected with schistosomiasis	3	38	7.60±0.57◆◆	18	25	6	1	4	2
+ SRF	6	31	6.20±0.63◆◆	15	23	3	----	5	----

Total number of examined metaphases= 500(100 metaphases/ animal,5 animals/group). R.T. =Robertsonian translocation, Frag. = Fragment, M.A. =Metaphases with more than one aberration.

** Highly significant $p < 0.01$ level (t-test).

◆◆ Highly significant compared to infected mice $p < 0.01$ level (t-test)

Table 10 illustrates that percentage of sperm abnormalities reached to 6.86±0.64 in the infected mice treated with 250 mg/kg b wt. SRF for 6 weeks and it was statistically significant ($p < 0.01$) comparing to 11.05±0.55 of the infected mice alone.

Table 9 - Percentage of chromosomal abnormalities induced in infected mice spermatocytes with *S.mansoni* and after treatment with SRF

Treatments mg/kg b.wt.	Time of Treatment (weeks)	A3bnormal metaphases		No. of different types of metaphases			
		No.	Mean % ± SE	X-Y univalent	Autosomal univalent	X-Y univalent+ Autosomal univalent	Chain(IV)
I- Control	-----	14	3.50±0.87	10	4	----	----
II- SRF	3	13	3.25±0.60	11	2	----	----
	6	16	4.0±0.69	11	5	----	----
III- Mice infected with schistosomiasis	8	55	13.75±1.21**	33	17	3	2
IV- Mice infected with schistosomiasis + SRF	3	47	11.75 ±0.50◆	31	15	1	----
	6	44	11.0 ±1.61◆	28	14	1	1

Total number of examined metaphases= 400(80 metaphases/ animal,5 animals/group). ** Highly significant compared to control $p < 0.01$ level (t-test).
◆ Significant compared to infected mice $p < 0.05$ level (t-test).

Table 10 - Number and mean percentage of sperm abnormalities induced in infected mice with *S.mansoni* and after treatment with SRF

Treatments mg/kg b.wt.	Time of Treatment (weeks)	No of sperm examined	Abnormal sperms		No. of different types of abnormal sperms				
			No.	Mean %±SE	Triangular	Banana shape	Amorphous	Without hook	Coiled tail
I- Control	-----	5112	158	3.09±0.50	37	6	84	18	13
II- SRF	3	5083	173	3.40±0.90	45	5	91	20	12
	6	5137	213	4.14.0±0.87	45	7	116	28	17
III- Mice infected with schistosomiasis	8	5201	575	11.05±0.55**	113	98	194	125	45
IV- Mice infected with schistosomiasis + SRF	3	5124	421	8.22±0.71◆◆	85	69	148	98	21
	6	5157	354	6.86±0.64◆◆	71	45	136	87	15

** Highly significant compared to control $p < 0.01$ level (t-test).

◆◆ Highly significant compared to infected mice $p < 0.01$ level (t-test).

The data obtained in this study demonstrate the potential mutagenicity of *S. mansoni* infection *per se* in mice. This result was in the same line with the previous studies which showed that schistosomiasis causes increase in the induction of chromosomal aberrations in somatic and germ cells and the frequency of SCEs. Also, schistosomiasis causes a decrease in the mitotic index and a cell cycle delay in human somatic cells (El-Ansary et al., 2010). The mechanism involved in these abnormalities is not clear. The most important explanation is that oxidation of DNA may lead to mutation (and hence to carcinogenesis); Free radicals can also damage DNA and result in mutations, altered capacity of cells to produce critical factors and derangement of the capacity to proliferate (Bendich, 1999).

It is worth mentioned, our data showed that SRF was safe and did not induce any mutations in somatic and germ cells. In the contrary it has the ability to reduce the percentage of aberrations in somatic and germ cells induced by schistosomiasis in a time dependant manner. On contrary, praziquantel as antischistosomiasis drug in its effective dose, was reported to induce chromosomal aberrations on somatic and germ cells in mice (Abouel Ela et al., 1996). The absence of genotoxic activity displayed by SRF is in agreement with other reports showing that the saponins extracted from different plants did not induced DNA damage in human or in experimental animal's test (Ait et al., 1996 and Scarpato et al., 1998).

