# USING OF *THYMUS VULGARIS* EXTRACTS TO CONTROL THE SNAIL VECTOR IN SCHISTOSOMIASIS *(*Part II)

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**ABSTRACT**

**The aim of this study is to determine the toxicity of *Thymus Vulgaris* aquatic plant extracts comparing with copper sulfates against *Bulinus truncatus* the vector of urinary Bilharziasis. Samples of the snails were collected from a site in Al-Rasheed district (30 km) southern of Baghdad. The isolation, identification, and acclimatization made in the laboratory. Many toxic parameters as NOEL (Non-Observed Effect Level), Threshold, EC (Effective Concentration) and LC (Lethal Concentration) levels were determined in this study. The EC50 of *T. vulgaris* and Copper sulfates to *B. truncatus* were (8.4, and 0.9g/L) respectively. The LC50 of *T. vulgaris* and Copper sulfates to *B. truncatus* were (18.7, and 2.2 g/L) respectively. The study showed that the *T. vulgaris* extracts were less effective than CuSO4. The results improved that the toxicity of extracts was concentration and time dependent. This work concluded the ability to use the target extracts in control of snails the middle host of the cause of urinary Schistosomiasis.**

**Keywords: Thymus Plant, Schistosomiasis, Snails**



# إستعمال مستخلصات الزعتر (*Thymus vulgaris*) في السيطرة على القوقع الناقل الحيوي في مرض البلهارزيا (Schistosomiasis) (الجزء II)

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**المستخلص**

**الهدف من الدراسة الحالية هو قياس تأثيرات المستخلصات المائية لنبات الزعتر *Thymus vulgaris* مقارنة مع كبريتات النحاس ضد القوقع *Bulinus truncatus* الناقل الحيوي للبلهارزيا الدموية. جمعت عينات القواقع من موقع في ناحية الرشيد (30 كم) جنوب بغداد. تم العزل والتشخيص والأقلمة لظروف المختبر. قيست عدة معايير للسمية مثل التأثيرات غير المشاهدة NOEL وحد العتبة Thresholdو التراكيز المؤثرة EC بعدة مستويات والتراكيز المميتة LC بعدة مستويات في هذه الدراسة. كانت التراكيز المؤثرة لمستخلص الزعتر ومادة كبريتات النحاس كانت تساوي (8.4 و0.9 غم/لتر) على التوالي. أما التراكيز المميتة لهما فكانت مساوية الى (18.7 و2.2 غم/لتر) على التوالي. أوضحت الدراسة ان مستخلصات نبات الزعتر كانت اقل سمية من مادة كبريتات النحاس للقوقع المستهدف. واثبتت الدراسة ان سمية المستخلصات تعتمد على التركيز ووقت التعرض ايضا. واستنتج من العمل الحالي ان هناك امكانية لاستخدام المستخلصات المستهدفة في السيطرة على القوقع المضيف الوسطي لمسبب مرض البول الدموي.**

**كلمات مفتاحية: نبات الزعتر، البلهارزيا، قواقع**

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## INTRODUCTION

Schistosomiasis (Bilharziasis) is considered as a big public pathological problem in the world. It affects 240 million people worldwide. Several millions of humans are suffering from severe morbid because of Schistosomiasis. The type parasitic worm *Schistosoma haematobium* is the causing of urogenital Schistosomiasis and the types *S. guineensis*, S. *intercalatum*, S. *mansoni*, S. *japonicum*, and S. *mekongi* are the causing of intestinal Schistosomiasis. Iraq is one of the countries suffering from urogenital Schistosomiasis. Baladruz is one of endemic distracts of Diyala province with Bilharziasis. Al-Bzania River in Baladruz is considered as a focus of disease vector. According to statistics of health associations and many studies in the region, 18% of Baladruz populations affects with Schistosomiasis. Many causes were affected by the prevalence of Schistosomiasis in the region as authorities' factors like using of river water as a wash place and swim especially with children whereas it specialized to palms irrigation (9, 17, 22). The life cycle of the worms is depending on factors such as presenting of *Bulinus truncatus* snail and contacting with water infected with cercariae. Cercariae are released from the vectors to the water then penetrate human skin through bathing, swimming, fishing, and agricultural activities. Adult worms are lives in the veins draining the urinary tract and intestines (17). Control of Bilharziasis might occur by chemical, physical, and biological. Chemical management may have several disadvantages as aspect effect, non-selectivity, represent treatment, not interference, and expensively. Now, the control of Bilharziasis became it doable by WHO ways. Severe morbidity because of bilharzia is often preventing by treatment with Praziquantel, Albendazole, and Ivermectin or by community education (2, 3). Biological control by cutting off life cycle of the parasite, management of the vector and eradication of disease agent before put down the body is a bear in mind to be higher than chemical control for previous causes. Materials utilized in biological management should study additional details to guard the surroundings and living communities (15). Plant extracts are victimization as molluscicides to regulate of Bilharziasis wide. The urge for the utilization of plant molluscicidal has augmented interest; because of it might be associate degree acceptable and cheap technology for snail management in endemic poor nations of the globe. There is still associated degree pressing would like extremely potent plant molluscicides to avoid the transmission of the parasitic unwellness Bilharziasis. Extracts of some plant molluscicidal as *Euphorbia splendens, phytolacca dodecandra, and Tetrapleura tetraptera* had been reportable to find the toxicity towards snails. It is also reported that the N-butanol extracts of plant molluscicidal as *Agave americanam Sapindus trifoliatusm, Balanites agyptica, Jatrapha gossypifolia, and Vaccaria pyramidata* are toxic against freshly arranged eggs of *L. luteol* ( 24, 25). Leaves of *T. Vulgaris* are containing a giant range of chemicals. For example, Trimethoxyflavone, Hydroxybenzoyl-glucose, Hydroxy-luteolin, Methoxy-Cirsilinol, Aluminum, Ascorbic-acid, Beta-pinene, Beta-sitosterol, Borneol, Caffeic acid, Boron, Calcium, Chromium, Cobalt, Iron, Labiatic acid, Luteolin, Magnesium, Niacin, P-coumaric acid, Phosphorus, Potassium, Riboflavin, Salicylates, Selenium, Silicon, Sodium, Tannin, Thiamine, Zinc (7). Copper sulfates used as molluscicides to the snail of *Biomphalaria alxandry* the middle host of *Schistosoma mansoni* in Egypt and Sudan, *B. truncatus* the middle host of Schistosoma haematobium in Iraq and *Lymnaea caillaudi* the middle host of *Fasciola hepatica* (8). The aim of the study is to determine the plant molluscicides to the snail of *Bulinus truncatus* to regulate the Bilharziasis with environmental safety.

