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RESEARCH ARTICLE

Schistosomicidal and Immunological Properties of Grape Seed Extract During Murine *Schistosoma mansoni* Infection

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ABSTRACT

Background: Many developing nations face a severe public health problem due to parasitic schistosomiasis. **Aim:** This study evaluates the use of grape seed extract with PZQ against *Schistosoma mansoni* in vivo. **Materials & Methods**: In this study, forty-eight male albino mice infected with *S. mansoni* were given grape seed extract (100 milligram/kilogram B.W., once daily for 10 days) and PZQ (250 mg/kg B.W.) orally, six weeks after infection. Parasitological aspects, antioxidant activity, and immunological parameters were determined following treatment. **Results:** The total worm burden and ova count/g in hepatic and intestinal tissue decreased after administration of PZQ or combined with grape seed extract. Concurrently, the number of dead eggs increased significantly. Serum AST and ALT activities were diminished after being exposed to grape seed extract. Increases in the antioxidant hepatic enzyme activities of catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) were observed in infected mice treated with grape seed extract, PZQ or a combination of both. The levels of vascular adhesion molecules-1 (VCAM-1) and tumour necrosis factor-alpha (TNF-α) were significantly lower in the grape seed extract and PZQ group compared to the *S. mansoni* infected group. **Conclusion:** Most of the effects observed when grape seed extract and PZQ are used together indicated that PZQ and grape seed extract work together to fight parasites, prevent fibrosis, and change the host's immune responses.

Keywords: Antioxidant activities, Grape seed extract, *Schistosoma mansoni*, TNF-α, VICAM-1

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INTRODUCTION

Trematodes infection of the genus *Schistosoma*, which lives in the circulatory systems of mammals, is the cause of schistosomiasis in humans. This hazardous disease is among the world's most disregarded tropical illnesses (McManus et al., 2018; Miranda et al., 2022). Approximately 250 million people are infected with *Schistosoma spp*., and 700–800 million individuals living in endemic areas such as Africa, Asia, and Latin America are at risk of this disease (WHO, 2020). *Schistosoma mansoni* eggs use the portal vessels in the liver to make their way from the intestine to the liver. Hepatic fibrosis is a treatment for the granulomatous inflammatory reactions caused by these eggs (Colley & Secor, 2014). Activated vascular endothelial cells express additional adhesion molecules when signaled to do so by inflammatory cytokines and chemokines. These granulomas are caused by the activation and release of reactive oxygen species (ROS) from inflammatory cells (Halliwell & Gutteridge, 2015; Elmalawany et al., 2022).

Inflammation, fibrosis, blocked blood flow, and low oxygen levels compound each other in a vicious cycle when eggs become lodged in the hepatic sinusoids (Beshay et al., 2019). Controlling the spread of schistosomiasis is a primary focus for the World Health Organization (WHO), which centres on the drug praziquantel (PZQ), at which point seen widespread consumption in the last 4 decadal in a fight against the disease's global prevalence and severity (Bergquist et al., 2017). The widespread and prolonged use of praziquantel has created praziquantel tolerance and impervious strains of the parasite *S. mansoni*

(Khalil et al., 2022); PZQ treatment alone, however, will not be sufficient to repair the worms' damage or prevent reinfection. Drug resistance has already developed in some pandemic zones where praziquantel has been used extensively (Melman et al., 2009; Lam et al., 2021). Finding a new drug to replace or enhance praziquantel has become a top priority because of the concern about developing disease tolerance. Extracts from natural products are crucial for this kind of research because of their wide range of chemicals and biomedical functions (Bergquist et al., 2017; Atanasov et al., 2021).

Grape seed extract (GSE) is an innovative discovery with significant potential as an antioxidant. Elevated levels of bioflavonoids and vitamins C and E make GSE a potent antioxidant (Morsi et al., 2020). Proanthocyanidins are abundant in grape seeds, and they have been demonstrated to have intense free radical scavenging activity (Pathiraja et al., 2023). Grape seeds are a multi-component template with 40 per cent dietary fibre, 16 per cent oil, 11 per cent proteins, and 7 per cent complex phenols such as tannins (Ma & Zhang, 2017). Regulation of cellular oxidative stress is the mechanism by which GSE exerts its protective effect (Kadri et al., 2021), reduced injuries to organs, improved balance between oxidisers and antioxidants (Sochorova et al., 2021), and decreased release of inflammatory mediators (Lazcano-Silveira et al., 2022, and Wang et al., 2023). Additionally, due to the high polyphenol content, GSE was reported to have anticarcinogenic effects (Homayoun et al., 2020). It contains enzymes that catalyse histamine release in the context of inflammation and allergies and their antioxidant activity (Ali et al., 2010).