Our results showed that time dependant inhibition in the DNA damage induced in the mice infected with schistosoma after treatment with SRF for 3 and 6 weeks was observed. The protective effect of saponins may be attributed to its antioxidant activity and its ability to scavenging of harmful species, free radicals or electrophiles, which damage DNA and other cell targets. This explanation was confirmed in this study where we found that SRF has antioxidant activity. Several authors reported about the antigenotoxic and antimutagenic activity observed by the saponins extracted from different plants against the damage induced by the mutagens (Zhang et al., 2009). This explanation is also supported by the previous study by El Ashaal et al. (2010). They attributed the antimutagenic activity of *B. aegyptiaca* fixed oils to its antioxidant activity.

Conclusion

The results indicated that saponin rich fraction content of the fruits derived from natural *B. aegyptiaca* L. plant has potent antischistosomiasis activity. Our study proofs and supports and the traditional use of the fruits as antischistosomiasis by popular. SRF induced improvement in liver enzymes and granuloma of liver infected mice and induced reduction of egg and worm count. The saponin rich fraction possesses antioxidant activity and antimutagenic effect against schistosoma induced mutation that could be regarded as possible mechanisms of action. In addition, the saponin rich

fraction could be considered as start material for producing natural antischistosomiasis remedy. We suggest prolong the duration of treatment to reach complete eradication of the worms. Additionally, plant tissue culture could be considered as an efficient tool for continuous and steady supply of the very important saponins as biological active phytochemicals for drug manufacture from *Balanites aegyptiaca* L. Plant. This could be achieved away from the problems of depletion of active ingredients due to climatic conditions, plant diseases and increased demand of the plant. This helps to stabilize future possible pharmaceutical industry from these secondary metabolites as active raw materials.

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References:

- Aboul-Ela, E. I., 2002. Cytogenetic studies on *Nigella sativa* seeds extract and thymoquinone on mouse cells infected with Schistosomiasis using Karyotyping. *Mutation Research* 516, 11-17.
- Aboul-Ela, E. I., Soliman, A. M., Faddah, L. M., 1996. Induction of chromosomal aberrations on the somatic and germ cells in mice treated with praziquantel. *Proceeding of the Third Congress of Toxicology in Developing Countries, Cairo, Egypt.* 2, 219-230.
- Al Ashaal, H. A., Farghaly, A. A., Abd El-Aziz, M. M., Ali, M. A., 2010. Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (*Balantiaceae*). *Journal of Ethnopharmacology* 127, 495-501.
- Al Ashaal, H. A., Fahmy, M. A., Melek, F. R., Aly, N. H., Hassan, Z. M., 2012. Effect of supplemented soybean (*Glycine max* L) diet and extracts on aluminum sulfate - induced genotoxicity. *Toxicological and Environmental Chemistry* 94, 965-686.
- Ali, S. A., Hamed, M. A. 2006. Effect of *Ailanthus altissima* and *Zizyphus spina-christi* on bilharzial infestation in mice: histological and histopathological studies. *Journal of Applied Science* 6, 1437-1446.
- Aly, H. F., Amany, S. M., 2012. Hepatoprotective Efficacy of *Schistosoma mansoni* or *Fasciola gigantica* worm homogenates mixed with saponin. *African Journal of Biotechnology* 11, 11713-11725.
- Aly, H. F., Mona, H. H., 2006. Evaluation of antioxidant effect of *Citrus reticulata* in *Schistosoma mansoni* infected mice. *Trand Mediciene Research* 11, 411-431.
- Angustin, J., Klevn, B.P., Barker, J.B., Venagepa, P.B., 1985. Vitamin E In: *Methods of Vitamin Assay.* 4th Edition, Academic Press, Inc., New York pp. 266-267.
- Babson, A. L., Babson, S. R., 1973. Kinetic colorimetric measurement of serum