## MATERIALS AND METHODS

**Collection of snails** : A collection of *Bulinus truncatus* snail's samples were conducted from June to August 2015 weekly. A site of samples collection was from Al-Rasheed district (30km) southern of Baghdad. The study area is including a station near of a street number 37 (arrived between Al-Rasheed districts and Tigris River). The coordinates of the area were (33º32'83) longitude and (44º25'37) latitude. The snails were collected from small irrigation canal beside the main canal called (Muhyii Canal). Zooplankton net and steel like spoon

were used to collecting the snails. A plastic containers (5 litter) were using to keep the samples. Snails were placed with a quantity of river water. The snails were feeding with the extracts of *Alfa alfa* plant leaves (10ml per 50L) daily and cultivated in the laboratory. The collected snails were isolated, identified according to stander keys of snails then they are acclimatized to laboratory conditions (T 25± 3) before testing for two days.

**Preparation of Aquatic Extracts and stock solutions (SS)**: The *T. Vulgaris* aquatic leaves extracts were prepared, concentrated, and dried. The leaves dried in a shade, shredded in a hand mill (Estrella®, model 41B) and in an electric mill (Moulinex®), then sifted through a mesh (number 30) to obtain a fine powder and left in a cool dry place as described by Guo-qing (10). A weighted amount of the extract made up to desired concentrations in water for analysis. We were macerate 5 and 10 gram of *T. Vulgaris* leaf powder in (1 liter) of distilled water for 24hr then placed in glass flasks. The macerate was filtered through cotton gauzes in a plastic funnel to get crude extracts. To prepare each extracts stock solutions, 5 and 10 grams of *T. Vulgaris* extracts were adding to (1 liter) of distilled water to produce a stock solution 5% and 10%. From this stock solution, a serial of dilutions was made. One gram of copper sulfates (CuSo4.5H2O) (Riedel-De Haen Ag Seelze-Hannover) was adding to (1 liter) of distilled water to get a stock solution 1% as a standard of comparators or positive control. From this SS, a serial of dilutions was made.

**Treatments and Bioassays**

A serial of 1-10% concentrations was prepared from each stock solution of the *T. Vulgaris* extracts (5g and 10g /l). All tests repeated three times with different times. Ten individuals of snail without any food were tested in each replicate and calculated as average. In addition, a stock solution (1g/L) of Copper sulfates (CuSo4) made as standard in comparisons. The W.H.O. method (II) for molluscicides testing was followed to monitor the susceptibility of snails, compare its potency with the extracts, exposure and recovery determined. The lethal concentrations and their 95% confidence determined using probit analysis (11). Bioassays experiments were conducted in the TBRU (Tropical Biological Research Unit, College of Science) laboratories. Bioassays evaluated by sub-acute NOEL, EC10, EC16, EC50, EC84, EC90, and EC100. Same parameters were performing to LC. These parameters were determined for each exposure period (96, 72, 48, 24 hours) in all concentrations (20). The results were recording at the end of each 24-hour exposure. The numbers of dead snails were removed and recorded at 24, 48, 72, 96 hr. after each application. The end point of dead individuals was considered when there was no movement, no response to stimulation by the glass rod, no recovery after 24 hr. of putting in clean water and lack of the ability to adhere. All recorded results were comparing with the control group for each period of exposure and all concentrations (25).

**Statistical analysis**

Regression analysis depending on the probit units used to calculate different levels of LC and EC by using the provider of SPSS (V. 21) and Biostat (V. 5) programs (15-17). The results corrected by Abbott equation, calculating with two analysis methods included Log of concentration with probit and concentration with percent of response plotted (5).

## RESULTS AND DISCUSSION

### 1- *T. vulgaris* Extracts

**1.1- Escaping activity (Concentrations 5 and 10%) :**The results of Probit Analysis for log concentration –escaping normal distribution showed that the escaping activity of snails is marketing in 24hr. of exposure to (5% and 10%) extracts. Snails exposed to 10% recorded lowest value of escaping activity as 6 (Probit 4.159) and highest value as 17 (Probit 5.168). Snails exposed to 10% recorded lowest value of escaping activity as 7 (Probit 4.272) and highest value as 20 (Probit 5.430). No significant differences recorded between the effect of concentrations on escaping activity (p-value 0.9) Table1. The snails that exposed to 5% and 10% (SS of *T. vulgaris*) extracts was marked the EC10, EC16, EC50, EC84, EC90, and EC100 for 24hours of exposure recorded in Table2. The Clear significant relationship was appearing at 24hr from exposed the snail *B. truncatus* to 5% and 10% of *T. vulgaris* extracts, as it is noticed in probit

analysis of concentration normal distribution (Figure 1).

