

Assessment of *Hyoscyamus niger* seeds alcoholic extract effects on acute and chronic pain in male NMRI rats

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A B S T R A C T

Background and Objective: Recent studies have shown that anticholinergic alkaloids compounds have strong analgesic effects. Due to presence of anticholinergic alkaloids in *Hyoscyamus niger* and its mentioned effects in Iranian traditional medicine, its analgesic effects were assessed. At first, acute and chronic pain thresholds in male NMRI rats with formalin test was evaluated, then oral and injection forms of alcoholic extract of *Hyoscyamus niger* seeds were assessed.

Materials and Methods: Male NMRI rats weighted 300-350 g were randomly selected. Alcoholic extracts of *Hyoscyamus niger* seeds with 500, 1000 and 2000 mg/kg of body weight were injected intraperitoneally (10 rats per group). Also, *Hyoscyamus niger* seeds was prescribed orally with a proportion of 1 to 14 in their standard food to another group of rats (n=8) within 2 weeks. After all, acute and chronic pain were evaluated in control group rats (n=8) and aforementioned rats with formalin test. Moreover its analgesic effects were compared with sodium salicylate.

Results: Statistical analyses show that injection of *Hyoscyamus niger* seeds alcoholic extract with the mentioned dosage reduces the acute and chronic pain induced by formalin test significantly ($P < 0.001$). Also, orally prescribed *Hyoscyamus niger* seeds significantly increased chronic pain threshold.

Conclusion: The results of this investigation revealed that injection of *Hyoscyamus niger* seeds extract with the above dosages have a significant analgesic effect on acute and chronic pain thresholds. Additionally, orally prescribed only affects chronic pain in formalin test, which indicates different mechanisms of parenteral and oral on acute pain.

Key words:

Pain

Hyoscyamus niger seeds rats

1. Introduction

Majority of stimulations which cause injury to body tissues are with pain sensation. Pain is necessary to aware the body from harmful stimulations. Also pain is an important symptom of many diseases and with respect to its quality and localization of pain sensation some of the diseases can be diagnosed and

treated consequently (1). Pain as one of the clinical complications of patients and also variety of causes triggering it, demands different treatment methods had led to a wide and complex field of research (2). Despite different palliative and curative methods which are used widely, still researches are prone to find new and better management routes of this physiological phenomenon. In spite of modern pharmacy and wide range of chemical drugs for pain control, patients

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still face unsuccessful therapy and adverse effects of these agents (3). Using herbal plants their cost effectiveness character and also less side effects and more availability are recommended (4).

Hyoscyamus niger is one of the herbal plants which is introduced as analgesic (5, 6). Investigations performed by researches showed that this plant contain anticholinergic alkaloids tropane (Atropin, scopolamine and hyoscine) (7, 8). Also available reports admit analgesic effects of anticholinergic compounds (9). According to recent studies which showed strong analgesic effects of anticholinergic compounds and presence of these in *Hyoscyamus niger* in a large quantity, it is proposed that this plant especially its seeds which is a great source of these compounds be able to show some analgesic effects. Therefore in this paper it is tried to evaluate interaperitoneal injection and oral prescription analgesic effects of alcoholic extract of *Hyoscyamus niger* and comparing it with a known analgesic drug.

2. Materials and Methods

2.1. Animals

In this paper, NMRI rats weighted 300-350 g were investigated in Medical faculty under normal circumstances (temperature of 2 ± 21 °C, light and darkness rhythm of 12 hours and 30-40% of humidity). 52 rats were divided into cages containing 4 rats. Standard food and water was given without any limitations.

2.2. Extract preparation

In order to prepare alcoholic extract of *Hyoscyamus niger*, after preparing seeds and excluding Impurities, 500 g of seeds was ground. Then was hold in ethanol 96% with a ratio of 1 to 5 in laboratory for 48 hours. After by using small and big filters extract filtrations of mixed was performed. Filtered liquid was concentrated in bain marie with 65 °C. Finally prepared honey like extract was mixed with normal saline to make different concentrations based on mg/kg of rats body weight.