Proanthocyanidins are found in the grape seed extract Gervital and many other plant foods like fruits, vegetables, nuts, flowers, wine, bilberries, ginkgo, and black and green tea (Bjørklund et al., 2022). Heart disease, arteriosclerosis, drug-induced hepatotoxicity,

and nephrotoxicity are all caused by free radicals, but Gervital can clear them out of the body and protect against their over-oxidative damage (Spranger et al., 2008; Mansouri et al., 2011; Salahuddin & Katary, 2017; Shan et al., 2019). Anti-fatigue, anti-radiation, anti-mutagenic, anti-tumour, and anti-thrombotic properties are among its many uses (Qin et al., 2006; Engelbrecht et al., 2007; El-Awdan et al., 2015). The concentration of reactive oxygen species and LDL oxidation are lowered due to the antibacterial, antiviral, and anticarcinogenic actions (Ibrahim et al., 2022); proanthocyanidins have properties that reduce inflammation and allergy symptoms (Dhalaria et al., 2020, and Sheng et al., 2020). A grape seed extract has been shown to improve liver function (Charradi et al., 2014) among its many other benefits for liver health (Kadri et al., 2019).

To learn how the human immune system reacts to schistosomes, infection models in mice have been very important. In particular, there are several similarities between schistosomiasis in mice and schistosomiasis in people. These commonalities shaped the immunological studies of schistosomiasis patients (Colley & Secor, 2014). As a result, we conducted this analysis to determine the effectiveness of grape seed extract against *S. mansoni* in albino mice, both on its own and in combined effect with PZQ, by measuring parasitological aspects, antioxidant activity, and immunological parameters**.**

MATERIAL AND METHODS

Material

Drugs: SEDICO Pharmaceutical Company's praziquantel (PZQ) drugs (600 milligrams/ tablet) were used in this study (6th October City – Egypt). PZQ pills were crushed and dissolved in sterile water to be taken orally via a stainless-steel bent feed needle to mice in only one dose of 250 mg/kg B.W (Eissa et al., 2020). Gervital tablet (Grape seeds extract) (250 milligrams) has been purchased from Mina Pharm for pharmaceuticals, Cairo, Egypt. According to Bagchi et al. (2001), mice were given 100 mg/kg of a suspension of ground Gervital tablets in distilled water.

Experimental mice and infection

Pathogen-free male CD-1 Swiss albino mice aged 6–8 weeks weighing 20 ± 2 grams were procured from the Schistosome Biological

Supply Center at the Theodor Bilharz Research Institute (SBSC, Imbaba, Giza, Egypt). *S. mansoni* cercariae were taken from snails of the genus *Biomphalaria alexandrina* (the Egyptian strain) infected at SBSC as part of an experiment. After four weeks of infection, the snails were placed in distilled water warmed to 28 °C for 2 hours under artificial light to prompt cercarial shedding (Tekwu et al., 2017).

Recently emerged cercariae were injected subcutaneously into the abdominal skin of each mouse, infecting them with 70 ± 5 cercariae (Liang et al., 1987). After being housed in a safe and secure setting with regulated weather (25 ± 2 °C) and moisture (70%), mice were moved to the Experimental Research Unit at the Faculty of Science in Menoufia, Egypt. An ethical approval (approval ID: MUFS/F/Pa/3/22) has been given by the Institutional Animal Ethical Committee at Menoufia University.

Seven groups of six mice were randomly assigned (n = 6), apiece as follows: Group I was a normal control group. Group II was the treatment given orally with praziquantel (one dose of 250 mg/kg B.W). Group III was administered orally ten doses of grape seed extract (Gervital) (100 mg/kg B.W., one dose every day for ten consecutive days) (Bagchi et al., 2001). Group IV was a positive control, infected with *S. mansoni* (70 ± 5 cercariae). Group V was *S. mansoni*-infected and, after six weeks, received oral praziquantel (one dose, 250 mg/kg B.W.). Group VI was infected with *S. mansoni* and given grape seed extract (Gervital) orally for ten doses (100 mg/kg B.W., one dose daily for ten consecutive days) after six weeks of infection. Group VII was infected with *S. mansoni* and treated orally with 10 doses of grape seed extract (100 mg/kg B.W., one daily for ten consecutive days) and one PZQ (250 mg/kg B.W.).

The experiment was finished ten days after administering the final doses of the medications put through the test.