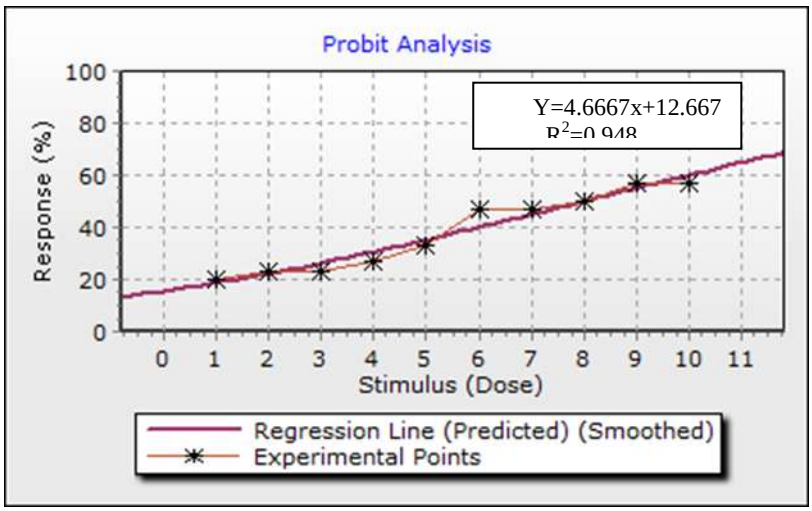
**Table 1. Escaping activity of *B. truncatus* exposed to *T. vulgaris* for 96hr with Probit Analysis - Finney Method (Normal Distribution)**

| Concentrations | Log10 con. | N | Response | %(R) | E(R) | Probit (R) | Chi-square |
|---|---|---|---|---|---|---|---|
| 5% stock solution (SS) | | | | | | | |
| 1 | 0.000 | 30 | 6 | 20.0 | 4.11 | 4.159 | 0.865 |
| 2 | 0.301 | 30 | 7 | 23.3 | 6.91 | 4.272 | 0.001 |
| 3 | 0.477 | 30 | 7 | 23.3 | 8.94 | 4.272 | 0.420 |
| 4 | 0.602 | 30 | 8 | 26.7 | 10.53 | 4.377 | 0.607 |
| 5 | 0.699 | 30 | 10 | 33.3 | 11.82 | 4.570 | 0.282 |
| 6 | 0.778 | 30 | 14 | 46.7 | 12.92 | 4.917 | 0.091 |
| 7 | 0.845 | 30 | 14 | 46.7 | 13.85 | 4.917 | 0.002 |
| 8 | 0.903 | 30 | 15 | 50.0 | 14.67 | 5.000 | 0.007 |
| 9 | 0.954 | 30 | 17 | 56.7 | 15.39 | 5.168 | 0.168 |
| 10 | 1.000 | 30 | 17 | 56.7 | 16.04 | 5.168 | 0.058 |
| 10% stock solution (SS) | | | | | | | |
| 1 | 0.000 | 30 | 7 | 23.3 | 3.96 | 4.272 | 2.328 |
| 2 | 0.301 | 30 | 7 | 23.3 | 7.23 | 4.272 | 0.007 |
| 3 | 0.477 | 30 | 7 | 23.3 | 9.67 | 4.272 | 0.738 |
| 4 | 0.602 | 30 | 7 | 23.3 | 11.58 | 4.272 | 1.814 |
| 5 | 0.699 | 30 | 11 | 36.7 | 13.14 | 4.660 | 0.348 |
| 6 | 0.778 | 30 | 16 | 53.3 | 14.43 | 5.083 | 0.170 |
| 7 | 0.845 | 30 | 16 | 53.3 | 15.53 | 5.083 | 0.014 |
| 8 | 0.903 | 30 | 17 | 56.7 | 16.48 | 5.168 | 0.016 |
| 9 | 0.954 | 30 | 19 | 63.3 | 17.31 | 5.340 | 0.165 |
| 10 | 1.000 | 30 | 20 | 66.7 | 18.05 | 5.430 | 0.212 |

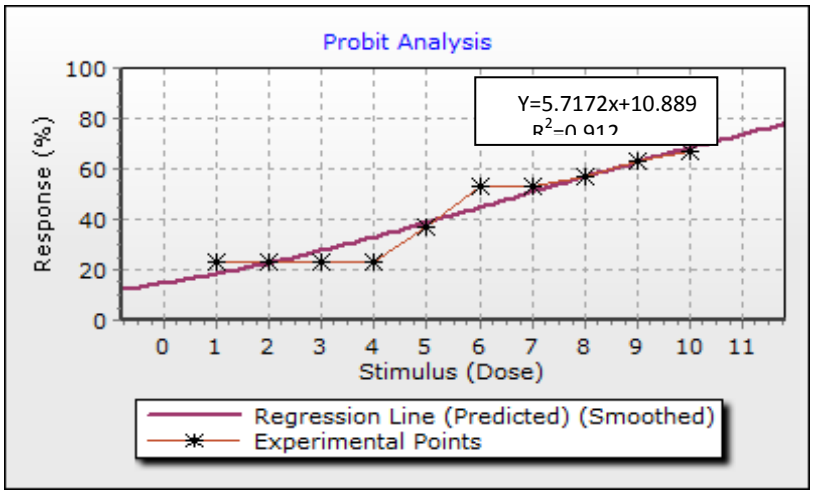
| Parameters | 5%SS | 10%SS |
|---|---|---|
| *Chi-square* | 2.5011 | 5.8119 |
| *Degrees of Freedom* | 8 | 8 |
| *p-level* | 0.9617 | 0.6683 |
| *Alpha value (for confidence interval) 0*.001 | | |

