Egypt. J. Chem. **Vol. 64,** No. 8 pp. 4557 - 4568 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In Vivo Assessment of the Antischistosomal Activity of *OPUNTIA FICUS-INDICA* Flowers and Their Chemical Constituents

Asmaa H. Sheded,[a] Maher A. EL-Hashash,[a] Sayed A. EL-Toumy,[b] Josline Y. Salib,[b*] Samia William,[c] Manal M. Hamed,[d]

[a].*Organic Chemistry Department, Faculty of Science, Ain-Shams University*
[b] *Chemistry of Tannins, National Research Center, Cairo, Egypt.*
[c]*Parasitology Department, Theodor Bilharz Research Institute, Ministry of Scientific Research, Warak El Hadar, Embaba, Cairo, Egypt*
[d] *Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza 1241, Egypt*

Abstract

Hepatic schistosomiasis is the most well-known form of chronic disease, with a wide variety of clinical symptoms. The aim of this study was to assess the schistosomicidal effect of the aqueous methanolic extract of *Opuntia ficus-indica* flowers *in-vivo* regarding disease progression in a comparative experimental study to praziquantel (PZQ). Phytochemical investigation of flowers extract resulted in thirteen phenolic and flavonoid compounds. Structures of these compounds were elucidated by UV and 1D⁄2D 1H⁄ 13C NMR spectroscopy and compared with the literature data. From the 49th day after infection, mice infected with *Schistosoma mansoni* were given the extract (200 mg/kg) orally every day for 5 days, whereby the Praziquantel (500 mg/kg) was used as reference drug. The parasitological parameters (total worm burden, tissue egg load and oogram pattern) per gram of liver or intestinal tissue of the infected and the treated mice were counted. A histopathological examination of the liver granuloma took place as well. *Opuntia ficus-indica* extract caused a substantial decrease in the number of worms and eggs (%); the extract also decreased worm and egg burdens moderately. The results demonstrated the flower extract as a promising antischistosomal activity due to the parasitological and histopathological changes induced, besides it highlights the importance of its polyphenolic constituents.

Keywords*: Opuntia ficus-indica;* flowers; flavonoids constituents; antischistosomal activity

1. Introduction

Schistosomiasis is widespread in 75 countries across Africa, Asia, South America, and the Middle East, making it one of the most important neglected tropical diseases [1, 2]. Schistosomiasis is a parasitic disease that has infected Egyptians since the pharaohs' period. The disease is caused by schistosome eggs embedded in the host liver, which trigger an immune response and cause hepatic granuloma and fibrosis in some patients [3]. Schistosomiasis is the second leading cause of morbidity in the world, second only to malaria in terms of socioeconomic significance, and the third most common parasitic disease in terms of public health importance, according to the World Health Organization (WHO) [4]. Until a viable vaccine is developed, the use of safe and effective drugs will remain the key control method for schistosomiasis. Praziquantel (PZQ) is the only anti-bilharzial drug that works against the four Schistosoma species that cause human disease [5]. Control of schistosomiasis using PZQ at a population level faces some problems. Resistance to PZQ treatment has been recently induced in Schistosoma by laboratory selection [6] whereby, Senegalese, Kenyan, and Egyptian patients have showed reduced cure rates and treatment failure post PZQ treatment [7, 8]. Therefore search for alternative antischistosomal drugs is an urgent need.

Opuntia, also known as prickly pear, is a flowering plant genus in the Cactus family (Cactaceae). Prickly pears, like most true Cactus plants, are native only to the Americas;

*Corresponding author e-mail: joslineysalib@gmail.com .; (Josline Y. Salib).
Receive Date: 27 May 2021, Revise Date: 25 June 2021, Accept Date: 25 July 2021
DOI: 10.21608/ejchem.2021.77763.3809

however they have since been introduced to many other parts of the world due to human actions. In Mexico, most of Latin America, South Africa, and the Mediterranean, the cactus pear (*Opuntia ficus-indica* L. Mill) is a common plant nowadays whereby the fruit is eaten [9]. The genus *Opuntia* consisting of about 200 species, thrives in South Western USA, Mexico and Mediterranean climates. *Opuntia ficus-indica* has various biological activities such as anti-allergic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, hepatoprotective, hypoglycemic, neuroprotective, and wound healing effects [10]. Previous studies on the chemical constituents of *O. ficus-indica* revealed the presence of alkaloids, flavonoids, terpenoids, polysaccharides, and organic acids [11].

Thus, the present study aims to evaluate the anti-schistosomal activity of aqueous methanolic extract from *Opuntia ficus-indica* flowers on *schistosoma manson*i infected mice *in vivo* in a biological mouse model. The study was intended to explore the parasitological and histopathological impact of the used natural remedies in different developing stages. Moreover the extract was subjected to phytochemical investigations using different chromatographic techniques to afford ten flavonoid compounds together with three phenolic acids. The compounds; protocatechuic acid, isorhamnetin-3-*O*-rutinoside and quercetin-3-*O*-α-L-rhamnopyranoside were isolated from the flowers of the Egyptian species for the first time.

2. Experimental

2.1. Plant Material

Opuntia ficus-indica flowers were collected from Abo-Zabel region, Egypt during May 2018 (flowering date). The samples were separately air-dried in shed, powdered and kept in tightly sealed round flasks and stored for biological and phytochemical studies. Identification of the plant was confirmed by Botany Department, Faculty of Science, Cairo University, Egypt [12]. Voucher specimens (T19) were deposited in the Herbarium of the National Research Centre, Dokki, Cairo, Egypt.

2.2. General methods and drugs

^{1}H (400 MHz) and ^{13}C (100 MHz) – NMR spectra were recorded on a Bruker 400 spectrometer; the chemical shifts were recorded in DMSO-d_6 and are given in ppm values. UV spectra were measured on Shimadzu spectrophotometer model UV-240; CC: was performed using Polyamide 6S and Sephadex LH-20; PC: was carried out on Whatman No.1 and 3MM using solvent systems (1) BAW (n-BuOH: HOAc: H_2O, 4:1:5); (2) H_2O; (3) 15 % AcOH (AcOH: H_2O, 15 : 85) and visualized under UV light using $AlCl_3$ and NA as spraying reagents; Aniline hydrogen phthalate was used as specific reagent for sugar analysis.

Praziquantel (PZQ) was obtained as tablets (Distocide, Egyptian International Pharmaceutical Industries Company, EIPICO) were freshly suspended in 2% Cremphore-EL (Sigma-Aldrich, St Louis, MO, USA) before use.

2.3. Extraction and isolation

1.2 Kg of the air dried flowers were reduced to a coarse powder and was first defecated by petroleum ether then successively extracted by maceration with 70% methanol (5x2L) at room temperature till exhaustion. The filtrates were collected, dried under vacuum at 40 °C to give 85 gm net weight (w/w).

Fifty grams of the methanolic extract was chromatographed on a Sephadex LH-20 column with water and water /ethanol mixtures as eluent to give seven fractions. Fractions were further separated and purified using Whatman 3MM papers and Sephadex LH-20 column to yield the 13 pure flavonoid and phenolic compounds [13]. Compounds **P1-P3** were isolated from fraction **II** (eluted by 20% EtOH) and further separated by preparative paper chromatography using 15% AcOH as solvent followed by purification on a Sephadex LH-20 column to afford the pure form of them. Compound **P4** was isolated from the column by 40% aqueous ethanol (fraction **III**) and was further purified on Sephadex LH-20 column by 50% ethanol. **P5-P6** were isolated from the fraction **IV** (50% EtOH) by applying repeated Sephadex LH-20 column fractionation, using ethanol for elution at a very low rate, which led to the successive desorption of these compounds. While fraction **V** (60% aqueous ethanol) gave rise to **P7-P9,** which were isolated by applying on a Sephadex LH-20 column and eluted by water followed by water/ethanol mixtures at a very low rate. From fraction VI (80% ethanol), **P10-P11** were isolated as an amorphous yellow powder as a mixture of two isomers in the ratio 2:1. Preparative paper chromatography of fraction **VII** (100% ethanol) using BAW as solvent, afforded two aglycones; **P12** and **P13**.