Sampling

Once the study was finished, the heads of all the animals were removed, and their peripheral blood was taken. Next, samples were centrifuged at 3000 rpm for 5 minutes to isolate the serum and kept in a freezer at -80°C until used to measure immune parameters like TNF-alpha, VCAM-1, and liver functions. All infected mice were infused after being dissected to allow access to the internal organs in the chest and abdomen. In order to carry out this procedure, a tiny cut had to be made in the liver's portal vein. A needle with a gauge of 20 was utilised to gain access to the descending aorta. The perfusion fluid, which consisted of a solution of 0.85% sodium chloride and 0.75% sodium citrate, was pumped through the needle. Petri dishes of suitable size were used to collect the perfusate (Duvall & DeWitt, 1967). The stages of egg development were studied, and the estimated worm load, egg loading into hepatic and intestinal tissues, and an oogram profile of egg development were all examined. The method of Lowry et al. (1951) was used to determine the total protein content of the liver homogenate. The samples were prepared by homogenising 0.5 grammes of liver from each mouse in 2.5 volumes (w/v) of cold potassium phosphate buffer (pH 6.5) and then centrifuged at 3500 rpm for 25 minutes at 4 degrees Celsius. The supernatants were frozen at -80°C until the antioxidant enzyme activities were measured; these included hepatic catalase (CAT), glutathione-S transferase (GST), and superoxide dismutase (SOD).

Methods

Investigation of parasitological parameters

The methodology employed for the perfusion of the portal and mesenteric veins involved the use of sterile physiological saline solution with a concentration of 0.9% NaCl (w/v), following the protocol established by Smithers and Terry in 1965. Worm burdens were determined by counting the number of males, females, and coupled worms recovered using a stereo microscope. The formula below was used to determine the level of protection or the percentage reduction in parasite load.

$$\%\ \text{reduction} = \frac{(\text{value of infected controls} - \text{value of treated mice})}{(\text{value of infected controls})} X\ 100$$

Table 1. Effect of PZQ and grape seed extract in parasitological parameters in *S. mansoni*-infected mice

	Total worm burden	Egg count/ g tissue		Oogram in intestinal tissue		
		Hepatic tissue	Intestinal tissue (Reduction rate)	Immature% (Reduction rate)	Mature% (Reduction rate)	Dead%
infected control (GIV)	16.3 ± 1.3	4156.1 ± 78	6900 ± 43	51.6 ± 4	46.1 ± 1.1	2.3 ± 0.8
Infected + PZQ (GV)	0.16 ± 0.4* (99.0%)	444 ± 41* (89.3%)	528.6 ± 22* (92.3%)	3.3 ± 1.3* (93.6%)	4.5 ± 0.5* (90%)	91.6 ± 1.6*
Infected + grape seed extract (GVI)	14.3 ± 1.6 (12.2%)	2918 ± 87 (29.7%)	5822 ± 136 (15.6%)	46.3 ± 17 (10.3%)	35.3 ± 1.7 (23.4%)	12.6 ± 0.8
infected+ PZQ + grape seed extract (GVII)	0.00± 0.00* (100%)	340 ± 28* (91.8%)	495 ± 11* (92.8%)	0.5 ± 0.5* (99%)	0.16 ± 0.4* (99.6%)	100 ± 0.75*
F-ratio	399.28	5254.51	13173.33	50.44	2500.67	13851.71
P-value	0.000	0.000	0.000	0.000	0.000	0.000

Data are expressed as mean ± SD, Number of mice in each group= 6, Mice were orally administered 100 mg/kg of grape seed extract for 10 consecutive days, sixth weeks post-infection. Mice were orally administered with only one dose of 250 mg/kg of PZQ six weeks post-infection. *Significant difference compared to the infected control group at ($P < 0.05$) by one-way ANOVA followed by a Dunnet Post Hoc test.

Table 2. Effect of grape seed extract and PZQ in antioxidant agents in mice infected with *Schistosoma mansoni.*

	CAT (U/mL)	SOD (U/L)	GST (mol/min/g wt tissue)
Normal control (GI)	33.6 ± 1.3	97.0 ± 1.0	0.67 ± 0.02
Normal + PZQ (GII)	30.1 ± 0.4	92.5 ± 1.0	0.46 ± 0.02
Normal + grape seed extract (GIII)	34.1 ± 1.3	95.3 ± 0.5	0.83 ± 0.01
Infected control (GIV)	24.0 ± 1.0$^{\$}$	64.5 ± 2.0$^{\$}$	0.16 ± 0.008$^{\$}$
Infected + PZQ (GV)	26.0 ± 0.6*	78.1 ± 0.75*	0.34 ± 0.01*
Infected + grape seed extract (GVI)	31.0 ± 1.4*	85.6 ± 3.0*	0.68 ± 0.008*
Infected + PZQ + grape seed extract (GVII)	32.8 ± 1.9*	93.5 ± 1.0*	0.78 ± 0.01*
F-ratio	56.681	324.292	1054.346
P-ratio	0.000	0.000	0.000