**Table 2. Different EC levels of escaping activity of *B. truncatus* exposed to *T. vulgaris* (Concentration-Response Analysis)**

| Parameters | Values | Parameters | Values |
|---|---|---|---|
| 5%SS | | | |
| *EC10* | -2.1019 | *Beta* | 0.127 |
| *EC16* | 0.116 | *Intercept* | 3.9853 |
| EC50 | 7.9884 | *Beta Standard Error* | 0.0526 |
| EC50 Standard Error | 0.6428 | *EC84* | 15.8609 |
| *EC50 LCL* | 5.8523 | *EC90* | 18.0788 |
| *EC50 UCL* | 10.1246 | *EC100* | 19.7971 |
| 10%SS | | | |
| *EC10* | -1.4963 | *Beta* | 0.1532 |
| *EC16* | 0.3426 | *Intercept* | 3.9475 |
| EC50 | 6.8701 | *Beta Standard Error* | 0.0529 |
| EC50 Standard Error | 0.533 | *EC84* | 13.3975 |
| *EC50 LCL* | 5.0989 | *EC90* | 15.2365 |
| *EC50 UCL* | 8.6413 | *EC100* | 16.6613 |




**A- 5% SS**




**B- 10% SS**

**Figure 1. Regression of Concentration-Response for escaping activity of *B. truncatus* exposed to *T. vulgaris* extracts**

**Mortality Rate (SS 5 and 10%)**

The results of the probit analysis of log concentration, and concentration normal distribution in this study showed that the mortality rate of snails was marketing in 48hr. of exposure to a stock solution (5and 10%) experiments. The lowest value of mortality was 0.25 (Probit 2.605) and the highest value was 6 (probit 5.430). For 10% experiments, the lowest value of mortality was 0.25 (Probit 2.605) and the highest value was 26 (Probit 6.110). The results found significant differences between effects of concentrations on mortality rates (p-value 0.) for both 5 and 10% experiments in Table3. A series of concentrations and their percentile needs to use to achieve the percentile of mortality that recorded in the study was limited in range (3.4-100.2 g/L) of the concentration 5% and (5.3-17.1g/L) of the concentration 10%. The concentration, which needs to achieve 50% mortality, is 18.7g/L for the SS 5% and 9.5g/L for the SS 10%. LC 50 (LCL-UCL) of *T. vulgaris* extracts for SS 5% to the snail *B. truncatus* was 18.7 (12.9-69.4) g/L with standard errors for concentration and log concentration (8.2 and 0.1) respectively. LC 50 (LCL-UCL) of *T. vulgaris* extracts for SS 10% to the snail *B. truncatus* was 9.5 (8.4-15.4) g/L with standard errors for concentration and log concentration (1.2 and 0.05) respectively, Table4. For SS 5%, the study record LC10, 16, 50, 84, 90, and 100 of *T. vulgaris* extracts to snail *B. truncatus* as 7.6, 9.1, 14.4, 19.3, 21.2, and 22.3 g/L respectively. The lower confident level of LC50 was 12.1 while upper one was 16.7 g/L. According to probit analysis for concentration used in the study, the Beta value of regression line was 0.1 with SE 0.08 and intercept 2.2. For SS 10%, the study record LC10, 16, 50, 84, 90, and 100 of *T. vulgaris* extracts to snail *B. truncatus* as 6.3, 7.1, 10, 13, 13.8, and 14.4 g/L respectively. The lower confident level of

LC50 was 8.8 while more than one was 11.3 g/L. According to probit analysis for concentration used in the study, the Beta value of regression line was 0.3 with SE 0.08 and intercept 1.5, Table 4. The Clear significant relationship between *T. vulgaris* extracts and *B. truncatus* response by mortality rates affect was recorded in this study. The results showed increasing of concentration extracts followed by increasing the response represented by mortality rates. As we note in the figure below, increasing the log of concentration led to increasing of the response percent (Figure 2).

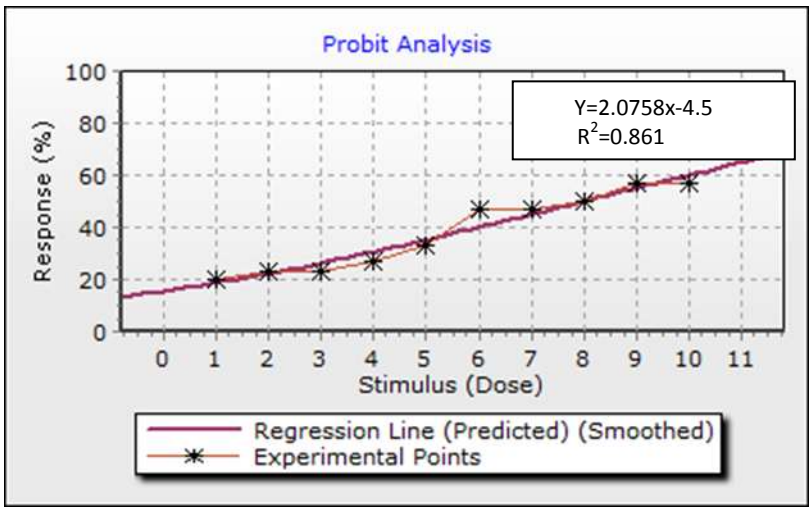
**Table 3. Mortality rates of *B. truncatus* exposed to *T. vulgaris* for 24hr with Probit Analysis - Finney Method (normal Distribution).**

