Ameliorative Effect of Dandelion Leaves Extract Against Liver Injury Induced by Schistosomiasis in Mice

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Abstract

The objective of this study is to examine the impact of dandelion leaf water extract (DWE) on Schistosomiasis. A total of thirty-five mature male albino mice, weighing between 20 and 25 grammes, were utilised and categorised into two primary groups. The first group, consisting of seven mice, functioned as a control group without any infection. The second group, including twenty-eight mice, was infected with S. mansoni. After 45 days following infection, the second primary group was divided into four subgroups. The first subgroup was infected with S. mansoni without receiving any treatment and served as the positive control group (IC). The second subgroup was infected with S. mansoni and then treated with praziquantel (PZQ). The third subgroup was infected with S. mansoni and then treated with DWE. The fourth subgroup was infected with S. mansoni and then treated with a combination of DWE and PZQ.

The findings demonstrated that the combined administration of DWE+PZQ is superior to either PZQ or DWE alone in all aspects. It notably resulted in a substantial decrease in parasitological parameters, such as the number and size of hepatic granulomas, as well as histopathological alterations in the liver, when compared to the untreated infected group. The results also revealed that the treatment of DWE+PZQ dramatically lowered AST and ALT activity and augmentation of serum albumin. The amelioration effects were found first in the group treated with DWE+PZQ, then in the group treated with PZQ alone, and finally in the group treated with DWE just. The study determined that the addition of dandelion leaf water extract, in combination with the anti-schistosomal medication praziquantel, has the potential to serve as a treatment for schistosomiasis.

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Keywords: Dandelion, Polyphenols, Liver, Schistosomiasis, Mice.

INTRODUCTION

Schistosoma infection has resulted in significant illness and death on multiple continents, ranking it as the third most devastating tropical sickness worldwide. The citation is from Rollinson et al. (2013). Schistosomiasis was previously believed to be a tropical disease that resulted in histological abnormalities in multiple organs. The citation is from Abu Almaaty et al., 2021. Organs such as the liver, intestines, bladder, and urethra can be infected by this parasitic condition, which is caused by bloodworms. Gene therapy, vaccinations, and Praziquantel (Nelwan, 2019) have the potential to eradicate these diseases. Microscopic histopathology examination is widely employed by investigators as a common scoring method. The references cited are Ibrahim et al. (2018) and Miao et al. (2010).

The liver is regarded as a crucial organ in the human body, playing a significant part in the control of physiological processes and other essential functions. Due to its unique anatomical position and physiological role, the liver is susceptible to a range of illnesses. The liver undergoes various pathological alterations, including the accumulation of fat, elevated levels of reactive oxygen species (ROS) or oxidative stress, inflammation of liver cells, cell death, impaired bile flow, fatty deposits, formation of granulomas and vascular abnormalities, fibrosis, increased levels of inflammatory markers, development of hepatocellular carcinoma, and ultimately cirrhosis, leading to portal hypertension and organ failure (Singh et al., 2011).

Schistosomiasis resulted in the deformation and degradation of hepatic cords, as well as necrosis and degeneration of hepatocytes. These modifications might occur from Kupffer cell hypertrophy and granuloma formation. Furthermore, the presence of significantly elevated lipid vacuoles and reduced carbohydrate levels were observed in the study conducted by Mostafa et al. (2011) and Mahmoud et al. (2016).

Praziquantel (PZQ) is considered the only appropriate medication for the treatment of schistosomiasis. Praziquantel (PZQ) is effective against all species of schistosomes. However, the emergence of resistance presents a potential challenge to the control of this disease. (Basha and Mamo, 2021). Praziquantel (PZQ), the sole preferred drug, has been widely utilised worldwide for an extended period. This has resulted in the finding of schistosomes that are tolerant to PZQ, which has raised worries on the potential emergence of Schistosoma strains that are resistant to the medicine.

The reference is from Danso-Appiah and De Vlas (2002). Furthermore, instances of parasite tolerance and resistance to this treatment have been recorded in specific situations. Due to the shortcomings of PZQ, there is a growing demand to develop a novel treatment method, particularly using natural plant derivatives (Abdulla et al, 2007; Mahmoud et al, 2014).

Several plant-derived compounds, such as resveratrol, curcumin, silymarin, silibinin, berberine, and dandelion, have demonstrated promising hepatoprotective activities (Ezhilarasan et al., 2012; Ezhilarasan et al., 2014). The dandelion (Taraxacum officinalis) is a perennial plant that belongs to the Asteraceae family. The traditional use of this plant has been recorded in various nations due to the presence of physiologically active compounds in its leaves, petals, and roots (Hu, 2018).

T. officinale is a conventional herbal remedy employed for the management of liver ailments, jaundice, and gallbladder conditions (You et al., 2010; Ahmed et al., 2013). The traditional medicines of Russia, India, and China have acknowledged the T. officinale's impact as a liver tonic. Roots and herbs have purportedly been used to heal many disorders, such as liver and gallbladder ailments. The reference is from Gulfraz et al. (2014). T. officinale is used with other herbs in traditional Chinese medicine to treat liver problems. The reference is from Modaresi and Resalatpour's study conducted in 2012.

T. officinale has been used as a traditional therapeutic medicine in ethnopharmacology due to its biological and pharmacological features, such as its hepatoprotective and hypoglycemic effects (Schutz et al., 2006). Dandelion possesses bioactive compounds such as polyphenols, vitamins, and terpenes, which exhibit antioxidant properties. These natural antioxidants play a crucial role in safeguarding organisms from oxidative stress, a significant contributor to the ageing process and the development of various diseases such as cancer, cardiovascular disorders, neurodegenerative conditions, and others (Nowak et al., 2019).

Dandelion contains a variety of beneficial compounds including lactones, chlorogenic acid, taraxerol, taraxasterol, and chicory acid. These components are safe to use and possess strong antioxidant, anti-inflammatory, and anti-rheumatic activities. The references cited are Arpadjan et al., 2008 and Yarnell and Abascal, 2009. Biologically active chemicals have been discovered in several components of this plant. The leaves include compounds classified as flavonoids, phenolic acids, coumarins, and vitamins, particularly vitamin A (Lis and Grabek-Lejko, 2016). The leaves of T. officinalis contain significant amounts of minerals, vitamins, fibre, and important fatty acids (Escudero et al., 2003). In addition, Schütz et al. (2006) reported that the extract from dandelion leaves contains a significant amount of polyphenols. The root extract has shown a higher level of hydrogen peroxide scavenging activity. The dandelion plant has garnered attention for its anti-inflammatory and antioxidant characteristics, as well as its ability to safeguard against cancer, obesity, and several cardiovascular risk factors. (Jeon et al., 2017). The dandelion aqueous extract has been found to have strong anti-tumor promoting effects in the two-stage carcinogenesis of mice skin tumours induced by an initiator and a promoter. In addition, the extract possesses analgesic, anti-inflammatory, and antiangiogenic effects, resulting in the reduction of cyclooxygenase 2 and nitric oxide synthesis, as well as the rate of reactive oxygen species (ROS) formation by activated macrophage cells. The reference is from Takasaki et al. in 1999.