Data are expressed as mean ± SD, Number of mice in each group= 6; Mice were orally administered 100 mg/kg of grape seed extract for 10 consecutive days, sixth weeks post-infection. Mice were orally administered with only one dose of 250 mg/kg of PZQ six weeks post-infection. *Significant difference compared to the infected control group ($P < 0.05$). $^{\$}$ statistically significant compared to the negative control group using a one-way ANOVA and Dunnett's post hoc test.

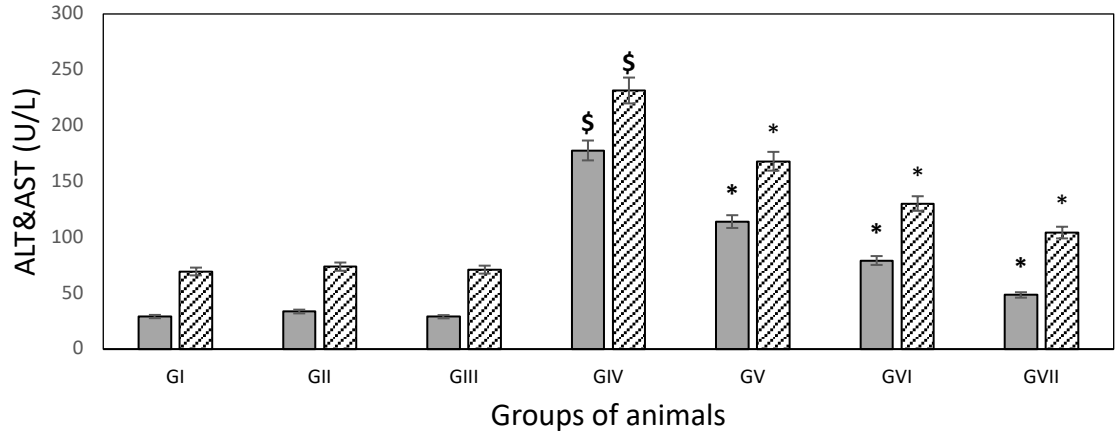

Figure 1. Effect of grape seed extract and PZQ on serum liver function in *S. mansoni*-infected mice. Data are expressed as mean ± SD. Number of mice in each group= 6. *Significant difference compared to the infected control group (P < 0.05). $ statistically significant compared to the negative control group using a one-way ANOVA and Dunnett's post hoc test.

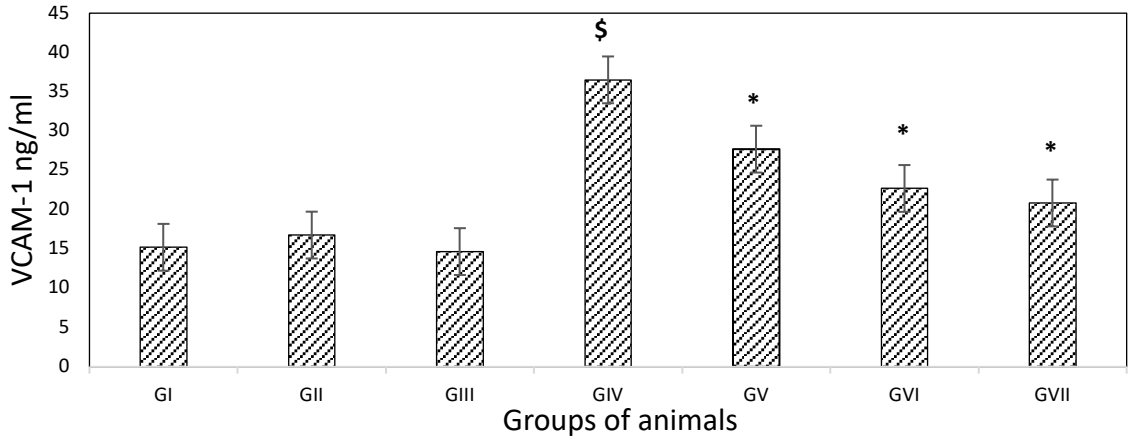

Figure 2. Effect of grape seed extract and PZQ on vascular adhesion molecules-1 (VCAM-1) in *S. mansoni*-infected mice. Data are expressed as mean ± SD. The number of mice in each group = 6, * Significant difference compared to the infected control group (P < 0.05). $ Statistically significant compared to the negative control group using a one-way ANOVA and Dunnett's post hoc test.