| Concentrations | Log10 con. | N | Response | %(R) | E(R) | Probit (R) | Chi-square |
|---|---|---|---|---|---|---|---|
| | | | 5% stock solution (SS) | | | | |
| 1 | 0.000 | 30 | 0.25 | 0.8 | 0.00 | 2.605 | 1578 |
| 2 | 0.301 | 30 | 0.25 | 0.8 | 0.02 | 2.605 | 2.65 |
| 3 | 0.477 | 30 | 0.25 | 0.8 | 0.29 | 2.605 | 0.005 |
| 4 | 0.602 | 30 | 1. | 3.3 | 1.27 | 3.718 | 2.316 |
| 5 | 0.699 | 30 | 1. | 3.3 | 3.21 | 3.718 | 0.014 |
| 6 | 0.778 | 30 | 1. | 3.3 | 5.92 | 3.718 | 1.444 |
| 7 | 0.845 | 30 | 3. | 10.0 | 9.05 | 4.569 | 0.099 |
| 8 | 0.903 | 30 | 4. | 13.3 | 12.23 | 4.832 | 0.047 |
| 9 | 0.954 | 30 | 4. | 13.3 | 15.24 | 4.832 | 0.329 |
| 10 | 1.000 | 30 | 6. | 20.0 | 17.91 | 5.430 | 0.241 |
| | | | 10% stock solution (SS) | | | | |
| 1 | 0.000 | 30 | 0.25 | 0.8 | 0 | 2.605 | 210217 |
| 2 | 0.301 | 30 | 0.25 | 0.8 | 0 | 2.605 | 99112 |
| 3 | 0.477 | 30 | 0.25 | 0.8 | 0.00 | 2.605 | 1122 |
| 4 | 0.602 | 30 | 0.25 | 0.8 | 0.00 | 2.605 | 7.753 |
| 5 | 0.699 | 30 | 1. | 3.3 | 0.14 | 3.165 | 5.022 |
| 6 | 0.778 | 30 | 2. | 6.7 | 0.94 | 3.498 | 1.170 |
| 7 | 0.845 | 30 | 3. | 10 | 3.21 | 3.718 | 0.013 |
| 8 | 0.903 | 30 | 3. | 10 | 7.17 | 3.718 | 2.429 |
| 9 | 0.954 | 30 | 5. | 16.7 | 12.17 | 4.032 | 4.226 |
| 10 | 1.000 | 30 | 26. | 86.7 | 17.17 | 6.110 | 4.540 |

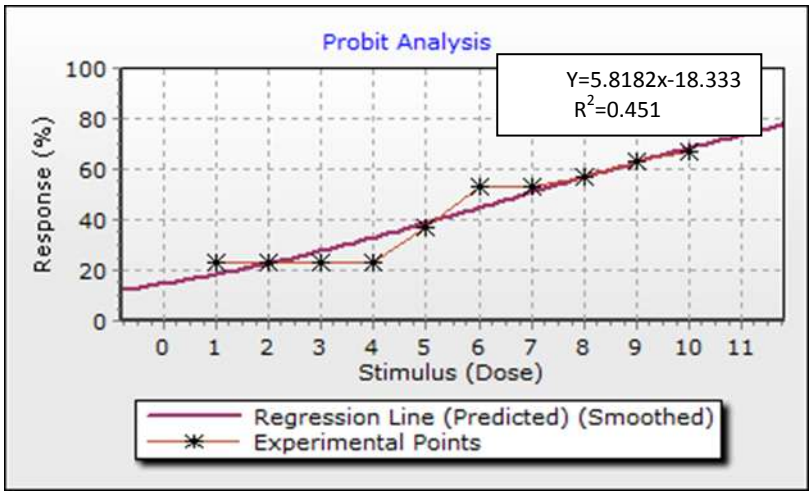
| Parameters | | 5%SS | 10%SS |
|---|---|---|---|
| *Chi-square* | | 87.715 | 21,021,725,048. |
| *Degrees of Freedom* | | 8 | 8 |
| *p-level* | | 0 | 0 |
| *Alpha value (for confidence interval)* | 0.001 | | |

**Table 4. Different LC levels of mortality rates of *B. truncatus* exposed to *T. vulgaris* (Concentration-Response analysis)**

| Parameters | Values | Parameters | Values |
|---|---|---|---|
| | | 5% | |
| *LC10* | 7.6417 | *Beta* | 0.1887 |
| *LC16* | 9.1343 | *Intercept* | 2.276 |
| LC50 | 14.4327 | *Beta Standard Error* | 0.0829 |
| LC50 SE | 0.684 | *LC84* | 19.731 |
| *LC50 LCL* | 12.1247 | *LC90* | 21.2237 |
| *LC50 UCL* | 16.7407 | *LC100* | 22.3802 |
| | | 10% | |
| *LC10* | 6.3639 | *Beta* | 0.3434 |
| *LC16* | 7.1841 | Intercept | 1.5326 |
| LC50 | 10.0958 | *Beta Standard Error* | 0.0851 |
| LC50 Standard Error | 0.3759 | *LC84* | 13.0074 |
| *LC50 LCL* | 8.8275 | *LC90* | 13.8277 |
| *LC50 UCL* | 11.3641 | *LC100* | 14.4633 |




**A- 5% SS**




**B- 10% SS**

**Figure 2. Regression of Concentration-Response for mortality of *B. truncatus* exposed to *T. vulgaris* extracts**

The study found a relationship between the log concentration of *T. vulgaris* used as 10% and escaping activity for the target snail. NOEL values did not consider in the lowest tested concentration (1%) because of marketing the escaping affects. In order to determine the NOEL values, we need to use low concentration than 1% or record the results after the first hours of exposure. As well as, the Threshold of effect is marked between (0-0.01) g/L for both SS-5 and SS-10 g/L experiments. Absence the marketing effect at 48, 72 and 96 hr. of exposure for SS-10%, may explain as losing the snails their ability to escape from the exposure media so that low concentration than 1% must use to record the EC50 values. No marketing of Concentration-response relationship recorded at 48, 72, and 96hr. of exposure for SS-10% because of the killing of all tested snail in treatment.

