

## ***In-vitro* Anti-Leech Effects of *Vitis vinifera* L., Niclosamide and Ivermectin on Mature and Immature Forms of Leech *Limnatis nilotica***

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**Abstract:** The grape (*Vitis vinifera* L.) posses several remedy effects. The methanolic extract of *Vitis vinifera* L., niclosamide and ivermectin were tested as anti-leech *limnatis nilotica*. The mean death time of leeches treated with niclozomide and ivermectin for mature and immature form were 15.4 and 11.2 and 10.1 and 11.2 minutes, respectively. The doses of 300 and 600 mg of methanol extract of *V. vinifera* L. against *L. nilotica* mature were ineffective but they exhibited death time with 260±63 and 200±50 minutes, respectively against the immature form of *L. nilotica*. It is concluded that the methanol extract of grape could be presented as a complementary treatment against *L. nilotica* immature form infestation in future.

**Key words:** Leech • *Limnatis nilotica* • Anti Leech Assay • *Vitis vinifera* L.

### **INTRODUCTION**

Aquatic leeches, particularly *Limnatis nilotica* may enter the body in drinking water. Some leeches may enter the excretory opening of the persons who bathe in infested waters. *L. nilotica* that inhabits lacks and streams of southern Europe, North Africa and the Middle East, attains length of up to 12 cm (4.75 inches). The young specimens are mostly entering the body [1]. Leech can cause haemoptesis, nose pleading, respiratory distress, vaginal bleeding [2-6]. The grape *Vitis vinifera* L. has several remedy effect such as antioxidant property. The compound proanthocyanidin in grape seed posses antioxidant property.

Abri *et al.* [7] showed this compound improve cardiovascular and kidney diseases. Also, in diabetic animals, proanthocyanidin could significantly reduce oxidative damage in brain, liver and digestive tract [7-9]. Also, the proanthocyanidin of grape seed posses similar activity induced by insulin [10]. Proanthocyanidin has protective effect against cataract, breast cancer and colon cancer [11, 12]. Yu *et al.* [13] showed the grape extract could reduce blood lipid concentration in rabbits suffering to hyperlipidemia.

The grape leaf is useful in nephrotoxicity with citrinin, anti-diarrhea and anti-vomiting property [14]. Jung *et al.* [15] showed grape has effective property in primary stage

of cancer. In another study, grape reduces risk of platelet aggregation and arthrosclerosis [16]. It also has indication in treatment of hypertension in human and diminishes protein oxidation in rats [17].

Several study accomplished at treatment of leech for example Bahmani *et al.* [18] evaluated plant extracts of *Quercus brantii*, *Achillea spp.*, *Scrophularia deserti*, *Artemisia kermanensis*, *Artemisia spp* with dose 600 mg for each and they did not give any anti -leech effect. However, *Artemisia spp.* extracts with doses of 1800 and 2400 mg was able to kill the leaches in an average time of 10 h. Moreover, *Artemisia kermanensis* extracts with doses of 1800 and 2400 killed the leeches in time of >10 h and < 4 h, respectively. Also, Farkhondeh *et al.* [19] found that the required time for killing leech (*L. nilotica*) treated with levamisole was 7min.

The effects of used herbal drugs depend on either traditional medicine or test them to know the effective. Therefore, according to the traditional medicine this study aimed to examine methanolic grape extract against leech *L. nilotica*.

### **MATERIALS AND METHODS**

The leaf and fruit of grape were collected from one Vineyard, in around of Dehloran city, East of Iran. In first, the morphological characters of grape were matched with

Table 1: Botanical information of the grape plant.

Scientific name	Family	English name	Persian name	Part use	Type of extract
<i>Vitis vinifera</i> L.	Vitaceae	Grape	Angour	Leaf and Fruit	Methanolic

references and determine species. The selected grape was *Vitis vinifera* L. The full information about this plant presented in Table 1.

In October 2011, methanolic extract of leaf and fruit of grape *V. vinifera* L. was prepared by maceration method. The leaves and fruits is dried in sun and powdered by the mixer (Molinox®, Italy). About 100 g of plant powder was soaked within 300 ml methanol 37% and shaken for 30 min. The methanolic extract incubated for 48 h at 50°C for evaporation of methanol. Finally, dried methanolic extract of *V. vinifera* leaves and fruits were obtained.

Niclosomide tablets (1250 mg) (an antiparasite) were investigated as a control. Also, injectable Ivermectin solution (1%) (Erfan-Daru co., Iran) was used and diluted in 10 ml distilled water.

In autumn of 2011, a total of 56 leeches (*Limnatis nilotica*) were collected from some water wells in Dehloran city in west Iran (Dehloran city, Ilam province).

For the anti-leech assay, the leeches were located individually in a glass container with 600 ml spring water.