2.3. Preparation of oral form of *Hyoscyamus niger*

In order to prepare special diet, some standard

food was powdered and mixed with pure water. *Hyoscyamus niger* seeds were ground and then mixed with standard food. Mixed formula changed into available form for rats using special instruments for producing foods. Special and standard diet and water was accessible for rats without any limitation. After 2 weeks rats were ready for performing pain tests.

2.4. Materials and Methods

At first rats were divided into groups each includes 6 rats as follows: group 1 control. 2, 3 and 4, receiving different doses of *Hyoscyamus niger* alcoholic extract intraperitoneally Group 5 receiving *Hyoscyamus niger* seeds orally and group 6 receiving the analgesic sodium salicylate drug. Then each rat was weighted. 16 rats were separated into 2 groups. One with standard diet and the other receiving food containing *Hyoscyamus niger*. Proportion of *Hyoscyamus niger* to standard food was 1 to 14 or 6/25.

Because this plant extract has been examined for the first time, we needed to design dose-response curve. In this research three groups of 8 to 10 rats for injection of *Hyoscyamus niger* alcoholic extract were selected. 500, 1000 and 2000 mg/kg of body weight concentrations for intraperitoneal injection were used. At first extract was injected then after 30minutes formalin test was performed.

A group of 8 rats were selected for comparison of *Hyoscyamus niger* analgesic effects with standard sodium salicylate. 300mg/kg of sodium salicylate based on rats body weight was diluted with 0.5 ml of normal saline and was injected intraperitoneally 30 minutes before formalin test.

2.5. Formalin test

In this test, 50 µl of formalin solution (2.5%) was used for inducing pain. Formalin with the mentioned dose was injected subcutaneously in the plantar surface of back foot. Rats were put in plexyglass which has a mirror beneath with a 45 degree in order to observe inside in detail. Pain rating was calculated as follow: if the animal put its injected foot on the surface it gets zero, if hesitates to put its foot on the surface score 1, if raises its foot score 2 and finally if because of pain licks or bites its foot score 3. Formalin test

is performed approximately in one hour. With measuring animal pain every 15 seconds and evaluating average of pain in each minute a score between zero to three is recorded for each minute. Test is completed in 60 minutes with 60 averages documented. It worth noting that control and test groups rats were adapted with the plexyglass 15 minutes before formalin test is done.

2.6. Statistical analysis

Results were calculated based on their averages and standard deviations. For comparing groups, averages together ANOVA was used and for comparing between two groups, Tukey test was

employed.

3. Results

3.1. Effect of oral prescription of *Hyoscyamus niger* seeds on pain threshold induced by formalin test

Statistical calculations indicate that in acute pain (according to figure 1 and table 1) there were no meaningful differences between control and test groups. Furthermore as shown in table 1 and 2, average of chronic pain induced by formalin test in test group which *Hyoscyamus niger* has been prescribed, was 1.86 ± 0.03 lower than 2.12 ± 0.03 in control group ($p < 0.001$).