In modern times, dandelion is used in medicinal preparations and as a readily available food supplement due to its qualities that stimulate bile production, increase urine production, promote growth, alleviate rheumatic conditions, fight against bacteria and cancer, and reduce inflammation (Park et al., 2010; Lis et al., 2018). There is a scarcity of scientific research on the anti-parasitic efficacy of dandelion leaf extract. Therefore, the objective of this study was to showcase the anti-parasitic and liver-protective properties of dandelion leaf extract in mice infected with S. mansoni, and compare it to the effectiveness of PZQ.

Materials and Methods

Plant Material.

The T. officinale (dandelion) leaves were gathered in February 2022 from the City of El-Mahalla El-Kubra, Egypt, and verified by the corresponding author.

Preparation of Extract:

The T. officinale sample was dehydrated at a temperature of 25 ± 5°C and subsequently pulverised into a fine powder. A quantity of 100 grammes of powdered dandelion was immersed in distilled water at a temperature of 100 degrees Celsius, using a ratio of 1 part dandelion to 20 parts water by weight/volume. The mixture was left to soak for a duration of 10 minutes. Subsequently, the aqueous solution was subjected to filtration using Whatman No. 1 filter paper and subsequently concentrated under vacuum conditions at a temperature of 40°C (Zanatta et al., 2021). The administration of dandelion water extract (DWE) commenced at a dosage of 500 mg/kg/day, starting on day 45 after infection and continued for a duration of two weeks.

Phytochemical Analysis:

The Food Safety and Quality Control Lab (FSQC) at Cairo University's Faculty of Agriculture conducted a phytochemical analysis to identify and quantify polyphenolic compounds. The analysis was performed using the methodology outlined in Agilent Application Note, publication number 5991-3801 EN, 2014. The Agilent 1260 Infinity HPLC series, manufactured by Agilent in the United States, was utilised. It was fitted with a Quaternary pump and the column utilised was a Kinetex® 1.7µmEVO C1850mmx2.1mm column, manufactured by Phenomenex in the United States. The HPLC system was operated at a temperature of 30°C. The separation is accomplished by employing a ternary linear elution gradient consisting of (A) HPLC grade water containing 0.1% H3PO4 (v/v), (B) acetonitrile containing 0.1% H3PO4 (v/v), and (C) methanol. The flow rate is 0.2 millilitres per minute. The volume that was injected was 20 µL. Detection: Utilising a variable wavelength detector (VWD) **configured to a wavelength of 280nm.**

Drug:

The drug used in the study was Praziquantel (PZQ), which was obtained from Egyptian International Pharmaceutical Industries Company (E.I.P.I.C.O.). It was prepared as a suspension in 2% Cremophore (EL, Sigma Chemical Company, St. Louis, MO, USA) and administered orally. The drug was given after 45 days post infection at a dose of 500 mg/kg for two consecutive days, as described by El-Lakkany et al. (2012).

Experimental animals:

The trials were conducted on a group of 35 adult male Albino mice, with an average weight of 20-25 grammes. These mice were grown at the Schistosoma Biological Supply Centre located at Theodor Bilharz Research Institute (TBRI). The studies were conducted in accordance with the "Principles of laboratory animal care." Before commencing the experiment, the mice were given a period of time to adapt to the laboratory circumstances, which included a temperature range of 26–28 degrees Celsius and a 12-hour cycle of light and darkness, for a duration of seven days. During this period, the subjects were provided with a regular meal and unrestricted access to tap water (Bishayee et al., 1995).

Infection of Mice:

The experimental animals were housed at the TBRI animal facility. A total of twenty-eight mice were infected with an Egyptian strain of S. mansoni by injecting an average of 80±10 cercariae per mouse subcutaneously (Peters and Warren, 1969).

Experimental Design:

The animals were randomly allocated into two primary groups. The first group, consisting of 7 mice, functioned as the control group and did not receive any infection or therapy. The second primary cohort, consisting of 28 mice, was infected via subcutaneous injection with an average of 80±10 cercariae per mouse. After 45 days post-infection, this cohort was divided into four subgroups, each containing 7 mice. The first individual was infected with S. mansoni and did not receive any treatment, serving as the positive control (IC). The second individual was infected with S. mansoni and subsequently treated with PZQ. The third individual was infected with S. mansoni and subsequently treated with DWE. The fourth individual was infected with S. mansoni and then treated with a combination of DWE and PZQ.

Sample collection:

Following the conclusion of the experiment, all animals were terminated through decapitation. Peripheral blood was obtained from each animal, and serum was isolated using centrifugation at 3000 rpm for 10 minutes. The serum was then stored at -20 °C until it was utilised for biochemical analyses. The livers were promptly excised from all animals and subsequently partitioned into two segments. The initial portion of the liver was preserved in a solution of 10% formalin for the purpose of quantifying the diameter of the granuloma and conducting histological examinations. The remaining portion was utilised to ascertain the developmental phases of schistosome eggs and determine the count of ova (Pellegrino et al., 1962). In addition, portions of the intestine were collected for the purpose of quantifying ova and conducting Oogram tests.

Biochemical Serum Analysis:

Serum samples were obtained in aliquots for the measurement of serum alanine transaminase. (ALT), serum aspartate transaminase (AST) (Henry et al., 1974), and serum albumin concentration (Pinnell and Northam, 1978).

Worm burden:

To measure worm burden and percentage of worm reduction, cold saline was perfused into the liver, portal, and mesenteric veins. Male, female and coupled worms were counted (Duvall and DeWitt, 1967). The worm burden was determined as the percentage of maturation of cercariae into adult worms (Freire et al., 2003).

The percentage reduction of worm burden in all infected groups was calculated according to the following equation:

$$\text{Worm burden reduction\%} = \frac{\text{Mean of worms from control group} - \text{mean of worms from treated group}}{\text{Mean of worms from control group}} \text{ x } 100$$

Egg Developmental Stages (Oogram Pattern):

Developmental stages of eggs were determined; the mature stage contains a fully developed miracidium; the immature stage contains abnormal miracidia. In addition, the dead ova were counted and recorded. The number of eggs/grams of small intestine tissue of infected mice was detected according to (Pelligrino and Goncalves, 1965).