2.4. Animals

30 Male Swiss albino mice (CD-1) were obtained from SBSC of TBRI, Giza, Egypt, weighing 18–20 g each, were housed under environmentally controlled room temperature of 20–22 °C, a 12 h light/dark cycle and 50–60% humidity with access to food and water *ad*

libitum throughout the acclimatization and experimental cycles. Mice were infected with *S. mansoni* cercariae (provided by SBSC) using body immersion [14] through exposure to 80 ± 10 cercariae/mouse. The entire experimental animal were conducted in accordance with the Laboratory Animals Guide and approved by the Institutional Review Board of TBRI.

The cercarial suspension (0.1 ml) will be gently mixed, stained with picric acid solution and counted.

2.5. Experimental design

In 2 percent of Cremophore-EL (Sigma-Aldrich, St Louis, MO, USA) *Opuntia ficus-indica* extract and PZQ were freshly suspended. Infected mice were divided into 3 groups, each composed of 10 mice at the beginning of the experiment. Plant extracts will be orally administered for 5 consecutive days at the 7th week post infection.

Group 1: Control group for untreated infected mice (IC).

Group 2: Infected mice were treated with PZQ in a dose of 200 mg/kg.

Group 3: Infected mice were treated with ***(OP)*** in a dose of 200 mg/kg.

2.6. Assessment of parasitological criteria of cure

All mice were sacrificed and perfused fourteen days after treatment, and the number of worms recovered (worm burden) was quantified and sexed [15]. We calculated the number of eggs per gram of liver or intestinal tissue [16]. The percentage of egg developmental stages (oogram pattern) has been studied [17], identifying and counting eggs at different maturity stages (from I to IV). Mature eggs and dead eggs (granular, dark, and semi-transparent) were also counted in three intestinal fragments and the mean number was calculated for each stage.

2.7. Histological examination

For light microscopy inspection, liver specimens were fixed in 10% formalin, processed to paraffin blocks, sectioned (4 µm thick) and coated with hematoxylin/eosin (H&E). The presence of hepatic granulomas and associated histological changes were examined for the liver sections. Only transverse sections (250 µm apart) of H&E stained granulomas showing a central, viable egg, were considered for measurements. Granuloma diameter (30/mouse), composition and egg viability were investigated.

2.8. Statistical analysis

The percentage reduction of worm/egg burden in each treated group was calculated according to the following equation: % reduction = [(No. of worms/eggs in control group) − (No. of worms/eggs in treated group)] / (No. of worms/eggs in control group) × 100. Results were expressed as mean ± SEM. A two-tailed Student's *t*-test was used to detect the significance of difference between the means of different groups. Results were considered significant when the P value is <0.05.

3. Results

The phytochemical investigation of the aqueous methanolic extract of *Opuntia ficus-indica* flower extract afforded ten flavonoid compounds; rutin (**P4**), isorhamnetin-3-*O*-rutinoside (**P5**), isorhamnetin-3-*O*-robinobioside (**P6**), quercetin-3-*O*-β-glucopyranoside (**P7**), quercetin-3-*O*-β-galactopyranoside (**P8**), quercetin-3-*O*-α-L-rhamnopyranoside (**P9**), isorhamnetin-3-*O*-β-galactopyranoside (**P10**), isorhamnetin-3-*O*-β-glucopyranoside (**P11**), quercetin **P12** and isorhamnetin **P13**, together with three phenolic acids; protocatechuic acid (**P1**), *p*-methoxy benzoic acid (**P2**) and isoferulic acid (**P3**) (Fig. 1). The structures were elucidated UV and 1D⁄2D ^{1}H⁄ ^{13}C NMR spectroscopy besides their chromatographic behaviors [18-20] as follows:

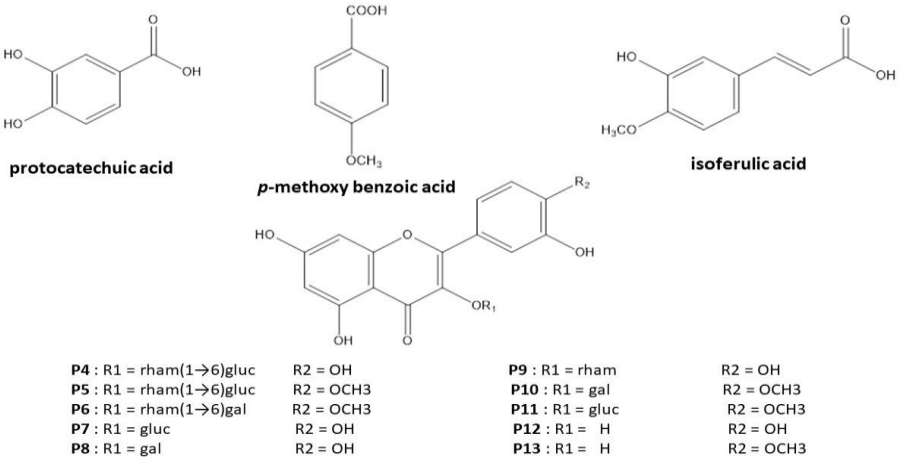

Fig 1. Chemical structures of the isolated compounds

Protocatechuic acid, (P1)

P1 was isolated as a white powder, gave a dark blue color under UV light with R_f values (x100): 81 (BAW), 59 (15 % ACOH), 57 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 240, 295, 325, +NaOMe: 345; ^{1}H-NMR ($CDCl_3$) spectral data: δ (ppm) at 7.333 (d, J = 2 HZ, H-2), 7.283 (dd, J = 8 and 2 HZ, H-6), 6.779 (d, J = 8 HZ, H-5); ^{13}C-NMR (CD_3OD) spectral data: δ (ppm) at 122.59 (C-1), 117.11 (C-2), 145.35 (C-3), 150.40 (C-4), 115.63 (C-5), 122.29 (C-6), 168.11 (C=O). P1 was isolated from the flowers of the Egyptian species for the first time.

p-methoxy benzoic acid, (P2)

It was obtained as a white powder with R_f values (x100): 92.6 (BAW) 56 (15% ACOH), 45 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 215, 270, 320; ^{1}HNMR ($CDCl_3$) spectral data: δ (ppm) at 7.91 (d, J = 8.8 Hz, H-2 & 6), 7.031 (d, J = 8.8 Hz, H-3 & 5), 3.828 (s, 4-OMe); ^{13}C-NMR ($CDCl_3$) spectral data: δ (ppm) at 131.80 (C-1), 123.44 (C-2 & C-6),114.27 (C-3 & C-5), 163.31 (C-4), 167.46 (C=O), 55.89 (OCH3).

Isoferulic acid, (P3)

It exhibited yellow crystals with R_f values (x100): 92 (BAW) 52 (15 % ACOH), 37 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 240, 295; ^{1}HNMR (CD_3OD) spectral data: δ (ppm) at 7.48 (d, J = 15 HZ, α-H), 7.34 (d, J = 2 HZ, H-2), 7.08 (dd, J = 2 and 8 HZ, H-6), 6.89 (d, J = 8 HZ, H-5), 6.28 (d, J = 15 HZ, β-H), 3.8 (s, OMe-4); ^{13}C-NMR ($CDCl_3$) spectral data: δ (ppm) at 167.9 (C-γ), 115.84 (C-β), 144.12 (C-α), 129.76 (C-1), 115.54 (C-2), 147.91 (C-3), 149.59 (C-4), 111.26 (C-5), 122.72 (C-6) and 55.70 (C-OMe).