- lactate dehydrogenase activity. *Clinical Chemistry* 19, 766-769. Bendich, A., 1999. Immunological Role of Anti oxidant Vitamins in: Antioxidants in human health and disease. CABI Publishing, New York, USA, CAB International pp 213.
- Bidawat, S., Nag, R., NAG, T. N., 2011. Antimicrobial principles from tissue cultures of *Balanites aegyptiaca*. *Romanian biotechnological letters* 16, 6120-6124 16.
- Bishnu, P., Chapagain, V. S., Zeev, W., 2008. Larvicidal activity of saponins from *Balanites aegyptiaca* callus against *Aedes aegypti* mosquito. *Bioresource Technology* 99, 1165-1168.
- Bodansky, O., Schwartz, M. K., 1963. Comparative effects of L-histidine on the activities of 5'-nucleotidase and alkalinephosphatase. *Journal of Biological Chemistry* 238, 3420-3427.
- Bogitish, B., Cheng, T., 1990. Human parasitology. Saunders College Publishing New York , pp. 193-210.
- Brown, G. W., Cohen, P. P., 1959. Comparative biochemistry of urea synthesis. I. Methods for the quantitative assay for urea cycle enzymes in liver. *Journal of Biological Chemistry* 234, 1769-1774.
- Buege, J. A., Aust, S.D., 1978. Microsomal lipid peroxidation. *Method of Enzymology* 52, 302-310.
- De-Duve, C., Wattiaux, R., Baudhuin, P., 1962. Distribution of enzymes: subcellular fractions in animal tissues. In FF Nord, *Advances in Enzymology*, Interscience Publishers, New York, London, pp 291.
- Doughari, J. M., Pukuma, M.S., De, N., 2007. Antibacterial effects of *Balanites aegyptiaca* L. Del. and *Moringa oleifera* Lam. on *Salmonella typhi*. *African Journal of Biotechnology* 6, 2212-2215.
- Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., Markova, L., Urban, M., Sarek, J., 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural Product Reports* 23, 394-411.
- El-Ansary, M. S., Khaled, I. A., Saleh, A. F., Mahmoud, O. M., Baioumi, E. A., Bakr, H. A., 2010. Cytogenetic Study of the Effect of *Schistosoma mansoni* Infection on Human Peripheral Blood Lymphocytes and the Role of a-Carotene and Vitamin E in Modulating this Effect. *Journal of American Science* 6, 191-202.
- El-Banhawey, M. A., Ashry, M. A., EL-Ansary, A. K., Ali, S. A., 2007. Effect of *Curcuma longa* or Parziquantel. On *Schistosoma mansoni* infected mice liver Histological and Histochemical Studies. *Indian Journal of Experimental Biology* 45, 877-888.
- El- Mekawy, M. A. M., Ali, M. A. A., Dawah, A. K., Hassan, H. M. S., 2012. Effect of some additives on micropropagation of *Balanites aegyptiaca* L. Explants. *World Journal of Agricultural Sciences* 8, 186-192.
- El-Sharkawy, I., Saleh, W., El-Alfy, N., 2003. Cytogenetics of acute and

- chronic *schistosomiasis mansoni*. Journal of Egypt Society of Parasitology 33, 341-352.
- El-Tahir, A., Satti, G. M. H., Khalid, S. A., 1999. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. *Phytotherapy Research* 13, 474-478.
- Evans, E. P., Breckon, G., Ford, C. E., 1964. An air-drying method for meiotic preparations for mammalian testes. *Cytogenetics* 3, 289-294.
- Evans, M., 2007. Pharaohs and the first prescriptions Citation: The *Pharmaceutical Journal*, Vol. 277, p735 | URI: 10005731.www.pharmaceutical-journal.com/.../10005731.article
- Frei, B., Stocker, R., Ames, B. N., 1988. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceeding of the National Academy of Science* 88, 9748-9752.
- Gad, M. Z., El- Sawahli, M. M., Ismail, M. F., El- Tanbouly, N. D., 2006. Bio Chemical Study of the anti-diabetic action of the Egyptian plants *Fenagreek* and *Balanites*. *Molecular and Cellular Biochemistry* 281, 173183.
- Gajakshmi, S., Vijayalakshmi, S., Devijayalakshmi, V., 2013. Pharmacological Activities of *Balanites aegyptiaca* (L.). *International Journal of Pharmacology and Science Review Research* 22, 117-120.
- Gella, F. J., Olivella, T., Cruz, P. M., Arenas, J., Moreno, R., Durban, R., Gomez, J. A., 1985. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxalphosphate. *Clinical Chemistry Acta* 153, 241-247.
- Ghareeb, A. M., El-Asmar, M. F., Kamal, M. A., El-Magd, A. A., 1979. Effect of bilharzial infection on liver and brain enzymes. *Ain-Shams Medicene Journal* 30, 59-64.
- Gnoula, C., Megalizz, V., De Neve, N., 2008. Balanitin-6 and -7: diosgenyl saponins isolated from *Balanites aegyptiaca* Del. display significant antitumor activity *in vitro* and *in vivo*. *International Journal of Oncology* 32, 515.
- Gour, V. S., Kant, T., 2006. Optimized callus production in *Balanites aegyptiaca* (L.) Del.: a potential source of diosgenin. *Journal of Phytological Research* 19, 53-57.
- Hall, J. B., Walker, D. H., 1991. *Balanites aegyptiaca* Del. -A Monograph. School of Agricultural and Forest Science, University of Wales, Banger, pp 1-12.
- Hamada, F. M. A., Abdel-Aziz, H. A., Badr, F. M., Moustafa, A., Metwally, A., 1992. The mutagenic effect of praziquantel in *S. mansoni* -infected mice. *Arab Journal of Laboratory* 18, 301-311.
- Hamed, M. A., Ali, H. F., Ali, S. A., El- Rigal, N., Rizk, M. Z., 2010. *Biomphalaria alexandrina* snails as immunogens against *Schistosoma mansoni* infection in mice. *Memorias do Instituto Oswaldo Cruz, Rio de Janeiro* 105, 879-888.
- Hamed, M. A., 2006. Excretort- secretory product of *Fasciola hepatica* worm