**2. The snail *B. truncatus* exposed to $CuSO_4$ (SS-1g/Litter)**

**2.1.: EC50 (Escaping activity)**The results of the study showed that the escaping activity of snails is marketing in 24hr. of exposure at the stock solution (1g/Litter) for $CuSO_4$ experiments. Lowest recorded number of escaping activity was 0.25 (probit 2.605) and highest number was 11(probit 4.659). The results of probit analysis of log of Concentration normal distribution cleared little differences between the real value of escaping number (R) that recorded in the study and expected number E (R) calculated according to the analysis. According to Chi-square values, there is a confidence of recorded results. No significant differences between the effects of concentrations on escaping activity (p-value 0.02), Table5. A serial of concentrations and their percentile needs to use to achieve the percentile of escaping activity was recorded in study. These concentrations limited in range (0.1-8.4 g/L) of the concentration 1g/Litter. The concentration, which needs to achieve

50% escaping activity, is 0.9g/L. According to regression analysis, correction must make to concentrations used in the experiments as it appears below with complete the series of concentrations. EC 50 (LCL-UCL) of CuSO4 extracts for SS 1g/L to the snail *B. truncatus* was 0.9 (1.7-0.07) g/L, Table 5. The study found that the EC10, 16, 50, 84, 90, and 100 of CuSO4 to snail *B. truncatus* were 4.3, 3.3, -3.4, -4.4 and -5.1 g/L respectively. The lower confident level of EC50 was 1.6 while more than one was -1.7 g/L, Table 6.

**Table 5. The escaping activity of *B. truncatus* exposed to CuSO4for 96hr with Probit analysis - Finney Method (normal Distribution).**

| Concentrations | Log10 con. | N | Response | %(R) | E(R) | Probit (R) | Chi-square |
|---|---|---|---|---|---|---|---|
| 1% stock solution (SS) | | | | | | | |
| 1 | 0.000 | 30 | 9. | 30 | 14.48 | 4.476 | 2.077 |
| 2 | 0.301 | 30 | 10. | 33.3 | 6.50 | 4.569 | 1.877 |
| 3 | 0.477 | 30 | 11. | 36.7 | 3.36 | 4.659 | 17.35 |
| 4 | 0.602 | 30 | 0.25 | 0.8 | 1.19 | 2.605 | 1.449 |
| 5 | 0.699 | 30 | 0.25 | 0.8 | 1.17 | 2.605 | 0.726 |
| 6 | 0.778 | 30 | 0.25 | 0.8 | 0.75 | 2.605 | 0.340 |
| 7 | 0.845 | 30 | 0.25 | 0.8 | 0.50 | 2.605 | 0.132 |
| 8 | 0.903 | 30 | 0.25 | 0.8 | 0.35 | 2.605 | 0.031 |
| 9 | 0.954 | 30 | 0.25 | 0.8 | 0.25 | 2.605 | 0.001 |
| 10 | 1.000 | 30 | 0.25 | 0.8 | 0.18 | 2.605 | 0.022 |

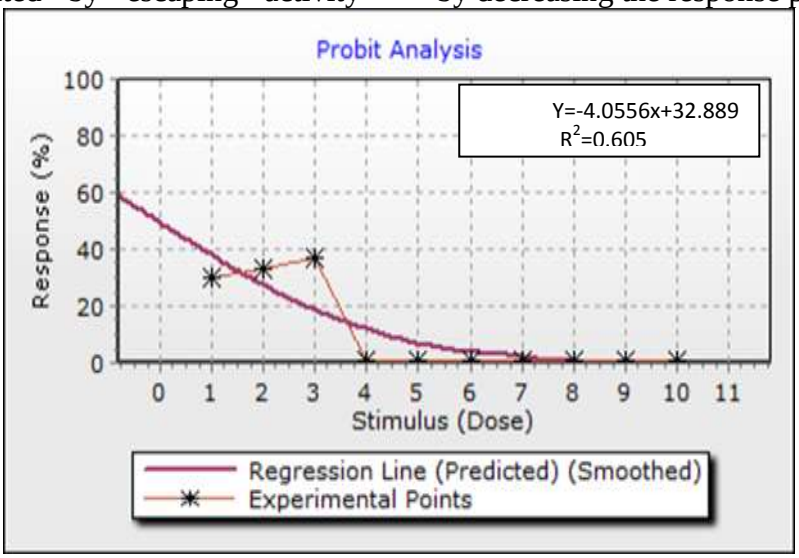
***Chi-square*= 24.0143** ***Degrees of Freedom*=8** ***p-level*=0.0023**

***Alpha value (for confidence interval)*** **0.001**

**Table 6. Different ED levels of escaping activity of *B. truncatus* exposed to CuSO4 (Concentration-Response analysis)**

| Parameters | Values | Parameters | Values |
|---|---|---|---|
| *LC10* | 4.341 | *Beta* | -0.2925 |
| *LC16* | 3.3777 | *Intercept* | 4.9878 |
| LC50 | -0.0416 | *Beta Standard Error* | 0.0811 |
| LC50 SE | -0.5097 | *LD84* | -3.4608 |
| *LC50 LCL* | 1.6931 | *LD90* | -4.4241 |
| *LC50 UCL* | -1.7762 | *LC100* | -5.1704 |

There are clear significant relationship between CuSO4 toxicity and *B. truncatus* response represented by escaping activity affect. The study showed increasing of extracts concentration and log of concentration follow by decreasing the response percent (Figure3).




**1%- SS**

**Figure 3: Regression of Concentration-Response and Log Concentration-Response for escaping activity of *B. truncatus* exposed to CuSO4.**

NOEL values of exposure the *B. truncatus* snail to CuSO4 were marked in concentration (>0.01) for 24-96hr. of exposure. The NOEL is marketing in concentrations less than 0.01g/Litter. Half-treated snails appeared to be able to escape from the exposure media in the concentrations 0.04 at 24hr. of exposure. No ability to escape marketing in the concentrations more than 0.03 (at 24 and 48hr), and 0.02 (at 72hr) of exposure respectively.

