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To cite this article: S S Shahatha *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1060** 012071

Effect of *Marlubium vulgare* Plant on Experimentally Infected Albino Mice with *Trichomonas muris* Parasite and Compare with Metronidazole Drug

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Abstract. This study was conducted to investigate the effect of Marlubium vulgare plant on albino mice experimentally infected with Trichomonas muris parasite and compared it with Metronidazole drug. The oral administration method was used to estimate the effect of the plant extract. where each group of mice was administered a different concentration of the plant alcoholic extract (500, 1500, 2500, 3500) mg/kg, and 30 mg/kg of metronidazole drug was administered to another group, while the control group was administered an equal amount of normal saline solution. The results showed a significant effect of the concentrations of alcoholic extract and the drug metronidazole in the treatment of infected albino mice and showed a high treatment efficiency of 100% with a variation in the time required for complete treatment and complete elimination of parasites. The highest concentration of 3500 mg/kg resulted in complete recovery in the affected albino mice on the fourth day after treatment, while the use of the drug led to a complete recovery on the fifth day after treatment, while the infection in the control group continued to the fifth day and then died. The conclusion of this study confirmed the high therapeutic efficacy of different concentrations of alcoholic extract of M. vulgare plant in treating T. muris parasite in mice experimentally infected with it, which is comparable to the effectiveness of Metronidazole drug.

Keywords. Albino Mice, Marlubium vulgare, Trichomonas muris,.

1. Introduction

Trichomonas muris parasite is one of the most common parasitic protozoa in rodents, the genus *Trichomonas* belongs to the class Trichomonadea and the family Trichomonadidae [1]. Many species belonging to this genus, including *T. tenax, T. vaginilis,* and*, T. hominis*, parasitize many vertebrate and invertebrate animals [2]. The *T. muris* parasite lives in the colon and caecum of rats and mice and feeds on white blood cells and bacteria [3]. The infection is transmitted through oral-fecal contamination [4]. The Trophozoite phase is pear-shaped, its length ranges between 10-16 micrometers and its width 5-10 micrometers, and it has one oval nucleus and three short flagella that extend forward and a fourth flagellum extends to the back, the cell envelope extends on the dorsal side to form the wavy membrane that helps in movement [5]. These parasites do not form cysts, but they work to round themselves and lose their flagella and are called the dormant phase [6]. Metronidazole is an appropriate treatment for trichomoniasis, where he succeeded in treating the injury by 95% [7].
Many medicinal herbs and plants have been used to treat many diseases, including parasitic diseases because they contain many effective compounds such as flavones, terpenes, alkaloids, glycosides,

resins, titans, phenols, volatile oils, and others [8]. The Qatina plant, or the *Marrubium vulgare* plant, belongs to the Labiatae family (Lamiaceae), which is characterized as an annual herb, its height ranges from 30-60 cm, and the stems are simple ascending, covered with white woolly or cotton wool, the leaves are elongated oval-circular in shape, the plant blooms from late April to early June. It is spread on slopes, low-lying areas, and on-road edges with dry calcareous soils. This species is found in clay or sandy soils of a gravel nature. It contains volatile oil, rich in sesquoturbine compounds, marrubenol bi-terpenes, flavones, resins, tannins, and glycosides, the most important of which are asteoside and marruboside. Arenarioside. The plant was used by the ancient Egyptians and Greeks to treat coughs, repellents for insects, and anti-venom of snakes and research indicates the importance of aqueous extract in expanding blood vessels as well as lowering cholesterol in the blood, experiments on mice have shown that it lowers blood sugar and killing worms and protozoa [9]. Among the studies conducted to treat the *Trichomonas* parasite, Masoudan's study [10] in which a cold aquatic extract of garlic and pomegranate peel was used to influence *T. muris* parasites in infected mice, in addition to Al-Ammash's study [11] which extracts of the *Artemisia herba-alba* plant were used to influence *T. muris* in albino mice, as well as a study by Al-Masoudi et al., [12], aquatic and alcoholic extracts of garlic and *Allium sativum* were used to kill *T. muris* parasites. Due to the lack of studies regarding finding appropriate treatment for *T. muris* parasite by using plant extracts as alternatives to medicinal drugs, this study was conducted using the alcoholic extract of *M. vulgare* plant to treat experimentally infected mice. This study is the first of its kind in which this plant is used to treat parasitic diseases.