Tissue egg load:

Samples of intestine (ileum) and liver were collected from each mouse and digested for 16 hours at 37 °C in 5% KOH. Ova were counted at x 40 under a light microscope. The number of eggs/gram tissues in the gut and liver was calculated according to (Herbert et al., 2010).

Histological studies:

The fixed specimens of liver were washed and transferred to 70% ethanol, dehydrated in ascending ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 5µ thickness. The sections were then stained with Harries Hematoxylin and Eosin (Delafield, 1984), and Masson's trichrome to demonstrate the collagenous fibers (Humason, 1972). Paraffin sections were used for the count of granuloma numbers and measuring of their diameter and histopathological studies.

Number and diameter of Granuloma

Using a multi-head microscope, the diameter of the granulomas was digitally measured and their number was recorded. Measuring was limited to granulomas with a single ovum. (Jacobs et al., 1997).

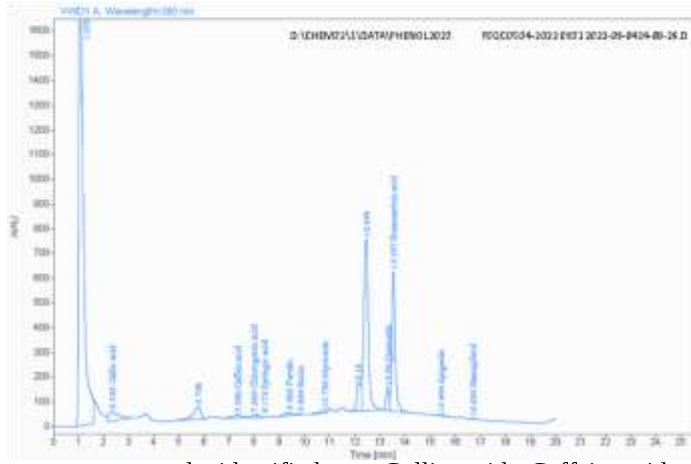
Statistical Analysis

The data was analysed using SPSS version 20.0, a statistical program. The quantitative data were represented as the mean value plus or minus the standard deviation (SD). One-way analysis of variance (ANOVA) was employed for tests that involved the comparison of multiple means. The Post Hoc test was used to do several comparisons between different variables. The results are considered statistically significant at a p-value of less than 0.05 for low significance, less than 0.01 for moderate significance, and less than 0.001 for high significance.

Results:

Phytochemical Analysis

The aqueous extract of dandelion (T. officinale) used in the present study was analyzed by FSQC-HPLC1260 (Agilent Technologies, USA) and carried out to obtain information about the qualitative and quantitative polyphenol contents in the samples of plant extract. By using the retention time from a reference test conducted under the same conditions, the polyphenolic components in the leaf extract sample were identified. The compounds identified are gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic, rutin, myricetin, quercetin, rosemarinic acid, apigenin and kaempferol. Rosemarinic acid was the major polyphenolic compound (16829.14243mg/kg at 13.537min), while the minor polyphenolic compound is rutin (49.25192mg/kg at 9.824min) (Fig.1, Table 1).



compounds identified are Gallic acid, Caffeic acid, Chlorogenic acid, Syringic acid, Ferulic, Rutin, Myricetin, Quercetin, Rosemarinic acid, Apigenin and Kaempferol.

Table 1: Composition of polyphenolic compounds in Taraxacum officinale leaves extract by HPL

Compound	RT [min]	Area	Amount [mg/kg]
Gallic acid	2.323	1109.0220	998.65228
Caffeic acid	7.338	357.2402	349.16572
Chlorogenic acid	7.969	166.3202	293.85249
Syringic acid	8.172	91.7083	64.39582
Ferulic	9.382	276.5160	438.85575
Rutin	9.824	41.8615	49.25192
Myricetin	10.796	256.7643	484.19328
Quercetin	13.320	756.7056	1334.32296
Rosemarinic acid	13.537	5500.1099	16829.14243
Apigenin	15.424	131.2235	175.68643
Kaempferol	16.695	73.7716	91.72130
	Max Conc. 16829.14243		

Parasitological parameters:

Worm Burden

The data in Table 2 showed a significant decrease in the average number of worms in each of the groups treated with DWE+ PZQ, PZQ alone, and dandelion extract (DWE), compared to the untreated infected group (IC). The reduction percentages were 100%, 96.84%, and 68.20%, respectively. This difference was highly significant ($p < 0.001$). However, when examining the average reduction in worm load between the PZQ + DWE group and the DWE group, a notable disparity was seen. The findings reported in Table 4 indicates that the groups treated with a combination of (DWE + PZQ), PZQ alone, or DWE alone exhibited a highly substantial ($p < 0.001$) reduction in both immature and mature ova compared to the control group (IC). Conversely, all groups that received treatment exhibited a remarkably substantial rise in the number of dead eggs, in comparison to the control group. The group treated with a combination of (DWE + PZQ) exhibited greater efficacy in eliminating mature and dead ova compared to the group treated just with PZQ.

Table 2. Effect of PZQ, DWE, and their combination on mean worm burden in S. mansoni-infected mice.

Mean worm burden +SD (liver and Porto-mesenteric)																	
	Male				Female				Couples				Total				% Total worm burden reduction
F test	35.200				1.000				188.559				340.288				
P value	0.001***				0.410				0.001***				0.001***				
	Mean ± SD	P1	P2	P3	Mean ± SD	P1	P2	P3	Mean ± SD	P1	P2	P3	Mean ± SD	P1	P2	P3	
IC	3.14 ± 0.90	-	-	-	0.14 ± 0.38	-	-	-	7.00 ± 1.15	-	-	-	17.14 ± 2.04		-	-	-
PZQ	0.29 ± 0.49	0.001***	-	-	0.00 ± 0.00	0.503	-	-	0.00 ± 0.00	0.001***	-	-	0.29 ± 0.49	0.001***	-	-	96.84
DWE	0.57 ± 0.79	0.001***	0.841	-	0.00 ± 0.00	0.503	1.0	-	2.57 ± 0.53	0.001***	0.001***	-	5.71 ± 0.95	0.001***	0.001***	-	68.20
PZQ+ DWE	0.00 ± 0.00	0.001***	0.841	0.368	0.00 ± 0.00	0.503	1.0	1.0	0.00 ± 0.00	0.001***	1.0	0.001***	0.00 ± 0.00	0.001***	0.966	0.001***	100

Data represented by mean ± SE (n = 7)

P1: Comparison with IC, P2: Comparison with PZQ, P3: Comparison with DWF.