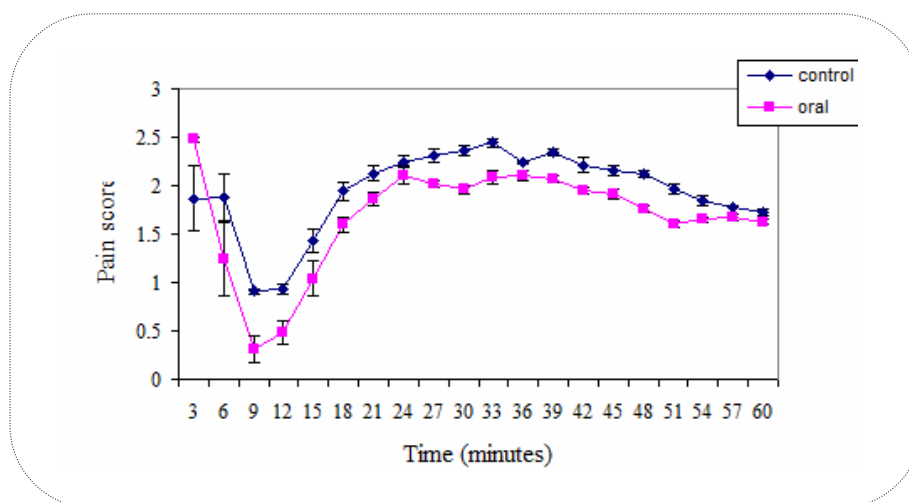


Figure.1. Curves comparing acute and chronic pain in control and treatment with *Hyoscyamus niger* performed with formalin test (n=10 in each group).

Table 1. Comparison of average pain in different groups in acute and chronic phase of formalin test

groups	Acute pain value	Chronic pain value
Control	13/0± 4/1	03/0 ±12/2
Oral <i>Hyoscyamus niger</i>	22/0±1/1	03 /0± 86/1
Injection of <i>Hyoscyamus niger</i> with the dose of 500 mg/kg	14/0 ±55/0	03/0±84/0
Injection of <i>Hyoscyamus niger</i> with the dose of 1000 mg/kg	11/0±39/0	04/0±78/0
Injection of <i>Hyoscyamus niger</i> with the dose of 2000 mg/kg	13/0±24/0	04/0±75/0
Injection of sodium salicylate	19/0±92/0	05/0±06/1

3.2. Effect of alcoholic extract injection in pain threshold induced with formalin

Findings from formalin test first stage (acute) based on figure 2 and figure 3 shows that aforementioned doses were able to reduce acute pain induced by formalin. Statistical analyses indicate

that average amounts of acute pain induced by formalin test in treated group receiving *Hyoscyamus niger* with the dose of 2000 mg/kg was 0.24 ± 0.13 which was meaningfully lower than control group 1.4 ± 0.13 ($p < 0.001$) (Tables 1 and 2). Moreover lower injection doses (500 and 1000 mg/kg) resulted in same consequences.

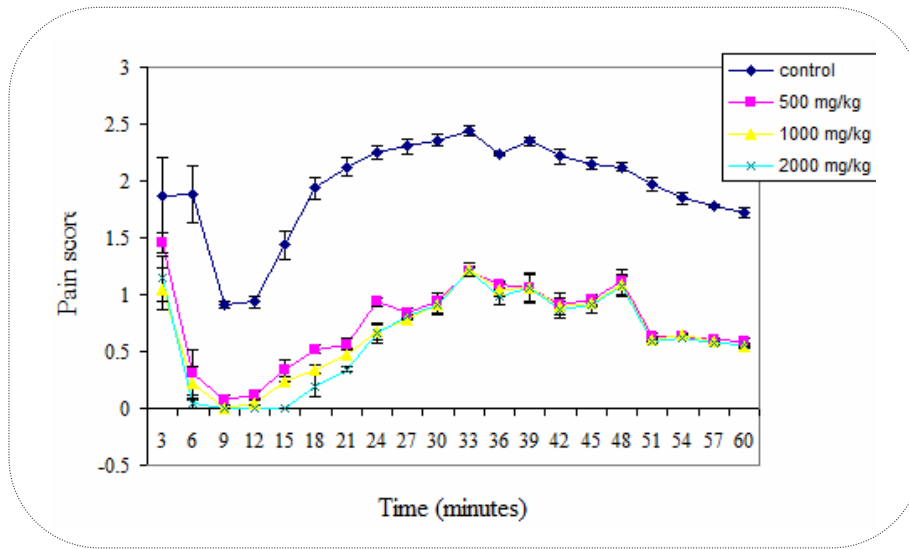


Figure 2. Curves comparing acute and chronic pain in control and treated injected with *Hyoscyamus niger* extract groups (n=10 for each group).

