

Comparing of Anti-Leech Effect of *Vitis vinifera* L. Plant and Niclosamide on Different Forms of *Limnatis nilotica*

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Abstract: The grape (*Vitis vinifera* L.) posses several remedy effects. In this study, the anti *limnatis nilotica* effect of methanolic extract of *Vitis vinifera* L. plant as well as niclosamide were investigated by anti-leech assay. Distilled water selected as placebo. The mean death time of leeches in groups treated with niclozomide for mature and immature form were 15.4 and 10.1 minutes, respectively. The treatment groups of *Vitis vinifera* L. methanol extract (300 and 600mg) on *L. nilotica* mature form and distilled water were ineffective but *vinifera* L. methanol extract (300 and 600mg) on *L. nilotica* immature form with the death time with 260±63 and 200±50 minutes, respectively (Effective treatment). The grape methanol extract could be presented as complementary treatment in *Limnatis nilotica* immature form infestation in future.

Key word: Leech • *Limnatis nilotica* • Anti leech assay • *Vitis vinifera* L

INTRODUCTION

Aquatic leeches, particularly *Limnatis nilotica* may enter the body in drinking water. Some may enter the excretory opening of persons who bathe in infested waters. *L. nilotica* which inhabits lackses and streams of southern Europe, North Africa and the Middle East, attains length of up to 12 cm (4.75 inches) but younger smaller specimens are most likely to enter the body [1]. Leech can cause haemoptesis, nose pleading, respiratory distress, haematemesis, vaginal bleeding and voice harchness [2 – 6]. The grape has several remedy effect such as antioxidant property. In grape seed proanthocyanidin posses antioxidant property.

Abri *et al.* (2005) showed this compound improve cardiovascular and kidney diseases [7]. 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 by insulin [10]. The protective effect of proanthocyanidin against cataract, breast cancer and colon cancer was stated [11, 12]. Yu *et al.* (2002) showed the grape extract could reduce blood lipid concentration in rabbits suffering to hyperlipidemia [13].

The grape leaf is useful in nephrotoxicity with citrinin. In herbal references mentioned the anti-diarrhea and anti-vomitting property of grape leaf [14].

The study of Jung *et al.* (2006) showed grape water have effective property in primary stage of cancer [15]. In another study characterized grape water reduces risk of platelet aggregation and arthrosclerosis [16]. The grape water has indication in treatment of hypertension in human. The consumption of grape water diminishes protein oxidation in rats [17].

Several study accomplished at treatment of leech for example Bahmani *et al.* (2010) evaluated *Quercus brantii*, *Achillea* spp., *Scrophularia deserti*, *Artemisia kermanensis*, *Artemisia* spp. extracted with dose 600 mg and results did not had any anti –leech effect but *Artemisia* spp. extracted with doses of 1800 and 2400 mg was able to kill the leaches in an average time of 600 and 601 min. *Artemisia kermanensis* extracted with doses of 1800 and 2400 was able to kill the leeches in an average time of 635 and 188 min [18]. Also, one study by Farkhondeh *et al.* 2011, the average time of killing of leech (*L. nilotica*) for levamisole was determined to be 7min and for garlet tablet were negative severity [19].

The access to treatment effects of herbal drugs is dependent to traditional medicine or test and error methods. For this, in this study according to the traditional medicine, the anti-leech effect of methanolic grape extract was examined.

MATERIALS AND METHOD

The leaf and fruit of grape were prepared in Ilam province, East of Iran. In first, the morphological characters of grape was matched with references and determine species. The selective grape was *Vitis vinifera L.* the full information about this inserted in Table 1.

In October 2011 prepared of leaf and fruit of grape and, the methanolic extract of *Vitis vinifera L.* was prepared by maceration method. First, the leaf and fruit valvated in sun then this plant was powdered by the mixer (Molinox®, Italy) and 100 gram of dried plant powder was soaked within 300 ml methanol 37% in perculator then and was for 48 hours in 50 degree in oven. Finally, dried extract was prepared of *Vitis vinifera L.*

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

The leeches were put individually in a glass container with 600 ml spring water. Then the extract and drugs were added and their effects were screened for 720 min and the time to paralyze and kill each leech was recorded. The examination was repeated for nine repeat. The evaluation of death of leech was based on immobility after stimulation with needle. The low average paralyzing and killing time of these compounds reflect anti leech properties [18, 20].

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

- 4+→ paralyze and death of each leech within 1-60min after addition of drug
- 3+→ paralyze and death of each leech within 61-120min after addition of drug
- 2+→ paralyze and death of each leech within 121-180min after addition of drug
- 1+→ paralyze and death of each leech within 181-240min after addition drug
- Negative→ paralyze and death of each leech within 241-720min after addition of drug [18, 20].

Table 1: botanical information of the grape plant.

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

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

Treatment	Dose(mg)	Mean ± SD	Severity
<i>Vitis vinifera L.</i>	300	720±0	-
grape methanol extract			
<i>Vitis vinifera L.</i>	600	720±0	-
grape methanol extract			
Distilled water	100	720±0	-
Niclosamide	625	15.4	4+

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

Treatment	Dose(mg)	Mean ± SD	Severity
<i>Vitis vinifera L.</i>	300	260±63	1+
grape methanol extract			
<i>Vitis vinifera L.</i>	600	200±50	1+
grape methanol extract			
Distilled water	100	720±0	-
Niclosamide	625	10.1	4+

The efficacy of drugs which could kill the leech within 1-60min after addition and reflect 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 treatment groups were analyzed using one-way ANOVA statistical method by Sigma State 2 software program.

RESULTS

The anti leech activities for treatments on *Limnatis nilotica* are shown in Table 2 and 3. The extract of *Vitis vinifera L.* showed anti *L. nilotica* immature activities. Among the treatments tested, niclosamide and grape methanol extract showed the good anti-Leech activity. The results mentioned in Table 2 and Table 3.

DISCUSSION

In this study, the methanolic extract of grape in dose of 300 and 600 mg has no anti-leech effect in immature form of leeches but in mature form of leeches observed proper anti-leech effect.

Procyanidin in grape seed lead 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 inhibit 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 be for this mechanism.

Bahmani *et al.* (2010) 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 [20].

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.* in 2011, 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 Metronidazole, methanol extract of *Allium sativum* L. and Levamisole was 718.77±66.3 min, 5.11±1.76 min and 144.55±57.217 min, respectively. Distilled water and garlic tablets at a dose of 400 mg were determined as the inert group [25]. 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 *L. nilotica* infestation.

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