Quercetin*-3-*O*-rutinoside, *(Rutin) (P4)

P4 was obtained as yellow amorphous powder and gave dark purple fluorescent spot turned to yellow on PC with ammonia vapor. R_f values (x100): 47 (BAW), 53 (15 % ACOH), 24 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 258, 266sh, 299sh, 360, +NaOMe: 272, 327, 416, +NaOAc: 270, 325, 393, +NaOAc/H_3BO_3: 262, 298, 387, +$AlCl_3$: 275, 303sh, 430, +$AlCl_3$/HCl: 271, 300, 364sh, 402; ^{1}H NMR (DMSO-d_6) spectral data: δ (ppm) 7.573 (d, J = 2.1 Hz, H-2'), 7.548 (dd, J = 9, 2.1 Hz, H-6'), 6.874 (d, J = 9 Hz, H-5'), 6.404 (d, J = 2.1 Hz, H-8), 6.2013 (d, J = 2.1 Hz, H-6), 5.375 (d, J = 7.5 Hz, H-1''), 4.40 (d, J = 2.0 Hz, H-1'''), 1.022 (d, J = 6.3 Hz, H-6''') 3.071-3.710 (m, the rest sugar of glucose and rhamnose); ^{13}C NMR (DMSO-d_6) spectral data: *aglycone moiety*: δ 156.6 (C-2), 133.3 (C-3), 179.4 (C-4), 161.3 (C-5), 98.7 (C-6), 164.0 (C-7), 93.6 (C-8), 156.4 (C-9), 104.0 (C-10), 121.2 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.4 (C-4'), 116.3 (C-5'), 121.6 (C-6'); 3-O-glucopyranoside moiety: δ 101.2 (C-1''), 74.1 (C-2''), 76.5 (C-3''), 70.0 (C-4''), 75.9 (C-5''), 66.9 (C-6''); 6''-O-rhamnopyranosyl moiety: δ 100.8 (C-1'''), 70.4 (C-2'''), 70.6 (C-3'''), 71.9 (C-4'''), 68.2 (C-5'''), 17.7 (C-6''').

Isorhamnetin-3-O-* rutinoside, *(P5)

It was obtained as a light-yellow amorphous powder. R_f values (x100): 58.3 (BAW), 69.7 (15 % ACOH); UV Spectral Data λ_{max} (nm): MeOH: 255, 268 sh, 303 sh, 357, +NaOMe: 272, 327, 414, +NaOAc: 274, 387 +NaOAc/H_3BO_3: 257, 266 sh, 306 sh, 361, +$AlCl_3$: 268, 298 sh, 365 sh, 407, +$AlCl_3$/HCl: 267, 297 sh, 357, 404; ^{1}H NMR (DMSO-d_6) spectral data: δ 7.825 (d, J = 2.0 Hz, H-2'), 7.489 (dd, J = 8.4, 2.0 Hz, H-6'), 6.88 (d, J = 8.4 Hz, H-5'), 6.39 (d, J = 1.6 Hz, H-8), 6.17 (d, J = 1.6 Hz, H-6), 3.79 (s, 3'-OCH_3); 5.15 (d, J = 7.2 Hz, H-1''), 4.53 (d, J = 1.2 Hz, H-1'''), 0.94 (d, J = 6.3 Hz, H-6'''), 3.8 – 3.028 (m, the rest sugar of glucose and rhamnose); ^{13}C NMR (DMSO-d_6) spectral data: *aglycone moiety*: δ 157.01 (C-2), 133.60 (C-3), 177.88 (C-4), 161.37 (C-5), 99.28 (C-6), 164.72 (C-7), 94.34 (C-8), 157.02 (C-9), 104.56 (C-10), 121.61 (C-1'), 115.80 (C-2'), 149.95 (C-3'), 147.45 (C-4'), 113.88 (C-5'), 122.84 (C-6'), 56.23 (3'-OCH_3); *3-O-glucopyranoside moiety*: δ 101.74 (C-1''), 74.83 (C-2''), 76.96 (C-3''), 70.66 (C-4''), 76.48 (C-5''), 67.38 (C-6''); *6''-O-rhamnopyranosyl moiety*: δ 101.42 (C-1'''), 71.15 (C-2'''), 70.68 (C-3'''), 72.35 (C-4'''), 68.82 (C-5'''), 18.22 (C-6'''). **P5** was isolated from the flowers of the Egyptian species for the first time.

Isorhamnetin-3-O-robinbioside, (P6)

It was obtained as dull yellow needles. R_f-values (x100): 52 (BAW), 58 (15% AcOH); UV Spectral Data λ_{max} (nm): MeOH: 255, 268sh, 303 sh, 357, +NaOMe: 272, 327sh, 415, +NaOAc: 274, 316, 387, +NaOAc /H_3BO_3: 257, 267sh, 307sh, 361, +$AlCl_3$: 269, 299sh, 365sh, 407, +$AlCl_3$/HCl: 275, 305sh, 361sh, 403; ^{1}H NMR (DMSO-d_6) spectral data: δ 7.86 (d, J = 2.0 Hz, H-2'), 7.54 (dd, J = 6.8, 2.0 Hz, H-6'), 6.97 (d, J = 6.8 Hz, H-5'), 6.51 (d, J = 1.6 Hz, H-8), 6.27 (d, J = 1.6 Hz, H-6), 3.84 (s, 3'-OCH_3); 5.43 (d, J = 6.8 Hz, H-1''), 4.43 (d, J = 1.2 Hz, H-1'''), 1.12 (d, J = 6.3 Hz, H-6'''), 3.057 -3.83 (m, the rest sugar of glucose and rhamnose); ^{13}C NMR (DMSO-d_6) spectral data: *aglycone moiety*: δ 157.00 (C-2), 133.47 (C-3), 177.75 (C-4), 161.06 (C-5), 99.29 (C-6), 164.75 (C-7), 94.34 (C-8), 156.88 (C-9), 104.40 (C-10), 121.43 (C-1'), 115.73 (C-2'), 149.89 (C-3'), 147.34 (C-4'), 113.70 (C-5'), 122.73 (C-6'), 56.14 (3'-OCH_3); *3-O- galactopyranoside moiety*: δ 101.56 (C-1''), 74.66 (C-2''), 76.28 (C-

3''), 70.54 (C-4''), 76.83 (C-5''), 67.4 (C-6''); *6''-O-rhamnopyranosyl moiety*: *δ* 101.31 (C-1'''), 71.04 (C-2'''), 70.68 (C-3'''), 72.23 (C-4'''), 68.69 (C-5'''), 18.1 (C-6''').

Quercetin 3-O-β-D-glucopyranoside, (P7)

Compound **P7** was isolated as a yellow amorphous powder, R_f-values (x100): 60 (BAW), 45 (15% AcOH), 08 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 253, 263sh, 294sh, 351, +NaOMe: 271, 328sh, 410, +NaOAc: 273, 321, 375, +NaOAc/H_3BO_3: 262, 300sh, 377, +AlCl3: 275, 305sh, 332sh, 435, +HCl: 275, 305sh, 361sh, 403; ^{1}H-NMR (DMSO-d_6) spectral data: *δ* (ppm) 7.67 (dd, *J* = 2.1 Hz and 8.6 Hz, H-6'), 7.58 (d, *J* = 2.1 Hz, H-2'), 6.82 (d, *J* = 8.6 Hz, H-5'), 6.40 (d, *J* = 1.8 Hz, H-8), 6.20 (d, *J* = 1.8 Hz, H-6), 5.39 (d, *J* = 7.6 Hz, H-1''), 3.28-3.69 (m, rest of glucose protons); ^{13}C-NMR (DMSO-d_6) spectral data: *aglycone moiety: δ* (ppm): 156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1'), 115.80 (C-2'), 145.80 (C-3'), 148.80 (C-4'), 116.20 (C-5'), 122.00 (C-6'), *sugar moiety: δ* (ppm): 101.20 (C-1''), 71.60 (C-2''), 74.40 (C-3''), 70.02 (C-4''), 77.70 (C-5''), 61.50 (C-6'').