* Significant p value < 0.05 , ** Significant p value < 0.01 , *** Significant p value < 0.001

Table 3. Effect of PZQ, DWE, and their combination on the hepatic and intestinal ova count in S. mansoni-infected mice.

Ova count	Mean egg count/gram Liver				Mean egg count/gram Intestine				Total ova/ mice				% Total ova reduction
F test	269.38				134.99				199.812				
P value	0.001***				0.001***				0.001***				
	Mean ± SD	P1	P2	P3	Mean ± SD	P1	P2	P3	Mean ± SD	P1	P2	P3	
IC	6139 ± 569.04	-	-	-	7288.9 ± 1225.4	-	-	-	13428 ± 1757.4	-	-	-	-
PZQ	585.14 ± 230.36	0.001***	-	-	501.71 ± 112.25	0.001***	-	-	1086.9 ± 238.42	0.001***	-	-	91.9
DWE	3446.6 ± 632.61	0.001***	0.001***	-	4028.4 ± 841.81	0.001***	0.001***	-	7475 ± 1385.6	0.001***	0.001***	-	44.32
PZQ+DWE	313.86 ± 58.191	0.001***	0.664	0.001***	439.57 ± 75.476	0.001***	0.999	0.001***	753.43 ± 92.267	0.001***	0.945	0.001***	94.39

Data represented by mean ± SE (n = 7)

P1: Comparison with IC, P2: Comparison with PZQ, P3: Comparison with DWF.

* Significant p value < 0.05 , ** Significant p value < 0.01 , *** Significant p value < 0.001

Table 4. Effect of PZQ, DWE and their combination on the oogram pattern in S. mansoni-infected mice (Immature, mature and dead ova).

% Egg developmental stages ± SD												
Ova count	**Immature ova**				**Mature ova**				**Dead ova**			
F test	392.398				197.650				744.610			
P value	0.001***				0.001***				0.001***			
	Mean ± SD	**P1**	**P2**	**P3**	**Mean ± SD**	**P1**	**P2**	**P3**	**Mean ± SD**	**P1**	**P2**	**P3**
IC	50.43 ± 1.27	-	-	-	44.71 ± 1.80	-	-	-	5.02 ± 0.79	-	-	-
PZQ	0.00 ± 0.00	0.001***	-	-	6.71 ± 1.80	0.001***	-	-	93.29 ± 1.80	0.001***	-	-
DWE	43.14 ± 2.12	0.001***	0.001***	-	41.29 ± 1.98	0.001***	0.001***	-	15.57 ± 1.27	0.001***	0.001***	-
PZQ+ DWE	0.00 ± 0.00	0.001***	1.0	0.001***	3.29 ± 1.80	0.001***	0.002*	0.001***	96.71 ± 1.80	0.001***	0.027*	0.001***

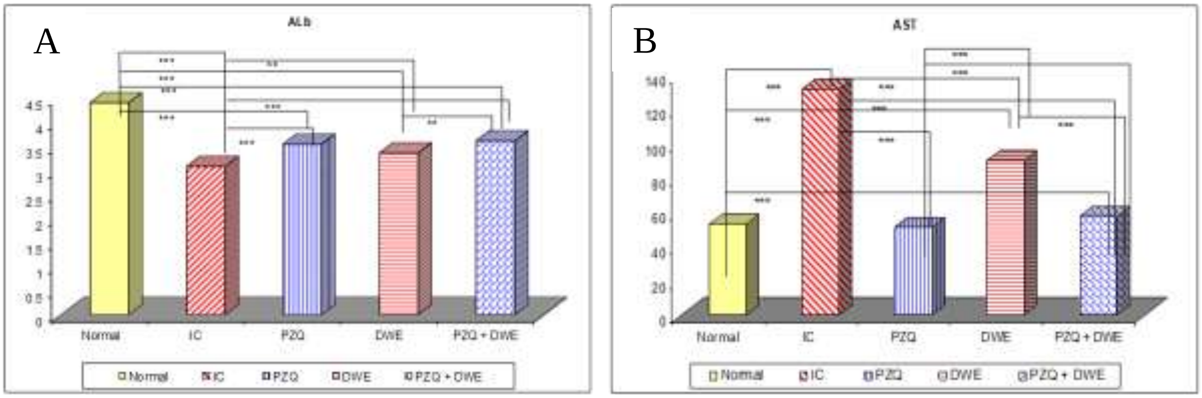
Data represented by mean ± SE (n = 7)
P1: Comparison with IC, P2: Comparison with PZQ, P3: Comparison with DWF.
* Significant p value < 0.05 , ** Significant p value < 0.01 , *** Significant p value < 0.001

Biochemical analysis:
The albumin levels in S. mansoni infected mice (3.08 ± 0.11) were considerably lower (P < 0.001) compared to the usual values observed in the uninfected control group (4.40 ± 0.15). The administration of a combination of DWE+PZQ, PZQ alone, or DWE alone to infected mice resulted in an increase in serum albumin concentrations, measured at (3.61 ± 0.13, 3.54 ± 0.10, and 3.36 ± 0.16) respectively, as compared to the untreated infected group.
A substantial and statistically significant rise in serum AST and ALT levels (131.57 ± 2.64, 160.29 ± 2.56 U/L) was seen in the group of infected individuals who did not get treatment, as compared to the control group of uninfected individuals (53.16 ± 1.43, 63.64 ± 3.01). Administering a combination of DWE+PZQ, PZQ alone, or DWE alone to infected mice resulted in a substantial decrease in the levels of blood enzymes AST and ALT, as compared to the infected group that did not receive any treatment. This finding is supported by data presented in Table 4 and picture 2. The groups treated with DWE+PZQ and PZQ exhibit greater effectiveness compared to the group treated alone with DWE.

Table 5. Effect of PZQ, DWE, and their combination on albumin, AST enzyme, and ALT enzyme in serum of S. mansoni infected mice.