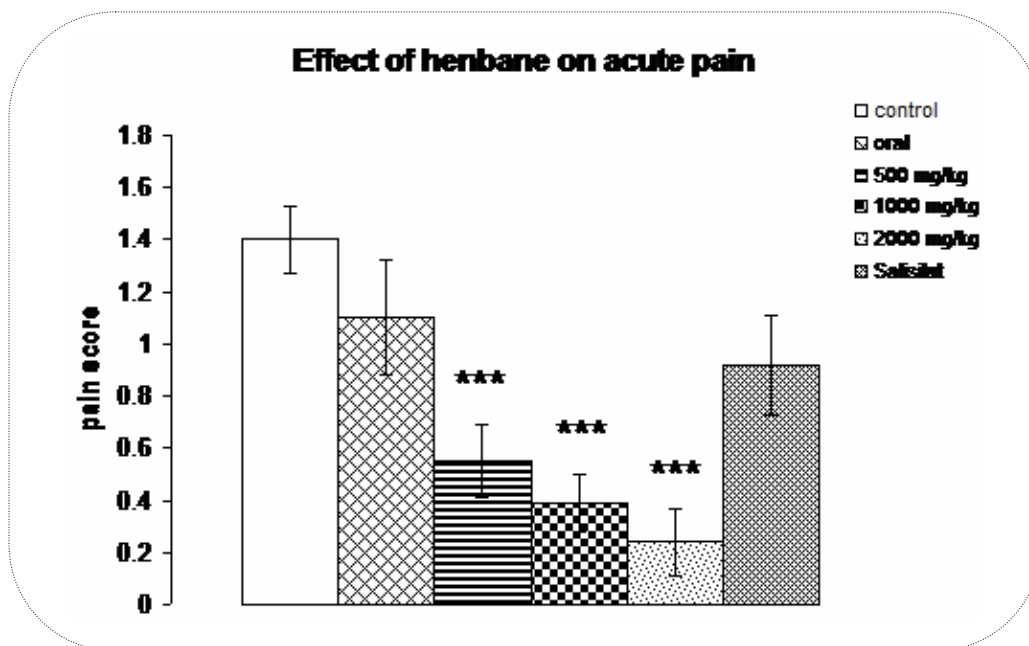


Figure 3. Comparison of acute pain in experiment groups. *** indicates significant difference with control group ($p < 0.001$)

Table 2. comparison of analgesic effects among different groups in acute phase of formalin test

Group 1	Group 2	P value
Control	Oral <i>Hyoscyamus niger</i>	>0/05
Control	Parenteral <i>Hyoscyamus niger</i> 500 mg/kg	<0/001
Control	Parenteral <i>Hyoscyamus niger</i> 1000 mg/kg	<0/001
Control	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	<0/001
Control	Sodium salicylate	>0/05
Oral <i>Hyoscyamus niger</i>	Parenteral <i>Hyoscyamus niger</i> 500 mg/kg	>0/05
Oral <i>Hyoscyamus niger</i>	Parenteral <i>Hyoscyamus niger</i> 1000 mg/kg	<0/05
Oral <i>Hyoscyamus niger</i>	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	<0/01
Oral <i>Hyoscyamus niger</i>	Sodium salicylate	>0/05
<i>Hyoscyamus niger</i> i.p 500mg/kg	Parenteral <i>Hyoscyamus niger</i> 1000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 500mg/kg	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 500mg/kg	Sodium salicylate	>0/05
<i>Hyoscyamus niger</i> i.p 1000mg/kg	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 1000mg/kg	Sodium salicylate	>0/05
<i>Hyoscyamus niger</i> i.p 2000mg/kg	Sodium salicylate	<0/05

Table3. Comparison of analgesic effects among different groups in chronic phase of formalin test