Quercetin 3-O-β-D-galactopyranoside, (P8)

Compound **P8** was obtained as a brownish material, brown color under UV light gave yellow color with ammonia vapors and faint yellow with $AlCl_3$ reagent; R_f-values (x100): 55 (BAW), 35 (15% AcOH), 09 (H_2O); UV Spectral Data λ_{max} (nm): MeOH: 258, 269sh, 360, +NaOMe: 272, 328sh, 405, +NaOAc: 274, 323sh, 380, +NaOAc/H_3BO_3: 262, 300sh, 377, +AlCl3: 275, 305sh, 332sh, 435, +HCl: 275, 305sh, 361sh, 403; ^{1}H-NMR (DMSO-d_6) spectral data: *δ* (ppm) 7.68 (dd, *J* = 2.5 and 8 Hz, H-6'); 7.55 (d, *J* = 2.5 Hz, H-2'); 6.84 (d, *J* = 8Hz, H-5'); 6.42 (d, *J* = 2.5 Hz, H-8); 6.22 (d, *J* = 2.5 Hz,H-6); *sugar moiety: δ* (ppm) 5.6 (d, *J* = 7.5 Hz, H-1''), 3.2-3.8 (m, rest of the galactose protons); ^{13}C-NMR (DMSO-d_6) spectral data: *aglycone moiety*: *δ* (ppm) 156 (C-2), 133.8 (C-3), 177.5 (C-4), 161.2 (C-5), 98.6 (C-6), 164.0 (C-7), 93.4 (C-8), 156.3 (C-9), 104 (C-10), 121.3 (C-1'), 115.3 (C-2'); 144.7 (C-3'); 148.3 (C-4'); 116.2 (C-5'); 121.8 (C-6'); *sugar moiety*: *δ* (ppm) 102.3 (C-1''), 71.3 (C-2''), 76.4 (C-3''), 68.0 (C-4''), 75.8 (C-5''), 60.8 (C-6'').

Quercetin-3-O-α-L-rhamnopyranoside, (P9):

yellow powder, gave dark purple fluorescent spot turned to orange yellow on PC with ammonia vapor .R_f-values (x100): 71 (BAW), 50 (15% AcOH), 20 (H_2O); UV λ_{max} nm: MeOH: 253, 263sh, 344; +NaOMe: 272, 322sh, 372; + NaOAc: 260, 300sh, 367; +NaOAc/H_3BO_3: 272, 382; +$AlCl_3$: 272, 304sh, 333sh, 430; +$AlCl_3$/HCl: 272, 303sh, 353, 401; ^{1}H-NMR (DMSO-d_6) spectral data: *δ* (ppm) 7.33 (d, *J* = 2 Hz, H-2'), 7.28 (dd, *J* = 2 Hz, and *J* = 8.5 Hz, H-6'), 6.90 (d, *J* =8.5 Hz, H-5'), 6.42 (d, *J* = 2 Hz, H-8), 6.23 (d, *J* = 2 Hz, H-6); 5.29 (d, *J* = 1.41 Hz, H-1''), 3.16-3.56 (m, rest of rhamnose protons), 0.85 (d, *J* = 6.07 Hz, CH_3 of rhamnose); ^{13}C-NMR (DMSO-d_6) spectral data: *aglycone moiety: δ* (ppm) 157.34 (C-2), 134.27 (C-3), 177.79 (C-4), 161.35 (C-5), 98.78 (C-6), 164.34 (C-7), 93.70 (C-8), 156.86 (C-9), 104.16 (C-10), 121.17 (C-1'), 115.52 (C-2'), 145.25 (C-3'), 148.50 (C-4'), 115.72 (C-5'), 120.80 (C-6'); *sugar moiety*: *δ* (ppm) 101.87 (C-1''), 70.43 (C-2''), 70.63 (C-3''), 71.25 (C-4''), 70.11 (C-5''), 17.54 (C-6''). P9 was isolated from the flowers of the Egyptian species for the first time.

Isorhamnetin-3-O-β-D-galactopyranoside and Isorhamnetin-3-O-β-D- glucopyranoside, (P10 & P11)

Compound **P10** and **P11** were isolated as an amorphous yellow powder as a mixture of two isomers**,** one major spot of brown color under UV light gave yellow color with ammonia vapors and shiny yellow with $AlCl_3$ reagent, R_f-values (x100): 62 (BAW), 58 (15% AcOH); UV Spectral Data λ_{max} (nm): MeOH: 254, 266sh, 301 sh, 356, + NaOMe: 273, 326sh, 412, + NaOAc: 274, 316, 387, + NaOAc /H_3BO_3: 254, 267sh, 305sh, 358, +$AlCl_3$: 268, 295sh, 360sh, 400, + $AlCl_3$/HCl: 268, 295sh, 360sh, 403.

the ^{1}H and ^{13}C NMR data of compound **P10** from the whole NMR spectrum showed the following data;^{1}H-NMR (DMSO-d_6) spectral data: *δ* (ppm) 8.039 (d, *J* = 2.0 Hz, H-2'), 7.52 (dd, *J* = 8.0, 2.0, H-6'), 6.92 (d, *J* = 8.0 Hz, H-5'), 6.46 (d, *J* = 2.0 Hz, H-8), 6.22 (d, *J* = 2.0 Hz, H-6), 5.527 (d, *J* = 7.8 Hz, H-1''), 3.86 (s, 3H), 3.14–3.72 (m, rest of the sugar protons); ^{13}C-NMR (DMSO-d_6) spectral data: *aglycone moiety: δ* (ppm)156.71 (C-2), 133.44 (C-3), 177.86 (C-4), 161.66 (C-5), 99.26 (C-6), 164.83 (C-7), 94.21 (C-8), 156.85 (C-9), 104.42 (C-10), 121.55 (C-1'), 122.35 (C-2'), 115.62 (C-3'), 149.87 (C-4'), 147.45 (C-5'), 113.97 (C-6'), 56.16 (C-OCH_3), *sugar moiety*: *δ* (ppm) 102.12 (C-1''), 71.75 (C-2''), 76.35 (C-3''), 68.42 (C-4''), 73.58 (C-5''), 60.79 (C-6'').

And after extracting and subtracting the ^{1}H and ^{13}C NMR data for compounds **P10** from the whole NMR spectrum, we can conclude that compound **P11** has the following data for the sugar moiety besides the isorhamnetin signals; ^{1}H NMR (DMSO-d_6) spectral data: *δ* (ppm) 5.58 (d, *J* = 7.5 Hz, H-1''), 3.83 (*s*, 3'-OCH_3), 3.14–3.72 (m, rest of the sugar protons); ^{13}C-NMR (DMSO-d_6) spectral data: *sugar moiety*: *δ* (ppm) 101.28 (C-1''),74.81 (C-2''), 76.87 (C-3''), 70.28 (C-4''), 77.85 (C-5''), 61.07 (C-6'').

Quercetin, (P12):

Amorphous yellow powder showed; R_f-values (x100): 71 (BAW), 07 (15% AcOH); UV Spectral Data λ_{max} (nm): MeOH: 253, 268sh, 297sh, 368, + NaOMe: 247sh, 321 (dec.), + NaOAc: 257sh, 274, 329, 390 (dec.), +NaOAc /H_3BO_3: 261, 303sh, 388, +$AlCl_3$: 272, 304sh, 333, 458, +$AlCl_3$/HCl: 265, 301sh, 359, 428; ^{1}H-NMR (DMSO-d_6) spectral data: δ (ppm): 7.69 (d, *J* = 2.1, H-2'), 7.55 (dd, *J* = 2.1 Hz, and 8.4 Hz, H-6'), 6.90 (d, *J* = 8.4 Hz, H-5'), 6.42 (d, *J* = 1.8 Hz, H-8), 6.20 (d, *J* = 1.8 Hz, H-6); ^{13}C-NMR (DMSO-d_6) spectral data: δ (ppm): 147.50 (C-2), 136.44 (C-3), 176.55 (C-4), 161.43 (C-5), 98.88 (C-6), 164.59 (C-7), 94.05 (C-8), 156.83 (C-9), 103.71 (C-10), 122.66 (C-1'), 116.31 (C-2'), 145.76 (C-3'), 148.40 (C-4'), 115.76 (C-5'), 120.68 (C-6').