2. Materials and Methods

30 Albino mice, Blab/c (Ages 8-10 weeks and weighs 28-46 g) were used in this study, and they were divided into 6 equal groups (5 mice per group), and they were placed in plastic cages for raising laboratory animals (Figure 1), the appropriate environment was provided in terms of temperature, lighting, ventilation, special feed, and sterile water.

Figure 1. A group of Albino mice used in this study.

2.1. Preparing the Alcoholic Extract of the Plant

Marrubium vulgare plant (Figure 2) was collected from Al-Baghdadi area west of Ramadi, and diagnosed in Anbar University herbarium. The parts of the plant were cleaned and dried and the extract was prepared according to Harborne [13] with a weight of 25 g of dehydrated plant parts and then dissolved in 350 ml of ethyl alcohol at a concentration of 70% at a 10: 1 ratio. After that the mixture was placed in a magnetic stirrer to mix homogeneously for 24 hours at room temperature, then the solution was filtered in a Buchner funnel, the filtrate was collected, and evaporated with a rotary vacuum evaporator at 60 °C to get rid of the solvent. The solution was filtered by filter paper to remove the chlorophyll pigment and return the extract to the evaporator to remove the water and obtain the concentrated extract. Then the extract is sterilized by dissolving 1 gram of it in 5 ml of DMSO (Dimethyl Sulfoxide), then the mixture is sterilized using the pasteurization process at 62 °C for 10 minutes, thus, the standard solution of the alcoholic extract of the plant was obtained, and from it, the concentrations (500, 1500, 2500, 3500) mg/kg used in this study were prepared.

Figure 2. The Qatina plant *Marrubium vulgare*

2.2. Microscopic Examination of Faeces Samples

The faeces samples taken from mice were examined visually by observing the texture and colour of the faeces, then microscopically examined by the direct smear method according to Price [14], by placing a drop of physiological saline 0.9 % on a clean glass slide and a quantity of faeces was taken by the size of the match stick by a stick of different areas, the sample was mixed well and covered with a cover slide, and another sample was prepared and stained with lugali iodine, and it was examined on the force 40X and 100X.

2.3. Events of Infection in Mice Experimentally and Treat with the Alcoholic Extract of the Plant and Metronidazole Drug

Many Mice were administered trophozoites of *T. muris* parasite by gastric tube for experimental infection events, then, the infected mice were mixed with healthy mice in plastic cages and the mice faeces were examined daily to ensure the transmission of infection, after ensuring that all mice are infected. It was divided into 6 equal groups (5 mice per group). where each group of mice was administered a different concentration of the plant alcoholic extract, the first group was administered a concentration of 500 mg/kg, whilst the second group was administered a concentration of 1500 mg/kg, the third group was administered a concentration of 2500 mg/kg and the fourth group of 3500 mg/kg. As for, the fifth group was administered a dose of 30 mg/kg of metronidazole drug, while the sixth group was administered a dose of 0.2 ml of normal saline solution 0.85%, the doses were given at once daily for 7 days.