- protects against *Schistosoma mansoni* infection in mice. Indian Journal of Experimental Biology 44, 554-561.
- Hamed, M. A., Hetta, M. H., 2005. Efficacy of *Citrus reticulata* and Mirazid in treatment of *Schistosoma mansoni*. Memorias do Instituto Oswaldo cruz 100, 771-778.
- Hamed, M. A., 2011. Potency of detergents in enhancing *Schistosoma mansoni* tegumental antigen. Journal of Infection in Developing Countries. 5, 209-215.
- Hara, A., Fukuyama, K., Epstein, W. L., 1981. Angiotensin converting enzyme and other enzymes in liver of mice with experimental schistosomiasis. Experimental of Molecular Pathology 35, 199-210.
- Hatem, A. S., Amira, H. E. N., Fathia, A. K., Omnia, M. K., 2012. Efficacy of methanolic extract of *Balanites aegyptiaca* fruits on *Toxocara vitulorum*. Veterinary Parasitology 183, 386-392.
- Heble, M. R., Staba, E. J., 1980. Steroid metabolism in stationary phase cell suspensions of *Discoreadeltoidea*. Planta Medica 40, 124-128.
- Hirsch, C., Zouain, C. S., Alves, J. B., Goes, A. M., 1997. Induction of protective immunity and modulation of granulomatous hypersensitivity in mice using PIII, an anionic fraction of *Schistosoma mansoni* adult worm. Parasitology 115, 21-28.
- Hirsch, H., Terano, A., Sugimoto, T., Harada, T., Razand, M., Ivey, K. J., 1994. Protective role of intracellular superoxide dismutase against extracellular oxidants in cultured rat gastric cells. Journal of Clinical Investigation 93, 331-338.
- Ip, S., Yiu, H. Y., Kos, K. M., 2000. Differential effect of schisandrin B and dimethyl diphenyl bicarboxylate (DDB) on hepatic mitochondrial glutathione redox status in carbon tetrachloride intoxicated mice. Molecular of Cell Biochemistry 205, 111-114.
- IUCN., 2005. *A guide to medicinal plants in North Africa*. Centre for Mediterranean Cooperation, International Union for Conservation of Nature and Natural Resources, Union internationale pour la conservation de la nature et de ses ressources. Printed by: Graficas La Paz, Torredongimeno, Jaen (Spain), ISBN:2-8317-0893-1.
- Jogata, S. K., Dani, H. M., 1982. A new colorimetric technique for the estimation of vitamin C (using Folin Phenol Reagent). Analytical Biochemistry 127, 178-182.
- Kamel, M. S., 1991. Phytochemical study of *Balanites aegyptiaca* and *Salvadoraperisica* as anti hyper glycemc plants. Ph. D. Pharmacological Science (Pharmacognosy), Faculty of Pharmacy, Assuit University.
- Karous, D., Tchacondo, T., Ouattara, L., Anan, K., Savadogo, A., Agbonon, A., Ben Attaia, M., de Souza, C., Sakly, M., Simpo, J., 2011. Antimicrobial, antiplasmodial, haemolytic and antioxidant activities of crude extracts of three selected Togolese medicinal plants. Asian Pacific Journal of Tropical Medicine 4, 808-813.