**2.2. Mortality rates**

The study showed that the expose of *B. truncatus* to a stock solution of (1g/Litter) CuSO4, the mortality rate was marked in the lowest concentration 0.1% continue increasing to complete death100% in 0.6% after 24hr. of exposure. After 48hr. of exposure, the mortality rate was marked in a concentration 0.1% continue increasing to complete death 100% in 0.5%. After 72hr. of exposure, the mortality rate was marked in a concentration 0.1% continue increasing to complete death 100% in 0.4%. After 96hr. of exposure, the mortality rate was marked in a concentration 0.1% continue increasing to complete death 100% in 0.3%, Table 7. The study found that the LC10, 16, 50, 84, 90, and 100 of *T. vulgaris* extracts to snail *B. truncatus* were -0.6, -0.01, 2.2, 4.5, 5.1, and 5.6 g/L respectively. The Lower confident level of LC50 was 0.8 while more than one was 3.6 g/L. According to probit analysis for concentration used in the study, the Beta value of regression line was 0.4 with SE 0.08 and intercept four, Table8.

**Table7: Mortality rates of *B. truncatus* exposed to CuSO4for 96hr with Probit Analysis - Finney Method (Log normal Distribution).**

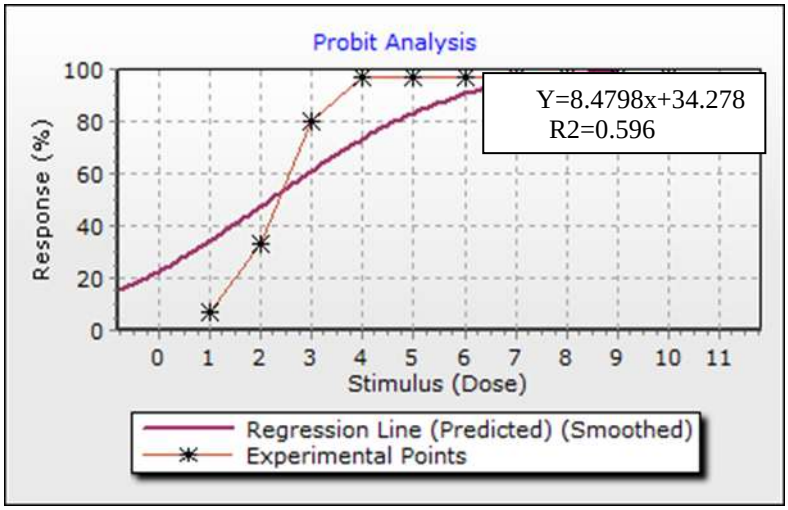
| Concentrations | Log10 con. | N | Response | R% | E(R) | Probit (Y) | Chi-square |
|---|---|---|---|---|---|---|---|
| | | | 1% stock solution (SS) | | | | |
| 1 | 0.000 | 30 | 9. | 30 | 2.83 | 3.498 | 0.244 |
| 2 | 0.301 | 30 | 10. | 33.3 | 13.38 | 4.569 | 0.855 |
| 3 | 0.477 | 30 | 11. | 36.7 | 21.30 | 5.841 | 0.340 |
| 4 | 0.602 | 30 | 0.25 | 0.8 | 25.54 | 6.834 | 0.466 |
| 5 | 0.699 | 30 | 0.25 | 0.8 | 27.67 | 6.834 | 0.063 |
| 6 | 0.778 | 30 | 0.25 | 0.8 | 28.75 | 6.834 | 0.002 |
| 7 | 0.845 | 30 | 0.25 | 0.8 | 29.30 | 6.834 | 0.003 |
| 8 | 0.903 | 30 | 0.25 | 0.8 | 29.60 | 6.834 | 0.012 |
| 9 | 0.954 | 30 | 0.25 | 0.8 | 29.76 | 6.834 | 0.019 |
| 10 | 1.000 | 30 | 0.25 | 0.8 | 29.86 | 6.834 | 0.024 |
| *Chi-square* | | | 1.857 | | | | |
| *Degrees of Freedom* | | | 8 | | | | |
| *p-level* | | | 0.985 | | | | |
| *Alpha value (for confidence interval)* | 0.001 | | | | | | |

**Table 8. Different LC levels of mortality rates of *B. truncatus* exposed to CuSO4 (Concentration-Response analysis)**

| Parameters | Values | Parameters | Values |
|---|---|---|---|
| *LC10* | -0.652 | *Beta* | 0.4429 |
| *LC16* | -0.0158 | *Intercept* | 4.007 |
| LC50 | 2.2423 | *Beta SE* | 0.0873 |
| LC50 SE | 0.4123 | *LC84* | 4.5003 |
| *LC50 LCL* | 0.8145 | *LC90* | 5.1365 |
| *LC50 UCL* | 3.67 | *LC100* | 5.6294 |

There is clear significant relationship between CuSO4 and *B. truncatus* response by mortality rates affect. The study showed increasing of extracts concentration follows by increasing the response represented by mortality rates. As we note in the figure below, increasing of the

log of concentration led to increasing of the percent of Response (Figure4).




**1%-SS**

**Figure 4: Regression of Concentration-Response and Log Concentration-Response for mortality of *B. truncatus* exposed to CuSO4**

The study showed that the complete death of snails exposed to CuSO4 was marked in all periods of exposure. Compete death was marked in concentration contagiously decreased through increasing of exposure time. In a summary of arrangement the effect of tested materials, the study found the scale: CuSO4> *T. vulgaris*. The EC50 of CuSO4, *T. vulgaris* to *B. truncatus* was 8.4 and 0.9respectively. In addition, in a summary of arrangement the toxicity of tested material, the study found the scale: CuSO4> *T. vulgaris*. The LC50 of CuSO4, *T. vulgaris* to *B. truncatus* was 18.7 and 2.2 respectively (Figure 5).