	Albumin g/dl					**AST (U/L)**					**ALT (U/L)**				
F test	95.469					2625.494					1082.3				
P value	0.001***					0.001***					0.001***				
	Mean ± SD	**P1**	**P2**	**P3**	**P4**	**Mean ± SD**	**P1**	**P2**	**P3**	**P4**	**Mean ± SD**	**P1**	**P2**	**P3**	**P4**
Normal	4.40 ± 0.15	-	-	-	-	53.16 ± 1.43	-	-	-	-	63.64 ± 3.01	-	-	-	-
IC	3.08 ± 0.11	0.001***	-	-	-	131.57 ± 2.64	0.001***	-	-	-	160.29 ± 2.56	0.001***	-	-	-
PZQ	3.54 ± 0.10	0.001***	0.001***	-	-	51.14 ± 2.27	0.24	0.001***	-	-	63.71 ± 0.76	1	0.001***	-	-
DWE	3.36 ± 0.16	0.001***	0.004**	0.095	-	90.33 ± 0.70	0.001***	0.001***	0.001***	-	93.57 ± 6.00	0.001***	0.001***	0.001***	-
PZQ+DWE	3.61 ± 0.13	0.001***	0.001***	0.852	0.009**	57.71 ± 1.11	0.001***	0.001***	0.001***	0.001***	65.43 ± 1.99	0.854	0.001***	0.872	0.001***

Data represented by mean ± SE (n = 7)
P1: Comparison with Normal, P2: Comparison with IC, P3: Comparison with PZQ, P4: Comparison with DWF.
* Significant p value < 0.05 , ** Significant p value < 0.01 , *** Significant p value < 0.001



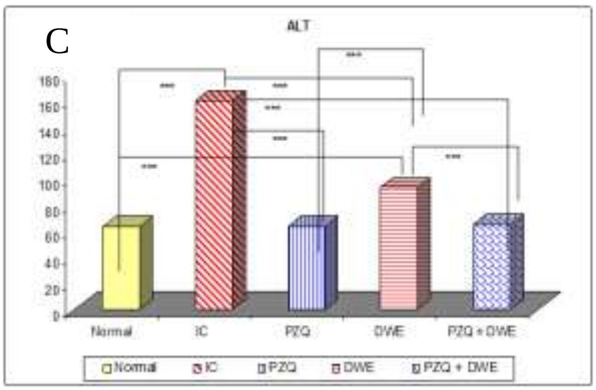


Fig. 2: Effect of PZQ, DWE, and their combination on: A; Albumin, B; AST enzyme, C; ALT enzyme in serum of infected mice by *S. mansoni* and compare differences between the groups. Data represented by mean ± SE (n=7).
*Significant p value < 0.05 ,
** Significant p value < 0.01 ,
*** Significant p value < 0.001

Histological study:

Diameter and number of Granuloma:

The groups treated with a combination of (DWE + PZQ), PZQ, or DWE exhibited a substantial decrease in both the diameter and number of Granuloma compared to the untreated infected group (IC). The mice treated with DWE+PZQ had the smallest granuloma diameter (151.57±25.22) and number (4.17+1.12), followed by the mice treated with PZQ alone (168.24±21.20, 5.64 +2.15). The mice treated with DWE only had a larger granuloma diameter and number (298.49±24.16, 9.98 +1.31) compared to their corresponding untreated infected group (349.32±22.17, 14.9 +3.11). The DWE+PZQ treatment group showed the largest decrease in both the average size and number of hepatic granulomas (56.61%, 72.01% reduction, respectively), followed by the PZQ group (51.84%, 62.15% reduction), and lastly the DWE group (14.55%, 33.02% reduction). The most efficacious treatment was DWE+PZQ, followed by mice treated with PZQ, and finally mice treated with DWE alone (Table 6). Furthermore, various forms of granuloma and the condition of S.mansoni eggs were identified, as demonstrated.

Table 6: Showing some Parameters of granuloma area in liver of S. mansoni infected mice and treated groups.

	Granuloma diameter				% Reduction in Granuloma	No. of granuloma				% reduction in successive power fields (10x10)	Types of Granuloma			% State of *S.mansoni* eggs	
F test	250.631					147.632									
P value	0.001***					0.41									
	Mean ± SD	P1	P2	P3		Mean ± SD	P1	P2	P3		Fibrous %	% cellular	Cellular %	Degenerated	Intact
IC	349.32±22.17	-	-	-	-	14.9±3.11	-	-	-		0	23	77	5	95
PZQ	168.24±21.20	0.001***	-	-	51.84	5.64 ±2.15	0.001***	-	-	62.15	0	45	55	46	54
DWE	298.49±24.16	0.001***	0.001***	-	14.55	9.98 ±1.31	0.001***	0.001***	-	33.02	0	35	65	12	88
PZQ+ DWE	151.57±25.22	0.001***	0.217	0.001***	56.61	4.17±1.12	0.001***	0.106	0.001***	72.01	0	48	52	65	35

Data represented by mean ± SE (n = 7)

P1: Comparison with Normal, P2: Comparison with IC, P3: Comparison with PZQ, P4: Comparison with DWF.

* Significant p value < 0.05, ** Significant p value < 0.01 , *** Significant p value < 0.001

Histopathological observations:

The liver tissues of the untreated infected group (IC) exhibited a chronic granulomatous inflammation, characterised by a significant presence of cellular granulomas and an abundance of eosinophil-rich inflammatory cells. The undamaged cellular miracidium is observed within the ovum. The presence of numerous granulomas disrupts the lobular structure of normal hepatocytes. The treated groups showed a clear improvement, with a small hepatic fibrocellular granuloma containing significantly degraded miracidium. The granuloma has a regular contour and is well-defined from the surrounding tissue, as seen in Figure 3. The Masson's trichrome staining reveals differences in the distribution of collagen fibres among the various treated groups. The untreated infected group exhibited an augmentation of collagen fibres surrounding, inside, and in the pre-granuloma of hepatic tissue. The group treated with DWE+PZQ had a significant decrease in collagen fibres, followed by the PZQ-treated group, and then the group treated with DWE alone.

The liver sections of the treated group with DWE+PZQ exhibited a reduction in fibrosis specifically localised around the trapped S. mansoni ova. This reduction was accompanied by a higher number of fibroblasts compared to the groups treated with PZQ or DWE alone (Fig. 4). In addition, the hepatocyte located next to the granuloma exhibited hydropic alterations, focal atypical hyperplasia, vacuolar degeneration, necrotic foci, and hypereosinophilia. The central and portal veins exhibited dilation and congestion, accompanied by infiltration of inflammatory cells. The number of Kupffer cells within enlarged hepatic sinusoids was elevated (Fig.5). The histopathological changes were diminished in all treated groups, with partial restoration of liver architecture and a majority of normal hepatocytes, particularly in the group treated with a combination of DWE and PZQ. The groups treated with either PZQ or DWE showed partial improvement in liver tissue (Fig.6).