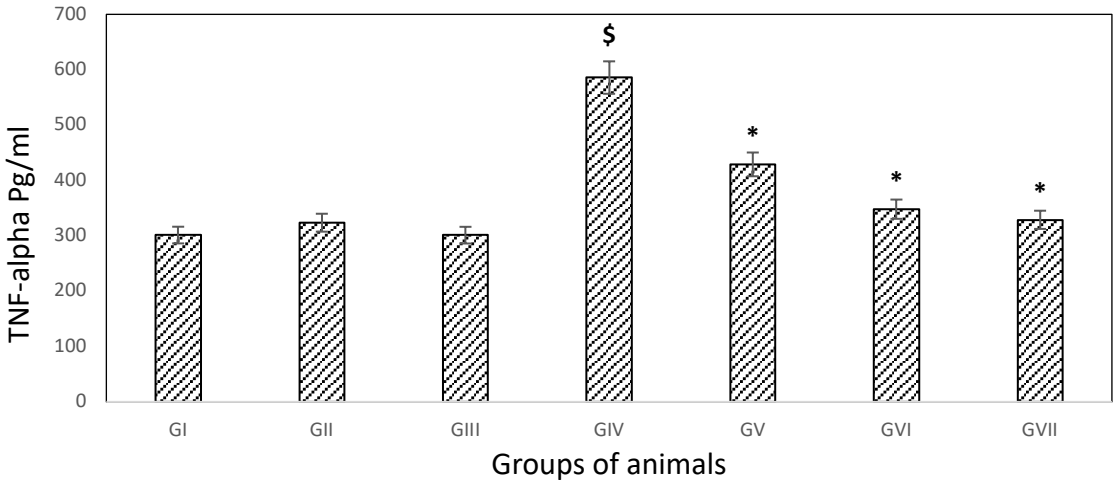

Figure 3. Effect of grape seed extract and PZQ on Tumor Necrosis Factor-alpha (TNF-alpha) in *S. mansoni*-infected mice. Data are expressed as mean ± SD. Number of mice in each group= 6. *Significant difference compared to the infected control group (P < 0.05). $ statistically significant compared to the negative control group using a one-way ANOVA and Dunnett's post hoc test.

Liver and intestine samples were gathered from perfused mice, weighed, and then digestible in 5% KOH at 37 °C for 16 hours. The eggs were calculated at a magnification of x40, and the average eggs/g of the hepatic and intestinal tissues were determined (Herbert et al., 2010). According to Mati and Melo (2013), the oogram pattern was examined. We washed three pieces of every animal's ileum in 0.9% saline solution before laying them out to dry on filter paper. In order to examine each piece under a microscope, we pressed it between two glass slides. The eggs were counted and sorted into three groups: immature, mature, and dead.

Immature eggs were defined as having a miracidium that took up lesser three-quarters of the shell. In contrast, mature eggs filled the entire shell, and dead eggs were described as opaque and having a retracted, granular miracidium (Pellegrino et al., 1962).

Determination of liver enzymes levels

Detecting liver disease is facilitated by measuring liver enzyme activities, also used as displays of liver function. Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is commonly calculated by utilising the method of Reitman and Frankel (1957).

Examination of oxidative status

Investigating oxidative status is a crucial aspect of understanding the role of oxidative stress in schistosomiasis. The catalase activity level was determined at 340nm by the (Colorimetric/Fluorometric) methods described by Góth (1991). According to the method of Nandi and Chatterjee (1988), SOD activity in hepatic tissue homogenates was measured by preventing the reduction of nitro blue tetrazolium (N.B.T., 0.025 mM) at 560 nm, using O2U generated as a side product of the xanthine/xanthine oxidase structure.

Fifty-millimetre sodium carbonate buffer containing 0.1-millimetre xanthine, 0.025-millimetre N.B.T., 0.1-millimetre EDTA, xanthine oxidase (0.1 U/mL), and 1-millimetre EDTA was used in the assay. A tissue homogenate dilution (1:20 v/v) initiated the reaction. The amount of SOD required to prevent 50% of the O2U N.B.T reaction has been defined as one activity unit for SOD. Following the method outlined by Habig et al. (1974), spectrophotometric analysis was used to determine the glutathione-S-transferase (GST) activity level using 1-chloro-2-4 dinitrobenzene (CDNB) and glutathione at the wavelength 340 nanometers, where the absorbance was determined.