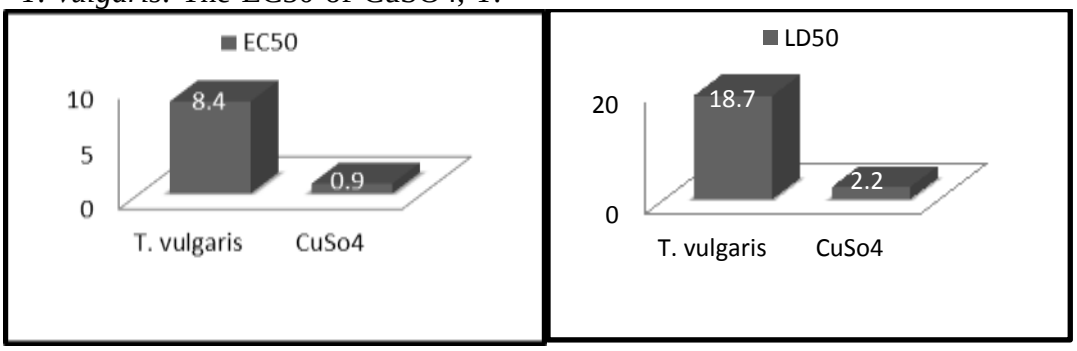



**Figure5. Summary of *T. vulgaris* and CuSO4 EC50 and LC50 against the snail *B. truncates***

Finally, the results of this study agreed with a histopathological study of *T. tetraptera* extract on *Bulinus* (Phyopsis) *globosus*, *Biomphalaria glabrata*, and *Physa waterlotti*. The effect of the extract on various snail tissues found to be time and concentration dependent. The current study was supported by another study which found that the LC50 values of CuSO4·5H2O treatment for 24h, 48h, 72h, and 96h were 2.596, 1.037, 0.690 and 0.400 mg/L respectively. That means increasing of death follow increasing concentrations from one side and increasing of death follow increasing of time of exposure from another side. Clear liner and Semi S-shape relationship between the concentrations of CuSo4 and mortality of tested snails was appearing at exposure with high correlation (4). The death of snails which marked in this study may explain by the mechanism of activity of these extracts that demonstrated by produced significant reductions in the glycogen and protein content and molluscicides action on the carbohydrate metabolism of the snail. As well as the mechanism of activity of extracts on the snails was included registration in many organs as kidney, hepatopancreas, and gastrointestinal tract. Further effects of T. *tetraptera* extracts to *B. glabrata* and *Lymnaea columella* snail as growth and egg production recorded in some

studies ( 29). The molluscicides effect of tested material in this study agreed with a study about molluscicides effect of nicotinanilide that evaluated and compared with niclosamide against different stages of the freshwater snail *Lymnaea luteola* eggs, immature, young mature, and adults and the calculated values of lethal concentration ($LC_{50}$ and $LC_{90}$) (21). In addition, the extracts of *S. officinalis* and *T. vulgaris* are known previously for their antioxidant, anti-inflammatory, antimicrobial, antileishmanial, antimalarial, antiprotozoal, insecticidal and molluscicides activities (16, 18). The subject of study was around the control of snails which depending on elimination or reduction of their population density under an explicit essential NOEL, laid to reduce transmission to a new people infection (13). The study used the molluscicides plant origin because the disadvantages of use synthetic molluscicides as Niclosamide which represented by high costs has the toxic effect to the non-target organism, and need complex organized at application (24).Therefore, we need to natural molluscicides from plants characterized with cheaper, environmentally friendly, biodegradable and immediately offered. Advantages of using the molluscicides plant origin are exhibiting low toxicity for snails' embryos (12). The study has targeted the vector because the snail vector of Bilharzia is characteristic by protective behavior pattern, hermaphroditic, capable of both sexual and asexual and capable of self-fertilization give it an epidemiological importance (6,26). Some studies mention different protective behavioral patterns of snails such ability to escaping of from the exposure media, avoid high Concentrations of toxicant, and enter into the shell (19). In addition, semis of these behavioral patterns were noticing in present study such as attempting to climb the beaker wall, pulling the body into the shell, secreting a protective slime over the aperture, and floating to the top of the containers. Thus, survival of a few individuals of snail can produce a large number of offspring. The study was chosen for these plants because the leave extracts contain saponins, which produce a foam in water causing a coating of the respiratory surfaces like lung and secondary gills which will impair respiration (19). Increasing of EC follows increasing of Concentration. Generally, the study reported that the increasing of stock solution concentration followed by increasing of mortality rates. As well as, the increasing of the period of exposure followed by increasing of mortality rates. The study reported that the complete death of snails was achieved in stock solution (5%) experiments in concentration 5% at 96hr. of exposure but the complete death was recorded in stock solution (10%) experiments with7% concentration at 48hr. of exposure. Therefore we can say there was a significant increase in the mortality rates of snails exposed to tested extracts compared to the control group. This finding agrees with finding which showed a marked reduction in the survival rate of snails treated with concentrations of different plant extracts compared to control (19, 20). According to results of this study, the use of *T. vulgaris* extracts, or their chemical derivative as molluscicides, must turn be applying in endemic areas with taking in account the cost estimates of extraction and requirements of the application? Moreover, continuous through surveillance is important to assess both the density of the snail hosts and the prevalence of Schistosomiasis and using of another plant origin. However, before this achieved, further work is needed to assess and confirm its effectiveness in the field as well as to determine the method of application and its biodegradability. The present work showed that both of *T. vulgaris* extracts were potent to snail of *B. truncatus*. The target snail was additional sensitive to CuSO4. The target extracts are often able to use as molluscicides to the snail of *B. truncatus*. These molluscicides are characterizing by potent, environmentally friendly, low-cost, simply applied, and biodegradable. The utilization of plant molluscicides may be one among the simplest ways that for the management of bilharzia is infections in Third World. The required analysis within the field of plant molluscicides ought to be inspired.

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