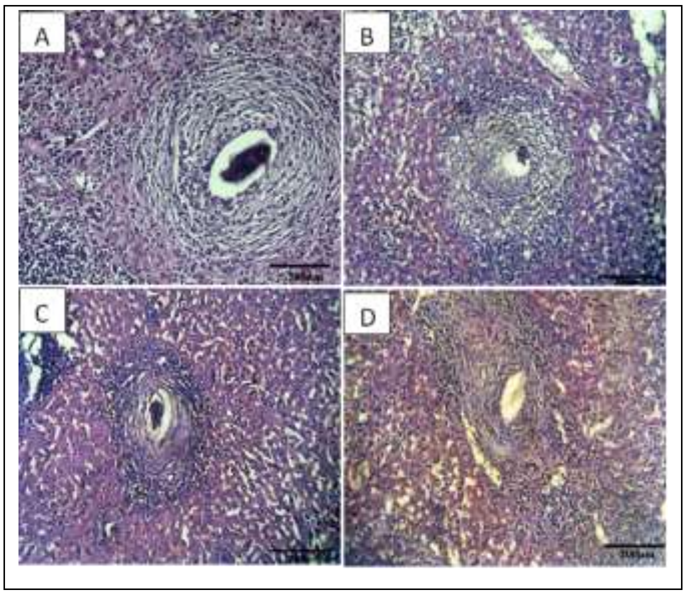


Fig. 3: Photomicrographs of liver granuloma in: (A) Mice infected with S. mansoni showed a massive cellular granuloma with an intact cellular miracidium inside the ovum. The granuloma was formed of a mixture of chronic inflammatory cells high in eosinophil. (B) representative of the PZQ group showing a small hepatic fibro cellular granuloma with markedly degenerated miracidium. (C): group treated with DWE showing a small hepatic fibro cellular granuloma with fewer inflammatory cells and markedly no degenerated miracidium. The granuloma contour is regular and well-demarcated from the surrounding tissue. (D): group treated with DWE+PZQ illustrating a fibro cellular granuloma with densely packed inflammatory cells and showing a small hepatic fibro cellular granuloma with markedly degenerated miracidium. H&E x100

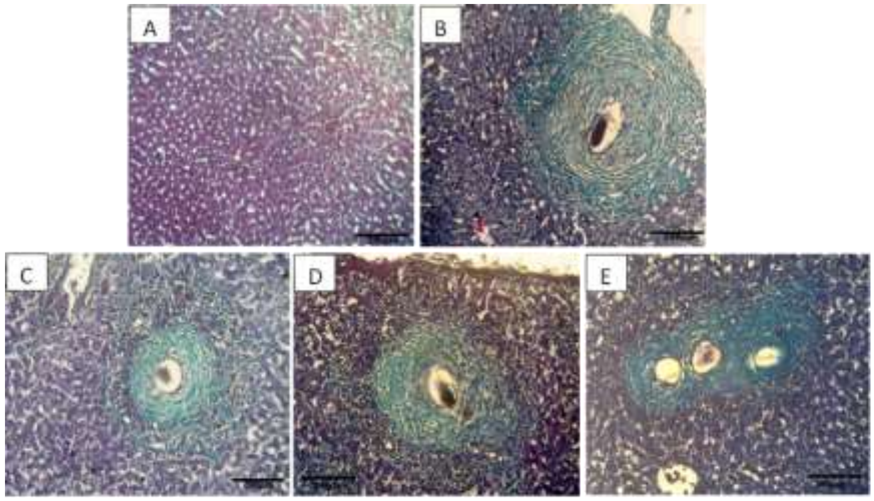


Fig. 4: Photomicrographs of liver sections stained with Masson trichrome from; (A) normal uninfected group showing normal minimal collagen fibers between hepatocyte, (B): infected group (IC) showing cellular extensive fibrosis around ova. (C) group treated with PZQ showing diminished cellular fibrosis around degenerated ova. (D): group treated with DWE showing moderate amount of collagen fibers around ova. (E) group treated with DWE + PZQ showing diminished cellular fibrosis around degenerated ova. H&E x100

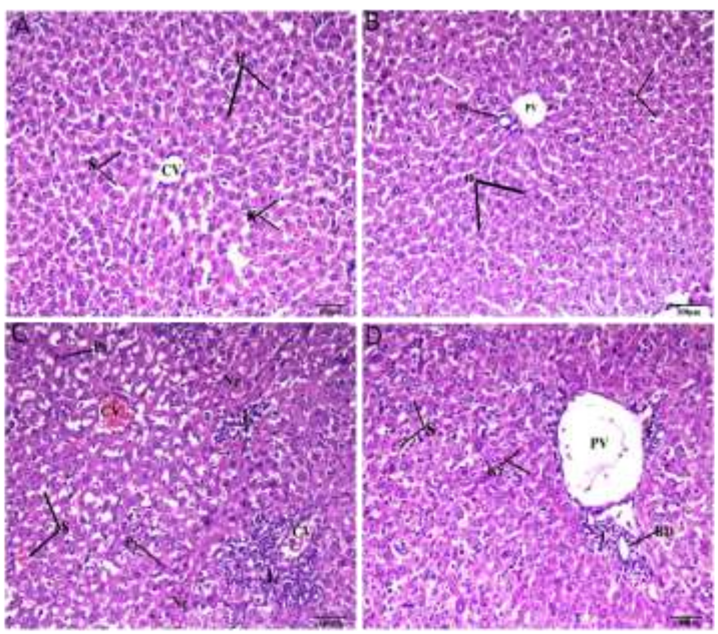


Fig. 5: Photomicrograph of Liver section from; (A, B) normal control group showing normal hepatic architecture, normal hepatocyte (H), central vein (CV), sinusoids (S), Kupffer cells (K), bile duct (BD) and Portal vein (PV). (C, D) infected mice with *S. mansoni* showing hydropic changes, necrotic areas (Ne) accompanied with inflammatory cell infiltrations (L), Pyknotic nuclei (Py) and few Karyolysis (K), dilation and congestion of sinusoids (S). Also, dilated and congested of central vein (CV) and portal vein (PV) surrounded by lymphocyte infiltration (L), proliferation of bile ductless (BD) are noticed. H&E, x200