Isorhamnetin, (P13):

Compound **P13** appeared on PC as a yellow spot under UV light which changed to deep yellowish green on exposure to ammonia fumes; R_f-values (x100): 74 (BAW), 04 (15% AcOH); UV Spectral Data λ_{max} (nm): MeOH: 253, 267sh, 306sh, 370, +NaOMe: 240sh, 271, 328, 435 (dec.), +NaOAc: 260 sh, 274, 393 (dec.), +NaOAc /H_3BO_3: 255, 270 sh, 306sh, 377, +$AlCl_3$: 264, 304sh, 361sh, 431, +$AlCl_3$/HCl: 242sh, 262, 271sh, 302sh, 357, 428; ^{1}H-NMR (DMSO-d_6) spectral data: δ (ppm): 7.86 (d, *J* = 1.6, H-2'), 7.73 (dd, *J* = 1.6 Hz, and 7 Hz, H-6'), 6.93 (d, *J* = 7 Hz, H-5'), 6.42 (d, *J* = 1.7 Hz, H-8), 6.19 (d, *J* = 1.7 Hz, H-6); ^{13}C-NMR (DMSO-d_6) spectral data: δ (ppm) 156.25 (C-2), 135.93 (C-3), 177.38 (C-4), 161.20 (C-5), 98.68 (C-6), 164.12 (C-7), 93.67 (C-8), 156.35 (C-9), 104.01 (C-10), 121.04 (C-1'), 122.00 (C-2'), 115.18 (C-3'), 149.37 (C-4'), 146.85 (C-5'), 113.46 (C-6'), 55.64 ppm (C-OCH3).

Treatment of *S. mansoni*-infected mice with *Opuntia ficus-indica* flower extract orally in a dose of 200 mg/kg and PZQ significantly reduced the worm burden (46.8% and 96.2%) of the treated mice altering the male and female worm ratio respectively, showing that the females were more sensitive. The mean total worms and couples in the liver, portomesenteric were 1.20 ± 0.37 and 0.0 ± 0.0, 0.0 ± 0.0 respectively for the PZQ group. In *Opuntia ficus-indica* extract group, the mean total worms and couples in the Liver, portomesenteric were 16.80 ± 2.95 and 0.60 ± 0.60, 6.40 ± 1.02 respectively. The treatments were more effective compared to the untreated control group (Table 1). A significantly reduction in the mean total tissue egg load (hepatic and intestinal) was found in animals given praziquantel at 200 mg/kg to *S. mansoni*-infected mice for five consecutive days, 7 weeks post infection (5.653±0.733 and 3.570±0.558). As well, reduction in the mean total tissue egg load was evident in mice given *Opuntia ficus-indica* extract (9.293±0.889 and 8.531±0.798).

The difference was statistically significant from infected untreated control (14551.41±1659.25 and 20818.29±2024.66) at $p < 0.01$ (Table 2).

S. mansoni infected groups received PZQ 200 mg/kg showed higher percentage of dead eggs when compared to infected untreated group. Data also showed complete disappearance of immature eggs in the treated group. Significant decrease in the percentage of total immature eggs for flower extract of *Opuntia ficus-indica* ($P<0.01$; 29.0%) as well as a noticeable rise in the proportion of mature ($P<0.01$; 60.0%) and dead eggs ($P<0.01$; 11.0%) compared with the infected untreated control (Table 2).

Microscopic examination of hepatic tissue of (infested positive control) group revealed the granulomas were of cellular and fibrous types. The cellular granulomas found displaying a viable egg and surrounded by dense inflammatory reaction and fibrosis.

Histopathological examination of liver sections from different examined groups (Fig. 2) showed a significant reduction in the number of egg granulomas in the PZQ treated group in relation to the other treated and the control groups ($p<0.01$). However, no significant reductions in granulomas count were detected between the control and *Opuntia ficus-indica* extract group examined.

As regards the granuloma diameter; there was significant reduction in granuloma size in the PZQ treated group compared to the control group. No significant difference in granuloma diameter was detected between the control and *Opuntia ficus-indica*. In addition; most of egg granulomas of different groups were fibrocellular with mild increase in the number of cellular granulomas in the control group compared to the other treated groups.

Considering the measurement of liver fibrosis in tissue sections, it was found that the PZQ treated group showed significant reduction in fibrosis (measured as area /LPF) compared to the control group. The *Opuntia ficus-indica* treated group showed less significant reduction in fibrosis compared to the control group.

Table (1):
Effect of PZQ and *Opuntia ficus-indica* flower extract (200 mg/kg/day for 5 days) on worm load and sex in *S. mansoni*-infected groups

Groups	Worm load and Sex							
	Liver			Portomesenteric			Total worms	Percent worm reduction%
	Total males	Total females	Total couples	Total Males	Total females	Total couples		
Infected control	1.40±0.24	1.20±0.20	4.80±0.37	1.00±0.44	.40±0.24	9.00±0.44	31.60±1.43	-
PZQ	1.20±0.37	0.00±0.00	0.00±0.00***	0.00±0.00	00±0.00	0.00±0.00***	1.20±0.37***	96.20%
Opuntia ficus-indica	1.60±0.24	1.80±0.44	0.60±0.60**	0.20±0.20	00±0.00	6.40±1.02**	16.80±2.95**	46.80%

PZQ and *Opuntia ficus-indica* flower extract were administered orally 7 weeks post *S. mansoni* infection in doses of 200 mg/kg/day for 5 days. Results are presented as mean ± SEM.
** Significant difference from infected control at P<0.01.
*** Significant difference from infected control at P<0.001.

Table (2)
Effect of PZQ and *Opuntia ficus-indica* flower extract (200 mg/kg/day for 5 days) on tissue egg load and percentage egg developmental stages in *S. mansoni*-infected mice sacrificed two weeks post treatment.

Groups	Tissue egg load		% of egg developmental stages		
	Hepatic count x10³	Intestinal count x10³	% immature	% mature	% dead
Infected control	14551.41±1659.2	20818.29±2024.6	58.8±3.92	34.20±3.62	7.00±0.54
PZQ	5.653±0.733**	3.570±0.558**	0.00±0.00***	4.00±2.44**	96.0±2.44**
Opuntia ficus-indica	9.293±0.889**	8.531±0.798**	29.00±5.09**	60.00±5.70**	11.00±1.00**

PZQ and *Opuntia ficus-indica* flower extract were administered orally 7 weeks post *S. mansoni* infection in doses of 200 mg/kg/day for 5 days. Results are presented as mean ± SEM.
** Significant difference from infected control at P<0.01.
*** Significant difference from infected control at P<0.001.

4. Discussion

Polyphenols are a group of organic molecules found in the plant kingdom. Their chemical structures are distinguished by the presence of many phenolic groups, which may be associated with more or less complex groups of chemicals, all of which are typically of high molecular weight, as indicated by their name. The majority of these compounds are byproducts of plant metabolism. The increasing prevalence in polyphenols results from their antioxidant potential, which is involved in health benefits [21]. *Opuntia ficus indica* is noted for its high polyphenol content, which results in high antioxidant activity and, as a result, beneficial biological activities. [22, 23]. Secondary metabolites or derivatives are gaining popularity as potential sources of new drugs for schistosomiasis control and treatment [24, 25]. From those potential platform we were interested to search for and separate the natural polyphenolic compounds namely; rutin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-robinobioside, quercetin-3-*O*-β-glucopyranoside, quercetin-3-*O*-β-galactopyranoside, quercetin-3-*O*-α-L-rhamnopyranoside, isorhamnetin-3-*O*-β-galactopyranoside, isorhamnetin-3-*O*-β-glucopyranoside, isorhamnetin and quercetin, together with three phenolic acids; isoferulic acid, *p*-methoxy benzoic acid and protocatechuic acid. Whereby, protocatechuic acid, isorhamnetin-3-*O*-rutinoside and quercetin-3-*O*-rhamnopyranoside were isolated from the flowers of the Egyptian species for the first time.