- Kela, S. L., Ogunsusi, R. A., Ogbogu, V. C., Nwude, N., 1989. Screening of some Nigerian plants for molluscicidal activity. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux* 42, 195-202.
- Koko, W. S., Abdalla, H. S., Galal, M., Khalid, H. S., 2005. Evaluation of oral therapy on Mansonial Schistosomiasis using single dose of *Balanites aegyptiaca* fruits and praziquantel. *Fitoterapia* 76, 30-3.
- Kusch, P., Deininger, S., Specht, S., Maniako, R., Haubrich, S., Pommerening, T., Lin, P. K., Hoerauf, A., Kaiser, A., 2011. *In Vitro* and *In Vivo* Antimalarial Activity Assays of Seeds from *Balanites aegyptiaca*: Compounds of the Extract Show Growth Inhibition and Activity against Plasmodial Aminopeptidase. *Journal of Parasitology Research*. 36, 86-92.
- Linton, S. N., Campbell, J. W., 1962. Studies of urea cycle enzymes in the terrestrial snail *Otala lactea*. *Archeive of Biochemistry and Biophysics* 97, 360-369.
- Liu, Y., Krepple, H., Liu, J., Choudhuri, S., Klaassen, C. D., 1994. Oleanolic acid protects against cadmium hepatotoxicity by inducing metallothionein. *Journal of Pharmacological Experimental Therapy* 266, 400-406.
- Loverde, P. T., 1998. Do antioxidants play a role in schistosome host- parasite interactions? *Parasitol Today* 14, 284-289.
- Maghraby, A. S., Hamed, M. A., Ali, S. A., Aly, H. F., 2010. The antischistosomal activity of *Fasciola gigantica* eggs influenced by Egyptian saponin extracted from *Atriplex nummularia*. *Journal of American Science* 6, 368-381.
- Mansor, N., Ismaila, D., Yaye, K., Gassama, D., 2003. *In vitro* multiplication of the semi-arid forest tree, *Balanites aegyptiaca* (L.) Del. *African journal of Biotechnology* 2, 421-424.
- Mohamed, A. M., Wolf, W., Spiess, W. E. L., 2002. Physical, morphological and chemical characteristics, oil recovery and fatty acid composition of *Balanites aegyptiaca* Del. *kernels*. *Plant Foods for Human Nutrition* 57,179189.
- Molla, E., Giday, M., Erko, B., 2013. Laboratory assessment of the molluscicidal and cercariacidal activities of *Balanites aegyptiaca*. *Asian Pacific Journal of Biomedicine* 3 , 657-662.
- Moron, M. S., Depierre, J. W., Mannervik, B., 1979. Level of glutathione, glutathionereductase and glutathione-S-transferase activities in rat lung and liver. *Biochimica Biophysica Acta* 582, 67-78.
- Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plant* 15, 973 - 97.
- Maurice, M. I., 1993. *Handbook of African medicinal plants*. Boca Raton u.a.: CRC Press. p. 129. ISBN 084934266X. Free encyclopedia; wikipedia
- Oliver, L., Stirewalt, M. A., 1952. An efficient method for the exposure of mice to cercariae of *Schistosoma mansoni*. *Journal of Parasitology* 39, 19-23.
- Osada, Y., Kumagai, T., Masuda, K., Suzuki, T., Kanazawa, T., 2005. Mutagenicity evaluation of *Schistosoma* spp. extracts by the umu-test and