Determination of immunological parameters

An enzyme-linked immunosorbent assay (ELISA) kit (Elabscience®; Cat. No. MBS70117) was adopted to quantify serum vascular adhesion molecule-1 (VCAM-1) levels (Gearing et al., 1992). Serum TNF- levels were measured using the method of Chan and Perlstein (1987), and the results were expressed in picograms per millilitre (Pg/ml) using the ELISA method by using a commercial ELISA kit (Elabscience®; Cat. No. # MBS825075).

Statistical analysis

SPSS 22.0 (IBM/SPSS Inc., Chicago, IL) was used to perform a statistical analysis of the data collected in this study. The mean ± standard deviation (S.D.) was used to summarise continuous variables. The ANOVA test was used to ensure the data satisfied the assumptions of normality and homogeneity when comparing multiple groups. On a significant difference in ANOVA, the Dunnet Post Hoc test was used to adjust for multiple comparisons with the control. In order to compare statistical results that had been reduced to just two groups, we used Student's t-test. The 0.05 cutoff for the P-values was used to determine statistical significance.

RESULTS

Parasitological parameters

Table 1 presents the influence of extract obtained from grape seed and PZQ on the number of *S. mansoni* worms found in the various groups of animals used in the study. The average number of total worms retrieved from the group treated with grape seed extract (14.3 ± 1.6) was not significantly less than that of the infected control group (16.3 ± 1.3). However, the average number of worms collected from PZQ alone (0.16 ± 0.4) or combined with grape seed extract (0.00 ± 0.00) was lower than those recovered from the positive group. As it turns out, Grape seed extract alone (GVI) was given to

mice infected with *S. mansoni*. This treatment did not induce a significant reduction rate in both the hepatic egg load (29.7%) and the intestinal egg load (15.6%) or a significant increase in the number of dead ova (12.6 ± 0.8) (table 1). However, PZQ alone (GV) or combined with grape seed extract (GVI) induced significant differences compared with the positive control. The highest percentage of tissue egg load was found in the hepatic (91.8%) and the intestinal tissues (92.8%) after administration of grape seed extract combined with PZQ (GVII). Additionally, there was a significant increase in the percentage of dead ova in groups treated with PZQ alone (91.6 ± 1.6) or in combination with grape seed extracts (100 ± 0.75%) compared with the positive control (Table 1).

Effect of grape seed extract and PZQ on liver functions

Liver enzyme activity showed significant elevations in serum ($P < 0.05$) in the *S. mansoni* infected control group (GIV) in contrast to the results obtained by the normal group (GI) (Fig. 1). Meanwhile, serum ALT and AST activities significantly ($P < 0.05$) decreased in *S. mansoni*-infected mice treated with grape seed extract (GVI), PZQ (GV), and the combination of both (GVII), compared to *S. mansoni* infected mice that were not treated.

Evaluation of the oxidative status

According to Table 2, *S. mansoni*-infected mice had significantly lower ($P < 0.05$) CAT, SOD, and GST activities than those in the control mice. Significant ($P < 0.05$) increases in CAT, SOD, and GST activities were observed in *S. mansoni*-infected mice treated with grape seed extract compared to an infected control group. The administration of mice infected with *S. mansoni* the grape seed extract and praziquantel resulted in a significant ($P < 0.05$) decrease in CAT, SOD, and GST activities compared to treatment with PZQ alone.

Furthermore, administering grape seed extract to normal mice showed a statistically significant increase in CAT, SOD, and GST activities compared to the normal control group.

Immunological results

Serum VCAM-1 level in *S. mansoni*-infected mice

The normal mouse treated with PZQ (G II) or grape seed extract (G III) did not have significant changes in VCAM-1 compared with the normal control group (G I) (Fig. 2). In the meantime, compared to the uninfected normal group, the level of VCAM-1 significantly increased ($P < 0.05$) in the infected mice. The VCAM-1 also significantly reduced ($P < 0.05$) in *S. mansoni*-infected mice treated with grape seed extract, praziquantel, or their combination. Similarly, the serum vascular cell adhesion molecule-1 concentration significantly reduced ($P < 0.05$) in *Schistosoma mansoni* infected mice treated with grape seed extract and praziquantel contrasted with infected mice treated with PZQ only.