Compounds **P10** and **P11** were isolated as an amorphous yellow powder as a mixture of two isomers from fraction VI by 80% ethanol. The UV spectrum in MeOH and in the different diagnostic reagents indicated that the compound belongs to the flavonol groups with 3, 3'-disubstition. No shift in band I of the compound was observed after the addition of $AlCl_3$ and $AlCl_3$/HCl. These UV data also indicated the absence of an ortho-dihydroxyl pattern in B ring and the presence of a free 7-OH group [18]. While the ^1H and ^{13}C NMR spectra in DMSO indicated the presence of a mixture of two compounds (**P10** and **P11**) which are isomers with approximately (2:1) proportion based on the integration for the signals especially which corresponding to the aglycone and the anomeric proton of the sugars form the ^{1}HNMR, whereas one hydrogen in compound **P10** was integrated to 1, while in compound **P11** integrated to approximately 0.52. The spectra showed that each compound was a flavonol with monosaccharide moiety, and the aglycone was

isorhamnetin and the sugars are galactose for **P10** and glucose for **P11**.

The ^{1}HNMR of **P10** demonstrated ABX signals at δ (ppm) 8.039 (d, J = 2.0 Hz'), 7.52 (dd, J = 8.0, 2.0) and 6.92 (d, J = 8.0 Hz'), assignable to H-2', H-6' and H-5', respectively. The presence of a methoxyl group was shown by a singlet signal representing three protons at δ 3.863 ppm. Thus the appearance of one-two doublets and their coupling constant values are further in agreement with the hydroxyl and the methoxyl groups at C-4' and C-3', respectively. The methoxylation of C-3' is confirmed by upfield shift of C-3'and downfield shift of C-4' at 115.62 (C-3') and 149.87 (C-4'), respectively in ^{13}CNMR than that of quercetin. The resonances of the signals observed in the low-field region in the ^{1}H NMR spectra at δ 5.53 (1H, d, J = 7.8 Hz, H-1") attributed to the anomeric protons of galactose. This was confirmed by the ^{13}C NMR spectrum which exhibited most of signals of monosaccharide between 60.8 and 77.5 ppm The β-anomeric configuration for the galactose was judged from its large coupling constants (J = 7.8 Hz) [19]. The NMR data of **P10** were identical with those of isorhamnetin 3-*O*-β-D-galactopyranoside [26].

Finally, by extracting and subtracting the ^{1}H/ ^{13}C NMR data for compounds **P10** from the whole NMR spectrum besides, the resonance of the glycosyl anomeric signals for **P10** and **P11** resonating at δ 102.5 and 100.75 ppm, respectively with their C-6 resonating δ 60.82 and 60.8 in the same integration values of the signals confirmed the isorhamnetin monosaccharide substitution mixture. We can conclude that compound **P11** is isorhamnetin β-D-glucopyranoside from the data shown in the experimental [27].

Praziquantel (a single medication used to treat schistosomiasis) is mostly successful against adult levels. Natural products for the treating of a wide variety of diseases, including schistosomiasis, have sparked a lot of interest in recent years. Many studies are looking into the anti-schistosomal function of various plant-derived substances [28]. Although herbal medicine has developed some highly successful malaria drugs, such as quinine and more recently, artemisinin [29], there have been few attempts to evaluate plants for schistosomiasis [30, 31]. Concerning *in vivo* antischistosomal activity of natural products, in randomized controlled clinical trials, Utzinger et al., [32] found that artemether, a methyl ether derivative of artemisinin, had antischistosomal properties.

Koko et al., [33] examined the effectiveness of oral therapy with *Balanites aegyptiaca* fruit mesocarp in mice infected with the Sudanese strain of *S. mansoni* at a dosage of 200 mg/kg body weight, and discovered a substantial reduction in egg count per gram of feces. Ramadan et al., [34] investigated the anti-*S. mansoni* effects of *Ferula assafoetida* in experimentally infected mice.

For these reasons, we were interested to look in this study for new medications having anti-schistosomal activity. In contrast to infected untreated controls, the data showed substantial reductions in total worm burden to 46.80 % after administration of *Opuntia ficus-indica* flower extract. While, in the infected mice treated with *Opuntia ficus-indica* flower extract, the intestinal egg count was decreased to 93.89 %, and in the hepatic egg count to 96.5 %, respectively.

These results were followed by a noticeable increase in the percentage of mature and dead eggs for *Opuntia ficus-indica* flowers. These results are in consistence with Pellegrino et al., [17] who claimed that in experimental schistosomiasis, if viable ova of any of the immature stages disappear after specific chemotherapy, the drug is considered active.

Still, this study showed that incubating *S. mansoni* with *Opuntia ficus-indica* flower extract reduced the survivability of adult worms and caused a considerable separation in worm pairs, as well as a reduction in egg count.

Seif el-Din et al., [35] and Ebeid et al., [36] discovered that giving the antioxidants - carotene or N-acetylcysteine (NAC) to *S. mansoni* worms resulted in a substantial reduction in worm burden, as well as a significant increase in the percentage of dead ova and a decrease in the percentage of mature ova stages. Furthermore, when used in combination with the anti-malarial medication artemether, Seif el-Din et al., [37] found that treatment with NAC alone raised the percentage of dead ova and improved the decrease in total number of worms and tissue egg loads.

In schistosomiasis, because of the granulomatous inflammatory reaction triggered by the immune response to egg antigens, the eggs are primarily blamed for the morbidity [38]. Lenzi [39] has described the granuloma as a diverse and complex system made up of eosinophils, neutrophils, lymphocytes, macrophages, giant cells, and fibroblasts surrounding schistosome eggs trapped in the liver.

Moreover, histopathological examination of liver sections from different examined groups (Figure 2) showed a significant reduction in the size of granuloma diameter in the liver tissue to (391.48±62.54 µm), however, no significant reductions in granulomas count were detected

between the control and *Opuntia ficus-indica* extract group examined.

In addition; most of egg granulomas of different groups were fibrocellular with mild increase in the number of cellular granulomas in the control group compared to the other treated groups. Considering the measurement of liver fibrosis in tissue sections, it was found that the PZQ treated group showed significant reduction in fibrosis (measured as area /LPF) compared to the control group. The *Opuntia ficus-indica* treated group showed less significant reduction in fibrosis compared to the control group (Table 3 & Fig 2). The granulomatous inflammatory reaction was suppressed by *Opuntia ficus-indica* flower extract in the current study, though this was not highly significant and the granulomas were fibrocellular relative to those in infected untreated controls.

Table 3

Hepatic granuloma size in *S. mansoni* infected mice treated with PZQ and Plant extract (200 mg/kg/day for 5 days) versus untreated control animals.

	Granuloma Egg			
Group	**Number (N/5 HPF)**	**Type**	**Diameter (µm)**	**Fibrosis (area µm²/LPF)**
Infected control	29	Fibrocellular 90% Cellular 10%	418.3±64.52	167.83±12.2
PZQ	13	Fibrocellular 95% Cellular 5%	287.18±55.14**	130.47±52.55**
Opuntia ficus-indica	23	Fibrocellular 95% Cellular 5%	391.48±62.54	147.00±43.75*