2.4. Determination of the Therapeutic Efficacy of the Alcohol Extract of the Plant And Metronidazole Drug

The numbers of parasites were calculated in 1 g of faeces of white mice that appeared in a pear shape containing three flagella extended forward, a fourth flagellum extended backward, and one oval or circular nucleus (Figure 3), to determine the therapeutic efficiency of the concentrations of the alcoholic extract of the plant and metronidazole drug, using the following equation [15]:

$$N=S\ (VOL \times Wt)$$

Whereas:
N = number of parasites in 1 gm of faeces
S = parasite numbers calculated in the white blood cell counting slide
VOL = volume of calculated sample (0.01 ml)
Wt = weight of faeces sample taken (1 g)
The parasite count was continued throughout the treatment period and the Eocene stain was used, which works to dye the dead parasites. As for the living parasites, they do not take the dye, and thus

the living parasites were distinguished from the dead [16], to measure the therapeutic efficacy of the alcoholic extract of M. vulgare plant and the drug metronidazole, the following equation was used:
Therapeutic efficacy % = rate of parasite numbers in 1 gm of faeces in the control group - rate of parasite numbers in 1 gm of faeces in the treatment group/rate of parasite count in 1 gm of faeces in the control group × 100.

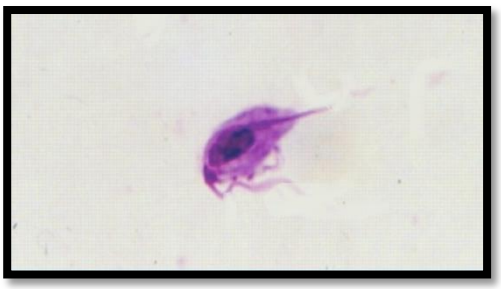

Figure 3. Trophozoite of *T. muris* in feces of albino mice (100 X**).**

2.5. Chemical Detection of Active Compounds in Plants [17]

2.5.1. Detection of Glycosides
Mixed 1 g of a dry plant extract with 10 ml of distilled water and filter the solution and add to it a few drops of fehlink's reagent, the appearance of the red colour indicates the presence of glycosides.

2.5.2. Detection of Flavonoids
10 grams of dry plant extract were dissolved in 50 ml ethyl alcohol 95% and filtered the solution (a solution was prepared by adding 10 ml of 50% ethyl alcohol to 10 ml potassium hydroxide 50%) and equal quantities of these two solutions were mixed, the appearance of the yellow colour indicates the presence of flavonoids.

2.5.3. Detection of Phenols
1 ml of dry plant extract was added to 1 ml of a 1% ferric chloride solution. The appearance of green or blue indicates the presence of phenols.

2.5.4. Detection of Terpenes
Dissolved 1 g of dry plant extract in 2 ml of chloroform and add a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid. The appearance of a brown precipitate is evidence of the presence of terpenes.

2.5.5. Detection of Saponins
3 ml of mercury chloride solution $HgCl_3$ 1% was added to 5 ml of plant extract, the appearance of a white precipitate indicates the presence of saponins.

2.5.6. Detection of Alkaloids
10 g of plant extract was boiled with 50 ml of distilled water acidified with 4% HCl, the solution was filtered after cooling and 0.5 ml was tested in an hour bottle with 0.5 ml of Meyer's reagent, the appearance of white precipitate evidence of the presence of alkaloids.

2.5.7. 7- Detection of Tannins
A few drops of a 1% lead acetate solution were added to 5 ml of the plant extract. The appearance of the white gel precipitate is evidence of the presence of tannins.

2.5.8. Detection of Fuocumarins
Two equal quantities of the plant extract were mixed with the alcoholic potassium hydroxide 1%. The appearance of a greenish-yellow colour indicates the presence of fuocumarins.

2.5.9. Detection of Resins

50 ml of 95% ethyl alcohol was added to 5 g of dry plant extract and heated in a water bath at 100 ^{o}C. The solution was filtered and 100 ml of distilled water acidified with 4% HCl was added. The appearance of turbidity is evidence of the presence of resins.

2.5.10. Detection of Volatile Oil

A few drops of plant extract were added to a filter paper to reduce saturation and exposure to ultraviolet rays, the appearance of Gray indicating the presence of volatile oils.

2.6. Statistical Analysis

Statistical analysis was performed using the analysis of variance (F-Test) by Minitab statistical program, the arithmetic means of the different coefficients were compared using the Duncan polynomial test under the probability level ($p \leq 0.05$) [18].