The mentioned extract and drugs were diluted and added to 600 ml water and their effects were screened for 720 min and time to paralyze, kill and death of each leech was recorded. The evaluation of death of a leech was based on immobility after stimulation with a needle. The low average paralyzing and killing time of these compounds reflects anti-leech properties [18, 20].

The severity effect of these compounds based on times needed to paralyze or kill the leeches was categorized in five groups as follows:

(1) 4+→ paralysis and death of each leech within 1-60 min after addition of the drug, (2) 3+→ paralysis and death of each leech within 61-120 min after addition of drug, (3) 2+→ paralysis and death of each leech within 121-180 min after addition of drug, (4) 1+→ paralysis and death of each leech within 181-240 min after addition of drug, (5) negative or no effect (0) → paralysis and death of each leech within 241-720 min after addition of drug [18, 20].

The efficacy of the drugs which were able to kill leeches within 1-60 min after addition reflects the anti-leech properties of these compounds and therefore, they may be used in the treatment of infestation with *L. nilotica* in the future [18, 20].

The differences between the control and treated groups were analyzed using one-way ANOVA and Sigma State 2 program.

## RESULTS

The results of this study revealed that the mean death time of mature leechs after treatment with Niclosomide and Ivermectin significantly lower than other groups ( $P<0.05$ ) but there are no significant differences between leechs treated with methanolic extract of grape and Distilled water. The anti-leech activities for treatments on mature *Limnatis nilotica* are shown in Table 2.

In immature leechs, there are significant differences between mean death time after exposure with methanolic extract of grape and other groups e.g. niclosomid, Ivermectin and Distilled water ( $P<0.05$ ) but the lower mean death time showed after exposure with Niclosomide and Ivermectin. The anti-leech activities for treatments on immature *Limnatis nilotica* are shown in Table 3.

## DISCUSSION

The methanolic extract of grape with doses of 300 and 600 mg has no anti-leech effect in mature form of leeches but in immature form of leeches observed partially proper anti-leech effect.

Procyanidin in grape seed leads to latent of endothelial muscles in aorta in human [21]. The ethanol extract of grape leaf prevent contraction of ileum following potassium chloride administration [22]. Also, Grape leaf inhibits contraction of uterus in rates following administration of oxytocine [23,24]. This study shown grape could have anti contractive property for muscles. Probably, the anti-leech effect of grape was for this mechanism.

Bahmani *et al.* [20] studied the anti-leech effects of *Nicotina tabacum* methanolic extract and also some other anti-parasite drugs such as mebendazole, metronidazole, triclabendazole, levamisole, niclosamide and succinylcholine. The results of this study showed that tobacco methanolic extract (600 mg/ml) was able to kill the leaches in an average time of 17 min.

Average death times for other drugs (triclabendazole, levamisole, niclosamide and metronidazole) were found to be 118.66, 7, 18.66 and 541.11 min, respectively.

Eftekhari *et al.* [25], the effects of the methanolic extract of *A. sativum* L. with levamisole and metronidazole as the control drugs were compared and distilled water was evaluated as the placebo group. The average time of paralysis and death of *Limnatis nilotica* for

Table 2: Treatment, Dose (mg), SD  $\pm$  Mean death time in mature leeches

Treatment	Dose (mg/600 ml)	Death time (min)	Severity
<i>Vitis vinifera L.</i>	300	720 $\pm$ 0 <sup>a</sup>	0
	600	720 $\pm$ 0 <sup>a</sup>	0
Niclosamide	625	15.4 $\pm$ 2.1 <sup>b</sup>	+4
Ivermectin	50	11.2 $\pm$ 1.5 <sup>b</sup>	+4
Distilled water	-	720 $\pm$ 0 <sup>a</sup>	0

\*The mean death time presented as Mean $\pm$ SD

<sup>ab</sup>Different words in each column represent existance of significant differences between groups

Table 3: Treatment, Dose (mg), SD  $\pm$  Mean death time in immature leeches

Treatment	Dose (mg/600 ml)	Death time (min)	Severity
<i>Vitis vinifera L.</i>	300	260 $\pm$ 63 <sup>b</sup>	+1
	600	200 $\pm$ 50 <sup>b</sup>	+1
Niclosamide	625	10.1 $\pm$ 1.4 <sup>c</sup>	+4
Ivermectin	50	11.2 $\pm$ 1.6 <sup>c</sup>	+4
Distilled water	-	720 $\pm$ 0 <sup>a</sup>	0

\* The mean death time presented as Mean $\pm$ SD

<sup>ab</sup>Different words in each column represent existance of significant differences between groups

Metronidazole, methanol extract of *Allium sativum L.* and Levamisole was 718.77 $\pm$ 66.3 min, 5.11 $\pm$ 1.76 min and 144.55 $\pm$ 57.217 min, respectively. Distilled water and garlic tablets at a dose of 400 mg were determined as the inert group. In this study it was demonstrated that *Vitis vinifera L.* plant can be effective in killing of leeches immature form and can presented as a complementary treatment in *I. nilotica* infestation.

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