**: Significant difference with the control group ($p<0.01$)
*: Significant difference with the control group ($p<0.05$)

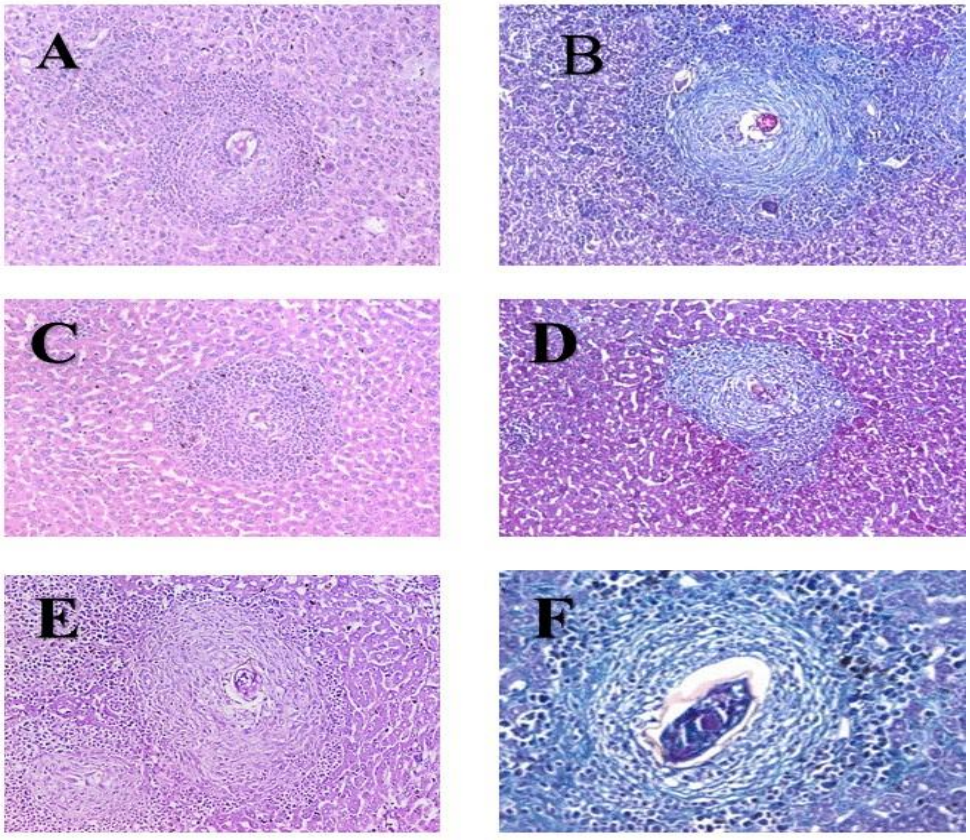

Fig 2. Section in mouse liver of the different groups tested

(A):control group showing an egg granuloma with central intact ova showing fibrocellular egg granuloma with central ova and peripheral condensation of neutrophils and concentric layers of fibrous tissue (H&E stain, X200). **(B)** (MT stain, X200). **(C)**: PZQ treated group showing a cellular egg granuloma consists mostly of neutrophils and mononuclear cells (H&E stain, X200). **(D)**: PZQ treated group showing a fibrocellular egg granuloma (MT stain, X200). **(E)**: *Opuntia ficus-indica* treated group showing an egg granulomas with central intact ova and peripheral mono and polymorphnuclear inflammatory cells (H&E stain, X200). **(F)**: *Opuntia ficus-indica* treated group showing an intact ova surrounded by fibrocellular tissue reaction (green) (MT stain, X200).

5. Conclusion

As plants have been used as drugs in curing several of diseases by humans since thousands of years ago and previous researches showed that many plants exerted antiparasitic effects in particular. We were interested in our research to study the *Opuntia ficus-indica* flower extract for antischistosomal ability *in vivo*, whereby it revealed a significant proof of concept. These results also confirm that its polyphenolic constituents are the main reason for their pharmacological activity. A total of 13 different compounds were isolated from the extract as well, 3 of them were isolated for the first time of the flowers of the Egyptian species. Future studies are needed to determine the bioactive properties of each flavonoid compound and promote the use of extracts derived from plants.

6. Conflicts of interest

There are no conflicts to declare.

7. References

[1] Hotez P.J. and Kamath A., Neglected tropical diseases in sub-saharan Africa: Review of their prevalence, distribution, and disease burden. PLoS Neglected tropical diseases, 3, e412, (2009).

[2] Gray D.J., Ross A.G., Li Y-S. and McManus D.P., Diagnosis and management of schistosomiasis. BMJ, 342, d2651, (2011).

[3] Shaker Y., Samy N. and Ashour E., Hepatobiliary Schistosomiasis. J Clin Transl Hepatol., 2(3), 212-6, (2014).

[4] Elbaz T. and Esmat, G., Hepatic and intestinal schistosomiasis: Review. J. Adv. Res., 4, 445–452, (2013).

[5] Gönnert R. and Andrews P., Praziquantel; a new board-spectrum antischistosomal agent. Z Parsitenkd., 52, 129-150, (1977).

[6] Fallon, P. G. and Doenhof, M. J. Drug-resistant schistosomiasis: Resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specifc. Am. J. Trop. Med. Hyg. 1994; 51, 83–88

[7] Fallon P.G., Mubarak J.S., Fookers R.E., Niang M., Butterworth A.E., Sturrock R.F. and Doenhoff, M.J., *Schistosoma mansoni:* maturation rate and drug susceptibility of different geographical isolates. Exp. Parasitol., 86, 29-36, (1997).

[8] Ismail M., Botros S., Metwally A., William S., Farghally A., Tao L-F., Day T.A. and Bennett J.L., Resistance to Praziquantel: Direct Evidence from Schistosoma Mansoni Isolated from Egyptian Villagers. Am. J. Trop. Hyg., 60(6), 932-35, (1999).

[9] *Majure L.C. Puente R., Griffith M.P., Judd W.S., Soltis P.S. and Soltis D. E., Phylogeny of Opuntia s.s. (Cactaceae): Clade delineation, geographic origins, and reticulate evolution. American Journal of Botany, 99 (5), 847–864, (2012).*

[10] Matias A., Nunes S. L., Poejo J., Mecha E., Serra A.T., Maderia P.J., Bronze M.R. and Duarte C.M., Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate recovered from *Opuntia ficus-indica* juice. Food Funct., 5(12), 3269-80, (2014).

[11] Lee, E., Kim H., Song Y., Jin C., Lee K., Cho J. and Lee Y., Constituents of the stems and fruits of *Opuntia ficus-indica* var. *saboten*. Archives of Pharmacal. Research, 26, 1018-1023, (2003).

[12] Boulos L. Flora of Egypt, Vol. 1. Al Hadara Puplishing, Cairo, Egypt. (1999).

[13] Mabry T.J., Markham K.R. and Thomas M.B., The Systematic Identification of Flavonoids, Springer-Verlag, p. 271, (1970).

[14] Liang Y.S., John B.I. and Boyd D.A., Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proceed 1st Sino-American Symp., 1, 34–48, (1987).

[15] Duvall R.H. and DeWitt W.B., An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Trop Med Hyg., 16, 483–6, (1967).

[16] Kamel I.A., Cheever A.W., Elwi AM, Mosimann J.E. and Danner R., *Schistosoma mansoni* and *S. haematobium* infections in Egypt: Technique for recovery of worms at necropsy. Am J Trop Med Hyg., 26, 696–701, (1977*).*

[17] Pellegrino J., Oliveira C.A., Faria J. and Cunha A.S., New approach to the screening of drugs in experimental *schistosomiasis mansoni* in mice. Am J Trop Med Hyg., 11:201–15, (1962).

[18] Markham K.R., Ternai B., Stanley R., Geiger H. and Mabry T.R., 13C NMR studies of flavonoids-III: naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahedron, 34, 1389–1397, (1978).

[19] Agrawal P.K., NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry, 31,

3307-3330, (1992)

[20] Markham K.R., Technique of Flavonoid Identification. Academic Press, London, 1982.

[21] Markham K. R. and Bloor S. J., "Flavonoids in Health and Disease" Eds. C. A. Rice-Evans and L. Packer, Dekker, New York, pp. 1-33, (1998).

[22] Butera D., Tesoriere L., Di Gaudio F., Bongiorno A., Allegra M., Pintaudi A.M., Kohen R. and Livrea M.A., Antioxidant activities of Sicilian prickly pears (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agric. Food Chem., 50, 6895–6901, (2002).

[23] Osuna-Martínez U., Reyes-Esparza J., and Rodríguez-Fragoso L., Cactus (Opuntia ficus-indica): A Review on its Antioxidants Properties and Potential Pharmacological Use in Chronic Diseases. Nat Prod Chem Res., 2(6), 1-8, (2014).