Group1	Group 2	P Value
Control	Oral <i>Hyoscyamus niger</i>	<0/001
Control	Parenteral <i>Hyoscyamus niger</i> 500 mg/kg	<0/001
Control	Parenteral <i>Hyoscyamus niger</i> 1000 mg/kg	<0/001
Control	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	<0/001
Control	Sodium salicylate	<0/001
<i>Hyoscyamus niger</i> i.p 500 mg/kg	Parenteral <i>Hyoscyamus niger</i> 1000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 500 mg/kg	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 500 mg/kg	Sodium salicylate	<0/001
<i>Hyoscyamus niger</i> i.p 500 mg/kg	Oral <i>Hyoscyamus niger</i>	<0/001
<i>Hyoscyamus niger</i> i.p 1000 mg/kg	Parenteral <i>Hyoscyamus niger</i> 2000 mg/kg	>0/05
<i>Hyoscyamus niger</i> i.p 1000 mg/kg	Sodium salicylate	<0/001
<i>Hyoscyamus niger</i> i.p mg/kg	Sodium salicylate	<0/001
Oral <i>Hyoscyamus niger</i>	Sodium salicylate	<0/001
<i>Hyoscyamus niger</i> i.p 1000 mg/kg	Oral <i>Hyoscyamus niger</i>	<0/001
<i>Hyoscyamus niger</i> i.p 2000 mg/kg	Oral <i>Hyoscyamus niger</i>	<0/001

3.3. Effect of intraperitoneal injection of sodium salicylate on pain threshold induced by formalin test

In the performed investigation, regarding table 1, averages of acute and chronic pain in the group treated with sodium salicylate were 0.19 ± 0.92 and 1.06 ± 0.05 . Statistical analyses showed meaningful difference ($p < 0.001$) between sodium salicylate received and control groups in chronic phase of pain induced with formalin test (table 3). Whereas no meaningful difference was seen between two mentioned groups in acute phase of formalin test (table.2)

Comparison between analgesic effects of *Hyoscyamus niger* and sodium salicylate:

Comparison between acute and chronic pain reduction treated with *Hyoscyamus niger* and sodium salicylate and statistical analyses revealed that, not only parenteral *Hyoscyamus niger*, affects on acute and chronic phases of pain induced by formalin test, but also has more

significant and meaningful effects in comparison with sodium salicylate which only affects on chronic phase.

Generally as seen in figure 3, in acute pain induced by formalin test, only i.p injection form of *Hyoscyamus niger* is effective in increasing pain threshold and sodium salicylate doesn't have any effect on acute pain control.

Finally as presented in figure. 4, about chronic pain induced by formalin test except henbane injection, its oral prescription and also sodium salicylate lead to a significant increase in pain threshold.

4. Discussion

Pain as a clinical complication of patients and variety of inducing reasons raises attention in order to more new and different methods of treatment, which has opened a wide filed of research as well (2).