3. Results and Discussion

The results of the current study appeared to the high efficiency of the concentrations of the alcoholic extract of *M. vulgare* plant and the drug metronidazole in treating albino mice experimentally infected with a *T. muris* parasite. The used concentrations (500, 1500, 2500, 3500) mg/kg resulted in a therapeutic efficiency of 100%, but varied in the time required to events full recovery from the infection, The concentration of 500 mg/kg led to complete recovery and treatment of the infection after the seventh day of treatment, and the concentration of 1500 mg/kg resulted in recovery after the sixth day of treatment, while the concentration of 2500 mg/kg resulted in recovery from infection after the fifth day of treatment, and the concentration of 3500 mg/kg had the greatest effect in bringing about a complete recovery from the infection and eliminating all *T. muris* parasites after the fourth day of treatment, while administered a dose of 30 mg/kg of metronidazole drug led to complete recovery from infection after the fifth day of treatment, significant differences ($p \leq 0.05$) were observed between the different concentrations and days of treatment (Table 1).

Table 1. Therapeutic efficacy of concentrations of the alcoholic extract for *M. vulgare* plant and metronidazole in mice experimentally infected with *T. muris* parasite

Groups	Therapeutic efficacy%				
Duration (day)	500 mg / kg	1500 mg / kg	2500 mg / kg	3500 mg / kg	Metronidazole 30 mg / kg
1	43	51	67	71	74
2	56	64	71	82	85
3	62	70	79	86	89
4	75	84	90	100*	99
5	81	91	100	100	100
6	92	100	100	100	100
7	100	100	100	100	100

*concentration 3500 mg/kg is the most effective in killing the parasite after the fourth day of treatment with the plant extract

This is consistent with the results of Al-Ammash [19], where it found high effectiveness of 100% for the alcoholic and aquatic extract of *Artemisia herba-alba* plant and the drug metronidazole in treating the infection with this parasite in albino mice. (Table 2) shows that the numbers of *T. muris* parasites in the treatment groups and the control group were similar at the beginning of the infection, as it ranged between 6770-8560 parasites / g faeces. and since the first day of treatment with the alcoholic extract concentrations of *M. vulgare* plant and the drug metronidazole, the parasites numbers average excretion began to gradually decrease until it reached zero and all parasites disappeared after the fourth day of treatment with a concentration of 3500 mg /kg of the alcoholic extract of the plant and after the fifth day of treatment with metronidazole drug. Significant differences ($p \leq 0.05$) were observed in the parasite numbers average in mice treated with different concentrations of the alcoholic extract of the plant during the treatment days. The parasites numbers average continued to increase during the first five days after infection until it reached 9200 parasites / g faeces after the fifth day of

treatment, After the sixth day, it decreased to 6240 parasites / g faeces, while after the seventh day it reached 3100 parasites / g faeces. This is consistent with Masoudan's study [10], using a cold aqueous extract of garlic and pomegranate peel to eliminate *Trichomonas* parasites in infected mice, and it recorded a significant effect on these parasites and a high efficiency in their elimination.

Table 2. Average of parasite numbers in the feces of infected mice and its comparison with the control group during the treatment period (number of parasites × 10^3).