[24] Ndjonka D., Rapado L.N., Silber A.M., Liebau E. and Wrenger C., Natural products as a source for treating neglected parasitic diseases. Int J Mol Sci., 14(2), 3395–3439, (2013).

[25] Keiser J. and Utzinger J. Antimalarials in the treatment of schistosomiasis. Curr. Pharm. Des., 18(24), 3531–3528 (2012).

[26] Sang S., Lapsley K., Jeong W., Lachance P., Ho C. and Rosen R., Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus Batsch*). J. Agric. Food Chem., 50, 2459–2463, 2002.

[27] Halim A. F., Saad, H-E. A. and Hashish N. E., Flavonol glycosides from *Nitraria retusa*. Phytochemistry, 40(1), 349-51, 1995.

[28] Moraes J.d., Almeida A.A., Brito M.R., Marques T.H., Lima T.C., Sousa D.P., Nakano E., Mendonça R.Z. and Freitas R.M., Anthelmintic activity of the natural compound (+)-limonene epoxide against *Schistosoma mansoni*. Planta Med., 79, 253–258, (2013).

[29] Frederich M., Dogne J.M., Angenot L. and De Mol P., New trends in anti-malarial agents. Curr Med Chem., 9, 1435–1456, (2002).

[30] Sparg S.G., Van Staden J. and Jager A.K., Efficiency of traditionally used South African plants against schistosomiasis. J Ethnopharmacol., 73, 209–214, (2000).

[31] Molgaard P., Nielsen S.B., Rasmussen D .E., Drummond R.B., Makaza N. and Andreassen J., Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. J Ethnopharmacol., 74, 257–264, (2001).

[32] Utzinger J., Xiao S., N'Goran E.K., Berg quist R. and Tanner M., The potential of artemether for the control of schistosomiasis. Int J Parasitol., 31, 1549–1562, (2001).

[33] Koko W.S., Abdalla H.S., Galal M. and Khalid H.S., Evaluation of oral therapy on mansonial schistosomiasis using single dose of *Balanites aegyptiaca* fruits and praziquantel. Fitoterapia, 76, 30–34, (2005).

[34] Ramadan N.I., Abde Same-Ekobo A., Lohoue J., Essono El-Aaty H.E., Abdel-Hameed D.M., El-Deeb H.K., Samir N.A., Mansy S.S. and Al Khadrawy F.M. Effect of *Ferula assafoetida* on experimental murine *Schistosoma mansoni* infection. J Egypt Soc Parasitol., 34, 1077–1094, (2004).

[35] Seif el-Din S.H., Ebeid F.A., Badawy A.A. and Ezzat A.R., Protective effects of b-carotene; N-acetyl-L-cysteine with and without praziquantel treatment in *Schistosoma mansoni*-infected mice. Egypt J Schistosomiasis Infect Endem Dis., 28, 67–90, (2006).

[36] Ebeid F.A., Ezzat A.R., Badawy A.A. and Seif el-Din S.H., Enhancement role of b-carotene against experimental schistosomiasis. Egypt J Schistosomiasis Infect Endem Dis., 29, 67–90, (2007).

[37] Seif el-Din S.H., Al-Hroob A.M. and Ebeid F.A., *Schistosoma mansoni*: N-acetylcysteine downregulates oxidative stress and enhances the antischistosomal activity of artemether in mice. Exp Parasitol., 128, 230–5, (2011).

[38] Pyrrho Ados S., Ramos J.A., Neto R.M., Silva C.S., Lenzi H.L., Takiya C.M. and Gattass C.R., Dexamethasone, a drug for attenuation of *Schistosoma mansoni* infection morbidity. Antimicrob. Agents Chemother., 46, 3490–3498, (2002).

[39] Lenzi J.A., Estudo do modelo Calomys callosus Renger, 1830 (Rodentia: Cricetidae) na infecao por *Schistosoma mansoni* Sambon, 1907. Parametros parasitologicos e histopatologicos. PhD thesis. Universidade Federal da Bahia, Salvador, Brazil, (1998).

التقييم الحى للنشاط المضاد للميكروبات لأزهار نبات اوبتينا فيكس انديكا و مكوناتها الكيميائية

تعتبر البلهارسيا منتشره في المناطق الأستوائيه وشبه الأستوائيه حيث انه يعيش 90% علي الأقل من الذين يحتاجون الي العلاج في افريقيا. ويعد البرازيكونتل العلاج الوحيد المضاد للبلهارسيا المتاح حالياً ولكن ظهور سلالات مقاومه ضد البرازيكونتل، بالاضافه الي انخفاض فاعليته ضد الديدان ,جعل البحث عن تطوير دواء جديد ضرورياً للغايه.

لذالك كان هدف هذه الدراسه تقيم التأثير المناعي للمستخلص الطبيعي لأزهار نبات اوبتينا فيكس انديكا ضد ديدان البلهارسيا ولذلك تم اصابة 30 من الفئران البيضاء تجريبيا ب80±10 من سركاريا البلهارسيا المعويه بواسطة تقنية الغمر في الجسم .تم تقسيم الفئران الي 3 فئات حيث تم علاج مجموعه باستخدام مستخلص أزهار الابنتيا فيكس انديكا ومجموعه اخري باستخدام البرازيكونتيل ومجموعه التحكم السلبيه وبعد الاسبوع التاسع من الاصابه تم قتل الفئران وتم قياس عبئ الدوده، عد البيض في أنسجه الكبد والامعاء وقياس نمط الأواجرام وعدد وكما تم قياس حجم الورم الحبيبي الكبدي وايضا تم قياس أشباه الجيوب الكبديه عن طريق تحليل الانسجه للتقييم.

كما تم تجميع الازهار و تجفيفها وطحنها حيث تم استخلاص المكونات الفينولية باستخدام 70% كحول ميثلى، ثم تم تجزئة المستخلص باستعمال العمود الكروماتوجرافى المحتوى على سيفادكس LH-20 كمادة ادمصاص واستعمال الماء ثم خليط من الكحول الايثلى و الماء حيث امكن الحصول على سبعة اجزاء من خلاصة النبات حيث اظهرت النتائج احتواء الجزء الاول من خلاصة النبات على سكريات حرة مثل جلوكوزو اربينوزو ورامنوز. ولقد تم التعرف على13 مركب فلافوني وبولي فينول وهم:

روتين، ايزورامنتين-3-ريتونيزيد، ايزورامنتين-3-روبينوبيوزايد، كويرستين-3-جلوكوزايد، كويرستين-3-جلاكتوزايد، كويرستين-3-رامنوزايد، ايزورامنتين-3-جلوكووزايد، ايزورامنتين-3-جلاكتوزايد، ايزورامنتين، كويرستين ، بروتوكاتشيوك و الباراميثوكسى بنزويك اسيد وايزوفيريوليك اسيد.

وتم التعرف على المركبات المفصولة بعد تنقيتها واثبات تركيبها الكيميائى بالطرق الكيميائية المختلفة مثل طريقة التميوء فى الوسط الحمضى، و باستخدام القياسات الطيفية المتعددة منها ^{13}C-NMR, ^{1}H-NMR , MS, UV بجانب الخواص الكروماتوجرافية.

حيث كانت نتائج البحث أن تسبب مستخلص الأوبنتيا فيكس انديكا لأنخفاض من أعباء الديدان بنسبة ضئيلة (46.8) . و لكن بنفس طريقه البرازيكونتيل، قلل المستختص من عدد البيض فى انسجة الكبد و الأمعاء بنسبة كبيرة (93.89% و 96.5%) على التوالى، كما تم دراسة الهستابثولجى لأنسجة الكبد.

وبذلك أظهرت النتائج أن مستخلص أزهار اوبتينا فيكس انديكا كان نشطاً واعداً كمضاداً لديدان البلهارسيا و ربما ذلك نتيجة للمحتوى الكيميائى من مركبات بولفينولية.