Serum level of tumour necrosis alpha

As observed in Figure 3, the pattern of TNF-α serum levels was similar to that of VCAM-1. In the present investigation, we found no statistically significant differences ($P < 0.05$) between TNF-α levels in the normal mice treated with PZQ (GII) and grape seed (GIII) compared with the normal control group (GI). TNF-α levels were discovered to be significantly greater ($P < 0.05$) in the *S. mansoni*-infected mice (585.8 ± 11.4 Pg/ml) when compared with the normal control group (301.3 ± 3.3 Pg/ml). The level of TNF-α in infected mice significantly decreased ($P < 0.05$) after treatment with PZQ, grape seed extract, or both, compared to the positive control group (429 ± 12.0, 348.0 ± 13.7, and 328.8 ± 4.5 Pg/ml, respectively). Meanwhile, when infected mice were treated with a mixture of PZQ and grape seed extract, the level of TNF-α significantly reduced ($P < 0.05$) compared with infected mice treated with PZQ only.

DISCUSSION

Even though the worldwide consequence and gravity of schistosomiasis, its treatment depends exclusively upon the PZQ. Inadvertently, *S. mansoni* strains resistant to PZQ have emerged due to the drug's widespread use.

As a result, research on new schistosomiasis treatments is highly encouraged. Herbal medicines may contain phytoconstituents with various pharmacological activities, including suppressing reactive oxygen species generation (Aborehab & Boshra, 2019). The current study indicated that the medication effects of grape seed extract (Gervital) alone against *S. mansoni* in experimental mice led to non-significant diminutions in worm count, hepatic, and intestinal egg load, with a non-significant increase in the ratio of dead eggs in the oogram pattern. PZQ was more effective than grape seed extract alone.

In contrast, when grape seed extract and PZQ were used together, the total number of worms and ova decreased, and the ratio of dead eggs increased compared to the positive control group. Grape seed extract may have an anthelmintic effect because of its secondary metabolites, which include flavonoids, polyphenols, and tannins (Sülsen et al., 2017; Hendiger et al., 2020; Soto-Sánchez, 2022; Ticona et al., 2022).

In addition, antischistosomal agents such as flavonoids, polyphenols, and tannins have been reported to ameliorate infection-related liver damage and oxidative stress (Mohammed et al., 2023). Grape seed extracts have antimicrobial and antioxidant activity (Andrade et al., 2019; Krasteva et al., 2023). More precisely, they inhibit the growth of some species of pathogenic bacteria such as *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis, Pseudomonas spp., Salmonella spp.,* and *Staphylococcus aureus* (Tsiviki & Goula, 2021; Ramli et al., 2021; Oun et al., 2022; Krasteva et al., 2023). Grape seed extracts' antimicrobial effects come from their concentrated effort of flavonoids and derivatives (Andrade et al., 2019). As opposed to that, Gupta et al. (2020) found that grape seed extract inhibits *Listeria monocytogenes* due to the presence of caffeic acid, quercetin, and quercetin-3-O-rutinoside.

According to the findings of this study, infection with *S. mansoni* seems to affect both the secretory and synthetic functions of the liver. Infection with *S. mansoni* impairs the liver's ability to secrete and synthesise substances. Because the eggs of *S. mansoni* get trapped in the liver, causing inflammation and damage to liver cells. Over time, this can lead to fibrosis and cirrhosis of the liver. Irrespective of this, the levels of both AST and ALT in mice infected with *S. mansoni* seemed to be significantly more elevated than those observed in the normal group. Grape seed extract, PZQ, or a combination of the two was given to mice infected with *S. mansoni*, and the results showed a significant improvement in the liver function parameters of the mice. These conclusions are in line with the outcomes of Abdel-Hafeez et al. (2012), Mohamed et al. (2014), and Elmalawany et al. (2022). They found that mice infected with *S. mansoni* had improved liver function after receiving PZQ. Consistent with prior research, Abdel-Ghaffar et al. (2015) found that grape seed extract secured against cytotoxic drugs in animal studies by lowering liver damage (CCL4). Ismail et al. (2016) also reported that the ability of grape seed oil to protect against liver damage brought on by CCl4 was the subject of a study in rats that have been irradiated. Furthermore, grape seed extract showed an abundant protective effect and was well tolerated at one hundred milligrams/kilogram (Long et al., 2016).