- V79/HGPRT gene mutation assay. *Parasitology International* 54, 29-34.
- Pascal, M., Abd-Allah, O. M., El-Wali, N. E., Mergani, A., Qurashi, M. A., Magzoub, M., De-Reggi, M., Gharib, B., 2000. Hyaluronate levels and markers of oxidative stress in the serum Sudanese subjects at risk of infection with *Schistosoma mansoni*. *Transactions of the Royal Society Medicine and Hygiene* 94, 66-70.
- Raafat, M., El-Gerzawi, S., Stich, H. F., 1984. Detection of mutagenicity in urothelial cells of bilharzial patients, micronucleus test. *Journal of Egypt National Cancer Institute* 1, 63-73.
- Rice, M. E., Shelton, E., 1957. Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. *Journal of Egypt National Cancer Institute* 18, 117-125.
- Rizk, M., Hafez, S., Aly, H. F., 2000. Measurement of urea cycle enzyme activities in mice livers under the influence of different stages of *Schistosoma mansoni* infection and *Cucuma longa* treatment. *Journal of Egypt and Germany Society of Zoology* 32A, 319-333.
- Rosalki, S. B., Foo, A. Y., Burlina, A., 1993. Multicenter evaluation of isoALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. *Clinical of Chemistry* 39, 648-652.
- Saad, S.I., 1998. *Ancient Egyptian Plants*. Egyptian academy of Science, Egypt 161-162.
- Scarpato, R., Pistelli, L., Bertoli, A., Nieri, E., Migliore, L., 1998. *In Vitro* genotoxicity and cytotoxicity of five new chemical compounds of plant origin by means of the human lymphocyte micronucleus assay. *Toxicology in Vitro* 12, 153-161.
- Shalaby, M. A., Moghazy, F. M., Shalaby, H. A., 2010. Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. *Parasitology Research* 107, 17-25.
- Shubber, E., 1987. Sister chromatid exchanges in lymphocytes from patients with *S. heamatobium*. *Mutation Research* 180, 93-99.
- Sokal, R. J., McKim, J. M., Goff, M. C., Ruyle, S. Z., Devereaux, M. W., Han, D., Packer, L., Everson, G. 1998. Vitamin E reduces oxidant injury to mitochondria and hepatotoxicity of taurochenodeoxycholic acid in rat. *Gastroenterology* 114, 164-174.
- Son, I. S., Kim, J. H., Sohn, H.Y., Son, K. H., Kim, J. S., Kwon, C. S., 2007. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea* spp.), on high-cholesterol fed rats. *Bioscience Biotechnology Biochemistry* 71, 3063-3071.
- Swanson, M.A., 1955. Glucose-6-phosphatase from liver. In *Methods in Enzymology* Academic Press, Inc., New York, 2, 541-543.
- Tredgold, M. H., 1986. *Food Plants of Zimbabwe*. Mambo Press Harare, p. 292-293.
- Treyvaud, V., Marston, A., Dyatmiko, W., Hostettmann, K., 2000.

- Molluscicidal saponins from *Phytolacca icosandra*. *Phytochemistry* 55, 603609. Unesco., 1960 NS.59/III. 17// in Medicinal plants of the arid zones - unesdoc - Unesco pp.69, 80. United Nations. Educational, Scientific and Cultural Organization. Place de Fontenoy, Paris-7e. Printed in France by Oberthur, Rennes unesdoc.unesco.org/images/0006/.../068198eo.
- Wyrobek, A. J., Bruce, W. R., 1978. The induction of sperm-shape abnormalities in mice and humans, In: Hallaender, A., De Serres, F. J. (eds.) *Chemical Mutagens: Principles and methods for their detection*. Plenum, New York 5, 257-285.
- Ya'u, J., Abdulmalik, U. N., Yaro, A. H., Chindo., B. A., Anuka, J. A., Hussaini, I. M., 2011. Behavioral properties of *Balanites aegyptiaca* in rodents. *Journal of Ethnopharmacology* 135, 725-729.
- Yosida, H., Amano, K., 1965. Autosomal polymorphism in laboratory bred and wild Norway rats, *Rattus norvegicus*. *Chromosoma* 16, 658- 667.
- Zhang, Q. H., Wu, C. F., Yang, J. Y., Mu, Y. H., Chen, X. X., 2009, Reduction of cyclophosphamide-induced DNA damage and apoptosis effects of ginsenoside Rb1 on mouse bone marrow cells and peripheral blood leukocytes. *Environmental Toxicology and Pharmacology* 27, 384-389.