Duration (day) Concentration		Parasites numbers average ± standard error (parasite / g feces)							
		infection beginning	1 day	2 day	3 days	4 days	5 days	6 days	7 days
Group 1	500 Mg / kg	7.30±0.22 a	0.18 5.90± b	±0.34 3.30 e	0.14 ± 2.80 f	±0.68 1.80 g	±0.80 1.10 g	±0.60 0.20 h	0.0±0.0 i
Group 2	1500 Mg / kg	8.56±0.24 b	±0.12 6.44 d	±0.18 4.00 c	0.88 ± 3.36 e	0.96 1.50± g	±0.16 0.77 h	0.0±0.0 i	0.0±0.0 i
Group 3	2500 Mg / kg	7.88±0.45 a	±0.86 5.12 b	±0.64 3.00 e	0.19 ± 1.14 g	±0.86 0.20 h	0.0±0.0 i	0.0±0.0 i	0.0±0.0 i
Group 4	3500 Mg / kg	6.90±0.26 b	±0.48 4.17 c	±0.11 2.00 f	0.70 ± 0.14 h	0.0±0.0 i	0.0±0.0 i	0.0±0.0 i	0.0±0.0 i
Group 5	Drug 30 mg/ kg	6.77±0.12 b	0.16 4.12± c	±0.17 2.05 f	0.84 ± 1.33 g	±0.68 0.64 h	0.0±0.0 i	0.0±0.0 i	0.0±0.0 i
Group 6	Control	7.10±0.15 a	±0.40 7.93 a	±0.66 8.18 a	0.80 ± 8.40 a	±8.98 0.46 a	9. 20± 0.21 a	±0.31 6.24 a	±0.45 3.10 a

*The different letters within one column indicate the presence of significant differences ($p \leq 0.05$) between the treated groups

The results showed the high efficiency of the alcoholic extract concentrations of *M. vulgare* plant in the treatment of *T. muris* parasite, which is comparable to the effectiveness of metronidazole drug, where no significant differences were observed ($p \leq 0.05$) in the parasites numbers average in mice treated with the highest concentration of alcoholic extract 3500 mg/kg and drug Metronidazole during the different treatment days, the efficiency of the plant extract is attributed to its containment of many active compounds such as glycosides, terpenes, flavones, resins, tannins and volatile oils (Table 3). As these compounds affect the parasite through their effect on its vital activities, for example, the volatile oils present in the plant work to inhibit the vital activities by entering it through the cell wall [20]. Also, other compounds such as flavonoids and glycosides form complexes with proteins, thus inhibiting the efficacy of proteins and non-oxidation of unsaturated fatty acids in the cell membrane, thus breaking this membrane and killing the parasite [21].

In addition to the plant's containment of terpenes and tannins, which have great effectiveness in affecting these parasites, [22] indicated the efficacy of these substances against parasites, bacteria, and fungi, he mentioned [23] the therapeutic properties of flavonoids and phenols, which are one of the compounds found in the *M. vulgare* plant, as they inhibit enzymes by interacting with specific groups in them or randomly interacting with these proteins. The efficiency of the alcoholic extract is also due to the compounds dissolved with ethyl alcohol, which greatly affect the parasite through its ability to extract the active compounds such as flavonoids, volatile oils, phenols, and others, and this is what he mentioned [24].

Table 3. Chemical reagents on active compounds in extract of M. vulgare plant.

Active Compounds	Type of Reagents	Reagent Guide	Result Reagents
Glycosides	Fehlink detector	Red color	+
Flavonoids	Alcoholic potassium hydroxide 10%	Yellow precipitate	+
Phenols	Ferric chloride 1%	A green or blue deposit	+
Terpenes	Concentrated sulfuric acid with chloroform	Brown deposit	+
Saponins	Mercury chloride 1%	White precipitate	–
Alkaloids	Meyer's reagent	White precipitate	–
Tannins	Lead acetate 1%	The white, gelatinous precipitate	+
Fuocumarins	Alcoholic potassium hydroxide 10%	Yellow precipitate	–
Resins	Detection of 4% HCl acid	Turbidity	+
Volatile Oils	Exposing a filter paper containing the extract to ultraviolet radiation	grey color	+

(+): Indicates the presence of the active compound, (**–**):Indicates the absence of the active compound.

Conclusions

Concludes from this study, the high therapeutic efficacy of concentrations of the alcoholic extract of *M. vulgare* plant in the treatment of *T. muris* parasite in albino mice experimentally infected with it, which is comparable to the effectiveness of metronidazole drug. The higher the concentration of the extract, the greater the effect. The higher concentration of 3500 mg/kg recorded the highest therapeutic efficacy.

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