Infection with *S. mansoni* led to significant oxidative stress in mice, as evidenced by a significant reduction in the hepatic activities of CAT, SOD, and GST compared to those of the normal group. Mohamed et al. (2014), Beshay et al. (2019), and Elmalawany et al. (2022) reported similar findings in mouse and human schistosomiasis. Mice infected with *Schistosoma mansoni* and later treated with grape seed extract, PZQ, or both, had significantly increased hepatic CAT, SOD, and GST activities compared with *S. mansoni*-infected controls. When PZQ and grape seed extract were administered to mice, antioxidant enzymes such as CAT, SOD, and GST were significantly higher than those in PZQ-treated mice alone. Considering these results, grape seed extract may have the antioxidant capacity to combat the production of reactive oxygen species (ROS) linked to schistosomiasis. Because of their high antioxidant ratios and ability to scavenge free radicals, polyphenols, and flavonoids found in grape seed extract have

attracted much attention (Georgiev et al., 2014; Gupta et al., 2020). Polyphenols, such as procyanidins and proanthocyanidins, were responsible for grape seed extract's antioxidant capacity, which has been shown to have a broad and potent effect on free radicals (Bagchi et al., 1997; Dulundu et al., 2007; Pešić et al., 2019). Nada et al. (2015) showed that grape seed extract enhances cellular antioxidant defence mechanisms, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Protecting the liver and other major organs from oxidative stress is possible thanks to reduced glutathione levels' ability to inhibit lipid peroxidation and recover diminished glutathione levels. Elmalawany et al. (2022) found that *Balanitis aegyptiaca* aqueous fruit extract and PZQ significantly increased GSH, CAT, SOD, and NO activity in *S. mansoni*-infected mice, giving support to this hypothesis.

B and T cells' cellular immune responses against the schistosome parasite are the primary pathology associated with schistosomiasis. In the case of *Schistosoma*, the immune system is directed at the egg that the female parasite deposits in the endothelium of the blood capillaries of small inferior mesenteric blood vessels surrounding the colon and the caecum (Sharaf et al., 2022). Zhong et al. (2022) provided further evidence that cytokines are essential for controlling fibrosis deposition and degradation by confirming that modifications in T-helper immune cell profile were observed throughout the induction of granuloma response. This study examined the proinflammatory cytokine VICAM-1 and TNF-α levels between the different groups. Mice infected by *S. mansoni* were significantly elevated ($P < 0.05$) TNF-α and VCAM-1 serum levels than uninfected mice. These findings are consistent with previous research by Azevedo et al. (2012), Mohamed et al. (2014), and Femoe et al. (2022). Mouse models of *Schistosoma mansoni* infection allowed for treatment by grape seed extract, praziquantel, or a combination of the two exhibited a significant reduction in levels of tumour necrosis factor-alpha and vascular cell adhesion molecule-1. In mice infected with *S. mansoni*, previous research found that administering either PZQ alone or the combination of the two led to significant reductions in the serum levels of TNF-α (Yu et al., 2012; Femoe et al., 2022); otherwise, a mixture of PZQ and blue-green algae (Mohamed et al., 2014). Oppositely, Ali Rajput et al. (2017) exhibit a natural tannin proanthocyanidin extract from grape seed with known antioxidant properties that differentially regulate TNF-α induced VCAM-1.

Further, Kuntz et al. (2015) recorded that grape extract significantly attenuated TNF-α stimulated VCAM-1 to reduce the inflammation-inhibited in vitro epithelial-endothelial co-culture model. Also underlined, Calabriso et al. (2022) documented that some studies indicated that pretreatment with grape seed proanthocyanidin extracts reduced tumour necrosis factor-α and down-regulated vascular cell adhesion molecule-1 expression but had no effect on ICAM-1 expression. Maybe grape seed extract may contain high antioxidant polyphenols, specifically proanthocyanidins, that can counteract chronic inflammatory symptoms.

In conclusion, grape seed extract reduced worm burden and tissue egg loads; in contrast, it increased the ratio of dead eggs in a mouse model infected with *S. mansoni*, but these results were not statistically significant. This improvement is linked to higher antioxidant activity and lower serum liver enzymes. PZQ's parasitological results were better than grape seed extract's, except for immunity and oxidative stress. Grape seed extract reduces schistosomiasis-related reactive oxygen species (ROS) and PZQ's side effects. Flavonoids, polyphenols, proanthocyanidins, and tannins in grape seed extracts are powerful antioxidants. Grape seed extract's potent antioxidant and immunological effects and PZQ's schistosomicidal effects produced the best results when used together. Thus, grape seed extract could be used with PZQ to boost its antiparasitic effects and prevent infection-induced fibrosis, cellular antioxidant, immune system improvement, and liver damage.

CONFLICTS OF INTEREST

There are no competing interests between the authors.

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AVAILABILITY OF DATA

The data that support the findings of this study are available from the corresponding author, [Elmalawany A.M.], upon reasonable request.

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