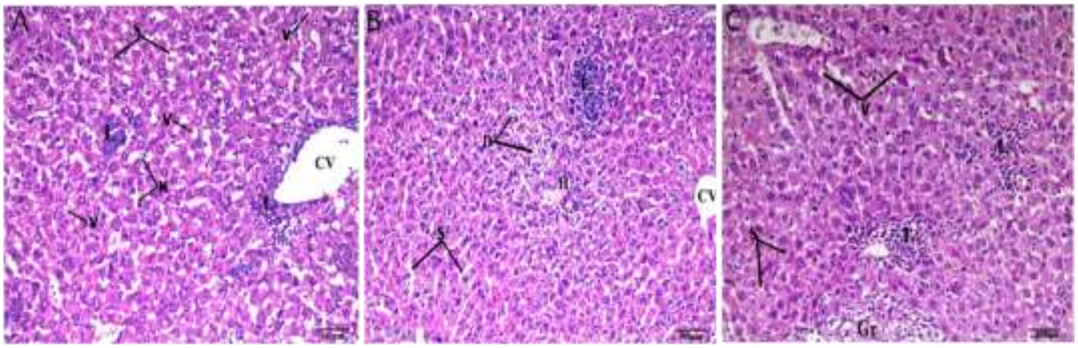


Fig. 6: Photomicrograph of Liver section from; (A) group treated with PZQ showing little vacuoles (v), proliferation of Kupffer cells (K), moderate congestion of hepatic sinusoids (s) and lymphocyte infiltration (L) between hepatocyte and around central vein (CV). (B) group treated with DWE showing partially improvement of hepatocyte, few lymphocytes infiltration (L) cytoplasm degeneration in some area of hepatic tissue (D) and hemolysis (H). Noticed normal hepatic sinusoids (s) and normal central vein (CV). (C) group treated with DWE+ PZQ showing normal hepatocytes architecture, normal hepatic sinusoids (s) little vacuoles (V) and lymphocytes infiltration (L) around blood vessels and between hepatocyte (L). Noticed part of Granuoloma (Gr). H&E, X 200

Discussion:

The current investigation evaluated the efficacy of a water-based extract derived from T. officinale leaves against schistosomiasis. The predominant phenolic acids and flavonoids found in the analysed sample were rosemarinic acid, quercetin, and gallic acid, in that order. Polyphenolic compounds, which are secondary metabolites, are commonly found in plant-based diets. Their significant levels of antioxidants and well-documented abilities to combat viruses, germs, inflammation, cancer, and dilate blood vessels have been reported (Ivanova et al., 2005). The presence of phenolic acids in plant extracts is typically attributed to their antioxidant effects (Ivanov et al., 2014; Diaz et al., 2018). Plants belonging to the Asteraceae family are widely recognised for their hepatoprotective effects. The references cited are Asadi-Samani et al., 2015 and Sharifi-Rad et al., 2018. Recent study suggests that T. officinale and its constituents possess anti-inflammatory and antioxidant characteristics, which can have diverse biological effects. The reference is from González-Castejon et al. (2012).

In this study, the group that received a combination of dandelion extract (DWE) and praziquantel (PZQ) demonstrated a highly significant reduction ($p< 0.001$) in worm burden, average egg count, and egg development (Oogram) compared to the group treated with only PZQ. The dandelion extract treated group (DWE) also showed better results than the infected untreated group (IC). The findings aligned with those of (Atwa et al., 2022), who observed that oral administration of dandelion root extract resulted in a partial anthelmintic effect. This was evidenced by a nearly 50% decrease in parasitological parameters (worm burden and ova count) compared to the untreated infected group. The decrease in egg tissue load observed in the treated mice may be attributed to reduced female productivity, a decline in the total number of worms after treatment, and a robust host tissue response that effectively eliminated the limited number of eggs produced. (Mostafa and Saved, 2001).

Vale et al. (2017) demonstrated that praziquantel (PZQ) is the most effective medication for treating schistosomiasis. The study conducted by Abdul Ghani et al. (2009) provided evidence of the efficacy of PZQ and artesunate derivatives in combating different stages of worm development. Praziquantel exerts a direct impact on schistosomes by inducing muscular constriction in the worm. The study conducted by Pax et al. in 1978 found that there was an increase in calcium entering the worms. Similarly, Wolde-Mussie et al. in 1982 saw that this influx of calcium occurred. Additionally, Bricker et al. in 1983 discovered that the tegument of the worms was disrupted. The main objectives of these drugs often involve maintaining the integrity of cell membranes, regulating microtubules, affecting DNA, and disrupting neural signal transmission in parasites. (Wink, 2012). Furthermore, the presence of the host immune system is essential for the elimination of the parasite within the living organism. The study conducted by Melhorn et al. in 1981 demonstrated outstanding therapeutic efficacy of the treatment, which was also favourably received by patients, as reported by Smith et al. in the same year.

An indication of schistosomiasis is the disturbance of the cellular antioxidant mechanism and the liberation of free radicals, which is associated with a confirmed infection. The host immune system generates oxidative stress and releases oxygen-derived free radicals as an initial defence response against the parasite's antioxidant defence system. (Aragon et al., 2008).

Panda and Luyten (2018) demonstrated that the Asteraceae family possesses a substantial quantity of phenols that repel a diverse range of parasites. The antiparasitic activity of the Asteraceae family can inhibit protozoan parasites such as intestinal worms, Trypanosoma species, Leishmania species, and Plasmodium species. Taraxacum officinale, often known as dandelion, belongs to the Asteraceae family and possesses diverse medicinal properties and applications. This natural cure exhibits potential for treating chronic liver diseases such as hepatitis and hepatic steatosis. (Devaraj, 2016).

Faria et al. (2019) and Aabideen et al. (2020) demonstrated that Taraxacum officinale leaves contain abundant polyphenols and have potent antioxidant activities. Taraxacum officinale is utilised for the treatment of inflammations and hepatic disorders. Additionally, it may possess antifungal, antiviral, antibacterial, and cytotoxic characteristics.

Rajamanickam and Muthuswamy (2008) stated that the liver plays a crucial part in overall metabolism, and any damage to the liver can impact the body's metabolic processes. Enzymes are essential for regular cellular metabolism. The hepatoprotective effects of dandelion in mice infected with S. mansoni were evaluated by measuring serum hepatic enzyme levels. The levels of serum AST and ALT increase dramatically due to S. mansoni infection, leading to the destruction of liver cells. The significant rise in the levels of the serum marker enzymes, AST and ALT, may be associated with the impairment of the cell membrane (Naik et al., 2011). The group treated with a combination of DWE and PZQ exhibited a substantial decrease in AST and ALT enzymes compared to the group treated with PZQ alone. These findings corroborate the findings of (Atwa et al., 2022) which indicated that the group that received both PZQ and dandelion root extract had the most significant decrease in blood ALT and AST levels. The enhancement in liver enzyme activity may be attributed to the absence of necrotic hepatic tissue and the reduction in size and fibrosis of hepatic granulomas in the treated mice afflicted with the disease. (Allam, 2009).The serum concentration of albumin in untreated infected mice was considerably decreased compared to the normal uninfected group. The decrease in blood total protein levels can be ascribed to the structural impairment of liver cells (Tag et al., 2020).