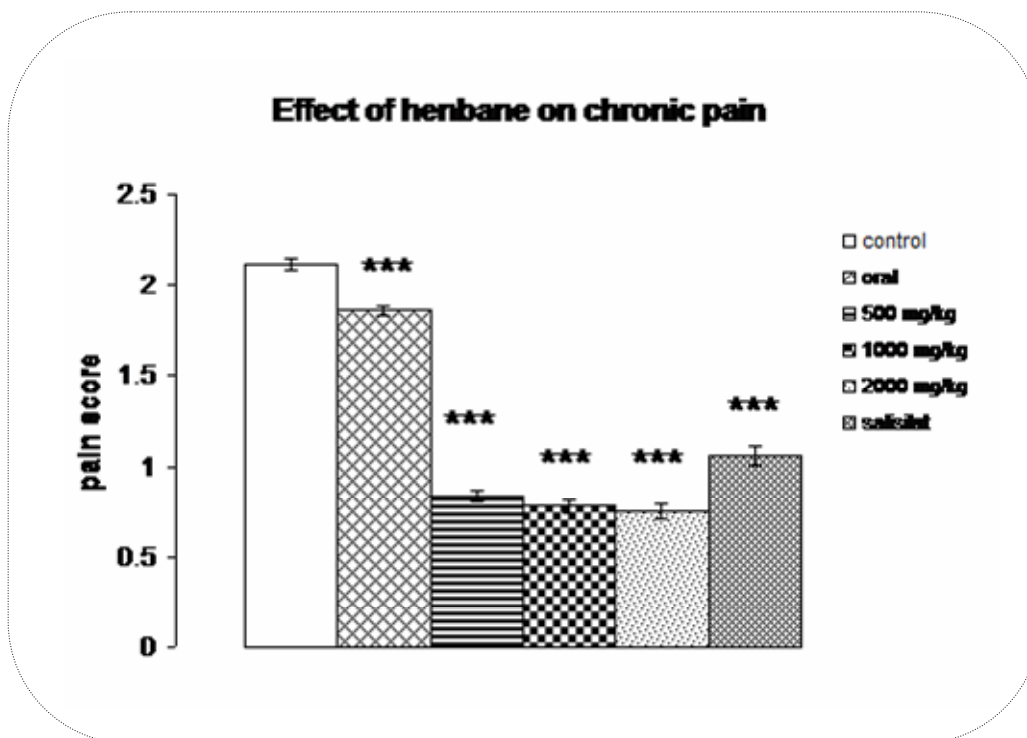


Figure.4. Comparison of chronic pain in experiment groups. *** indicates significant difference with control group ($p < 0.001$).

Despite different routes of alleviating and treating pain which have been used widely, still researches seek for better management of this physiological phenomenon. Moreover modern pharmacy and abundant of chemical compounds in controlling pain, unsuccessful treatment and variety of side effects have put patients in trouble (3). Therapeutic plants because of their cost effectiveness and also their low side effects and more availability are recommended (4). *Hyoscyamus niger* is an effective analgesic herbal plant containing compounds with alkaloids characteristics especially anticholinergic as the most important substance. Using this plant as hallucinogen and narcotizing has been habitual in many societies (10, 11). Whereas some side effects have been reported from consumers of the drug, these include increase of secretory activity of the GI, xerostomia, tachycardia, dysphoria and delirium (12). Investigators reports have shown that these side effects and also hallucination is due to its antimuscarinic compounds (13). In the present study data acquired from formalin test shows meaningful analgesic effects due to prescription of *Hyoscyamus niger* according to the formalin test which is used for both diagnosis of acute and chronic pain, it can be concluded that *Hyoscyamus niger* extract can reduce acute and chronic pain induced by formalin test. Because phasic pain acts mostly via central mechanism (14, 15), results of reduced pain in acute phase of formalin test indicate probable mechanism of the plant via central mechanism. Effect of the plant on the second phase or inflammatory of pain can be due to probable role in modulating peripheral mediators. But based on the reports which an inhibition of second phase (inflammatory) following first phase (acute) cessation has been reported (16), probably extract direct effect in inflammatory phase, is with modulating local pain mediators and indirectly followed by acute pain reduction. About probable mechanism of *Hyoscyamus niger* extract with respect to increasing the analgesic effects of morphine with cholinergic antagonists (17), and this action controversy is done on CNS on the afferent roots of pain (18) and presence of a cholinergic synapse in pain cessation opioid root (19), it can be concluded that presumably *Hyoscyamus niger* alkaloids compounds act with interaction with opioids mechanism to lessen pain. Therefore acute formalin pain inhibition with central mechanism is significant. But with

respect to peripheral pain (chronic) alleviation followed by acute pain or first phase inhibition with central mechanism (16), we can consider central inhibition of acute pain as a reason for chronic pain mitigation. In a study performed on analgesic effects of cholinergic drugs in male rats revealed that these drugs with muscarinic receptors are able to act as spinal analgesics, and it's against our findings which indicate analgesic effects of anticholinergic, this issue may due to drug mechanism in which cholinergic drugs act directly with stimulation of muscarinic receptors while anticholinergic proceed indirectly with interaction with opioid system (20). On the other hand in other study anticholinergic compounds of *Datura Stramonium* plant presented as analgesic which due to similarity of *Hyoscyamus niger* and *Stramonium* compounds is a confirmation for our study (21).

Finally it is concluded from this paper that in formalin pain test, *Hyoscyamus niger* seeds alcoholic extract is able to diminish pain significantly which is probably due to alkaloids compounds of this plant and central interaction with opioids system. To investigate this issue more detailed needs further works about interaction with opioids and cholinergic and the compounds of the plant.

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