The study conducted by Hfaiedh et al. in 2016 The hepatoprotective properties of dandelion leaf extract were verified in rats with liver injury produced by sodium dichromate. The daily oral treatment of a hot water extract of dandelion leaves (500 mg/kg) over a period of 30 days resulted in a decrease in the levels of total cholesterol, triglycerides, AST, ALT, lactate dehydrogenase, MDA, and chromium concentration in the blood and liver of rats. The consumption of dandelion leaves extract has been linked to an elevation in the activities of antioxidant enzymes such as GSH, SOD, catalase, and GPX, as well as a decrease in lipid peroxidation, GST, the development of atherosclerotic lesions, and DNA fragmentation (Choi et al., 2010; Hamzawy et al., 2015). The hepatoprotective properties of this substance may be attributed to the up-regulation of antioxidant enzymes in the liver.

The aqueous extracts of dandelion roots and leaves have been utilised as hepatoprotective agents among various components of dandelion extracts. Various constituents may contribute to its hepatoprotective properties. The primary constituents of the hot aqueous extract of dandelion leaves that possess the capability to eliminate free radicals include total phenolics, flavonoids, tannins, polysaccharides, and ascorbic acids (Hfaiedh et al., 2016).

In this study, the group that received both DWE and PZQ treatment shown the most substantial decrease in the number and size of hepatic granulomas. The PZQ treated group exhibited a lesser drop, followed by the DWE treated group, as compared to the group that did not get any treatment for the infection. The results demonstrated the significant therapeutic effect of dandelion extract combined with PZQ in the histological observations. Kabuyaya et al. (2018) asserted that praziquantel, despite its potent antiparasitic properties, is ineffective in treating liver fibrosis linked to schistosomiasis. The decline in the number of eggs and tissue is the cause of the decrease in the number of hepatic granulomas. However, the abundant presence of sesquiterpene lactones and phytosterols in dandelion may have beneficial effects in reducing inflammation and fibrosis. The citation is from Atwa et al. (2022).

The histopathological examination of the infected group showed a significant inflammatory response with the presence of massive granulomas surrounding the ovum. In contrast, the treated group exhibited a reduction in the size of these granulomas.

The primary cause of schistosomiasis symptoms is the formation of granulomatous inflammation surrounding the eggs of the parasite (Abath et al., 2006). Granulomas form around the eggs within several host tissues, including the liver. (Elbaz and Esmat, 2013). Granuloma production serves as a defence mechanism for the host against the harmful effects of antigens released from parasite eggs. However, it can also lead to fibrosis in the periportal regions due to an excessive accumulation of collagen and extracellular matrix proteins. The reference is from Morais et al. (2008). Granuloma production is a protective process that safeguards the host by containing dangerous and antigenic substances, ultimately eliminating the egg and removing any leftover debris. Granulomas can result in localised tissue damage and the initiation of fibrosis, among other harmful consequences. Granulomas consist of macrophages, epithelioid cells, giant cells, eosinophils, lymphocytes, and a small number of mast cells. Fibroblasts generate the collagenous framework upon which the inflammatory cells reside. This matrix displaces the normal organ parenchyma. The source is cited as Weinstock (1992).

Although the ongoing experiment determined that PZQ had a slight antifibrotic effect, Liang et al. (2011) discovered that PZQ exhibited both hepatoprotective and antifibrotic properties in mice infected with S. japonicum. Their outcomes were obtained after undergoing continuous PZQ treatment for a duration of 30 days. The restoration of normal hepatic organisation and hepatocyte appearance in the group treated with DWE + PZQ may be attributed to the strong antioxidant properties of dandelion and its capacity to eliminate free radicals (Sakr and Sabre, 2007).

Moreover, this phenomenon can be elucidated by the suppression of Th1 and Th2 cells, resulting in a decrease in related cytokines and playing a pivotal role in the formation of granulomas (Wynn and Cheever, 1995). The T. officinale aqueous extract has been found to have strong antiangiogenic, anti-inflammatory, and antinociceptive effects. This implies that it has the ability to decrease the synthesis of nitric oxide (NO) and cyclooxygenase 2 (COX-2), as well as the rate of reactive oxygen species (ROS) generation in activated macrophage cells. The reference is from González-Castejón et al. (2012). In addition to its demonstrated antioxidant activities and anti-fibrotic properties, dandelion aqueous extract has been shown to deactivate hepatic stellate cells and improve the regenerating capacity of the liver (Colle et al., 2012).

Al-Malki and Abo-Golayel (2013) discovered that supplementing with DLWE significantly improved portal inflammation, fibrosis, necrosis, and steatosis in rats treated with CCl4. This improvement was shown at the conclusion of the sixth week of the trial and was statistically significant ($P < 0.01$). Polyphenols, flavonoids, and polysaccharides are responsible components for hepatoprotective properties of dandelion. Consequently, the standardisation of dandelion extracts can be achieved by focusing on one of these chemicals (Mahboubi & Mahboubi, 2020).

The antiparasitic effects of flavonoids and isoflavones against Cryptosporidium parvum and Encephalitozoon intestinalis were further examined by Mead and McNair (2006). Furthermore, these investigators found that apigenin and quercetin exhibited activity against E. intestinalis. The presence of schistosomiasis causes oxidative stress at the site of granulomatous inflammation, resulting in the production of lipid peroxidation (LPO) products. These products are believed to have a significant impact on the development of schistosomiasis-related disease (Aly et al., 2010). The products of LPO induce cellular damage and necrosis by compromising the fluidity and integrity of the cell membrane (Dkhil et al., 2014).

Qiao et al. (2005) reported that rosmarinic acid effectively decreased the levels of nitric oxide (NO) and inducible nitric oxide synthase (iNOS) protein generated by lipopolysaccharide. Rats administered with rosmarinic acid essential oil exhibited hepatoprotective effects against carbon tetrachloride-induced liver damage. This was achieved by restoring the activity of antioxidant enzymes such as catalase, peroxidase, glutathione reductase, and glutathione peroxidase in the liver tissue. Additionally, the essential oil effectively eliminated free radicals by scavenging mechanisms (Raskovic et al., 2014). Numerous biological effects of rosmarinic acid include liver protection, blood clot inhibition, anti-inflammatory characteristics, and inhibition of HIV-1, cancers, and hepatitis. (Tepe, 2008; Aldoghachi et al., 2021). The use of medicinal plants for anti-helmintic therapy shows great potential as an effective treatment option. The citation is from Liu et al. (2020).

Conclusion:

Based on our findings, the usage of dandelion water extract alone yielded less successful results. However, when paired with PZQ, the most effective outcomes were observed across all parameters. The combination of dandelion leaf water extract and the anti-schistosomal drug praziquantel could potentially serve as an effective treatment for schistosomiasis.

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