Original Article

Antinociceptive activity of methanol extract of *Hyoscyamus reticulatus L.* in mice

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ABSTRACT

In the present study acute toxicity and possible antinociceptive effect of the metanolic extract of *Hyoscyamus reticulatus L.* was investigated in Swiss - albino mice of either sex. To assess *Hyoscyamus reticulatus L.* extract's acute toxicity it was given intraperitonelly to the mice (6 in each group) in dose 50, 100, 200, 400, 800, 1200 and 1600 mg/kg body weight. After probit analyze of mortality at 24, 48 and 72h after administration, 25, 50 and 100 mg/kg were determinate as safe doses for antinociceptive evaluation. Two models were used to study the effects of the extracts on nociception, acetic acid-induced writhing test and hot plate test in mice. *Hyoscyamus reticulatus L.* extract was administered intraperitoneally 30 minutes prior to pain induction. The metanolic extract in 50 mg/kg dose showed significant ($p<0.05$) analgesic activity comparable with diclophenac sodium, evidenced by increase in the reaction time by hot plate method and significant ($p<0.05$) reduction in acetic acid - induced writhings in mice in 100mg/kg dose with a maximum effect of 35.56 % reduction. These effects were compared with the control and standard drug, diclophenac sodium (50 mg/kg, p.o). The results indicate that metanolic extract of *Hyoscyamus reticulatus L.* possesses a significant antinociceptive activity in both central and peripheral pain models in mice and therefore, it can be used as supplemental therapy in acute or chronic pain conditions.

Keywords: Antinociception, Methanol extract, *Hyoscyamus reticulatus* L., Hot plate, Writhing test.

INTRODUCTION

Pain is a protective mechanism for the body, which is one of important health problems because of its prevalence and disabilities it can induce. In fact, every one experiences pain at least once in his lifetime.[1] It is believed that current analgesia inducing drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects and low potency. As a result, the search for other alternatives seems necessary and beneficial.[2]

Medicinal plants are important sources of new chemical substances that potentially have strong therapeutic effects.[3] Natural products of plant origin as alternative sources of drugs are still a major part of traditional medical systems in developing countries. In developed countries as well, the use of traditional plant extract in the treatment of varies diseases has been attracting more attention because of its minor side effects, especially to treat pain processes.[4]

Hyoscyamus reticulatus L. (Solanaceae), commonly known as henbane, is a medicinal plant rich in tropane alkaloids, mainly hyoscyamine and scopolamine, widely used for their mydriatic, antispasmotic, anticholinergic, sedative and analgesic properties. In addition, the plant has traditional uses and claimed benefits in asthma, gastric ulcers and Parkinson's disease, however, erroneous use of this plant would be toxic.[5] Other secondary metabolites detected in Hyoscyamus species include: flavonoids, chlorogenic acid, tannins and coumarins.[6] Plant materials which contain tannins, alkaloids, flavonoids, and phenolic acids bring out antinociceptive effects on experimental animals.[7-8] The purpose of the present study is to determine the antinociceptive effects and acute toxicity of *Hyoscyamus reticulatus L.*

MATERIALS AND METHODS

Animals

The study was carried out on Swiss - albino male mice (30-40 g), maintained under standard laboratory conditions of food and water. Animals were housed at room temperature of 22 ± 2 °C with 12h light / dark cycle. The animals were housed in groups for a minimum of 3 days prior to pharmacological experiment. The experimental protocols have been approved by the Local Ethical Committee on Animal Experimentation of the Yuzuncu Yil University, Van, Turkey. The minimum number of animals and duration of observation required to obtain consistent data were employed. After experiment was completed animals were kept under observation for 7 days for acute or subacute toxicity.

Plant Material

Specimens of *Hyoscyamus reticulatus L.* were collected from from Bulanik, Mus (eastern Turkey) in May 2010. The taxonomic identity of plant was confirmed in Faculty of Biology, Yuzuncu Yil University, Van, by Assist Prof. Dr. Fazlı Öztürk. Aerial parts of plants were dried in shed at room temperature, ground and kept in amber glass bottles.

Preparation of the metanolic extract

Dried aerial parts of plant were extracted with methanol in a soxhlet extractor for 48 h. Methanol at the end of the extraction method purged by rotary evaporator. The aqueous extract was then filtered, concentrated under reduced pressure and finally freeze-dried at -40°C. The yield of this process was 17.2 %. To assess acute toxicity of obtained extract, it was dissolved in 1% Tween 20 solution just before use and given orally to mice at different doses.

Experimental Design

Acute toxicity

Swiss albino mice of either sex were housed two animals per cage for one week to acclimatize. An approximate LD50 was initially determined in a pilot study using a small number of mouse (2 for each dose) and increasing doses of *Hyoscyamus reticulatus L.* Seven doses were then chosen for the determination of intraperitoneal LD50 in mice. Mice were then randomly divided into eight groups of 6 animals each. Group I served as control (normal saline 0, 2 ml per animal), and the other seven groups were intraperitoneally treated with 50, 100, 200, 400, 800, 1200 and 1600mg/kg body weight single dose of metanolic extract of *Hyoscyamus reticulatus L.* The mortality in each group was assessed at 24, 48 and 72h after administration of *Hyoscyamus reticulatus L.* extract and analyzed by probit method. The methanol extract of *Hyoscyamus reticulatus L.* was toxic at doses above 200 mg/kg. Based on probit analyze 25, 50 and 100 mg/kg body weight of metanolic *Hyoscyamus reticulatus L.* extract were chosen for assessment of antinociceptive effect.

Antinociceptive activity

Five groups of male and female Swiss albino mice were used to evaluate antinociceptive effect of *Hyoscyamus reticulatus L.* Control group (Group I) treated with normal saline, group II treated with diclophenac sodium (50 mg/kg) as standard drug and groups III, IV and V, treated with extract solution (25, 50 and 100 mg/kg of *Hyoscyamus reticulatus L.* metanolic extract respectively). All drugs were administrated intraperitoneally, half an hour before the onset of pain stimulus in different models of nociception in albino mice.

Hot Plate Test

Hot Plate analgesia meter (Commat Ltd., Turkey) was used to determinate the central component of nociception. Mice were placed individually on a hot plate set to 52.5 ± 0.5 °C and the time between placement of the mouse on the platform and shaking or licking of the paws or jumping was recorded as the reaction time or latency of the pain response. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 30 sec (cut-off time). Hot plate test was performed on all animals individually in 30^{th}, 45^{th} and 60^{th} minutes after treatment.

Writhing Test

Acetic acid – induced writhing test was used to evaluate the antinociceptive activity against chemical noxious stimulus and peripheral analgesic activity of herbal extract. Abdominal contractions were induced by 0.6 % acetic acid solution (15 ml/kg, i.p.) in mice pretreated with normal saline, diclophenac sodium or metanolic extract of *Hyoscyamus reticulatus L.*. Five minutes after the injection of acetic acid, the number of abdominal contractions and stretches during the following 10 min was counted. Writhing movement was accepted as contraction of the abdominal muscles together with stretching of the hind limbs. Antinociceptive effect was expressed as the reduction of the number of writhing between control and pretreated mice. The percentage of the inhibition of writhes was calculated as:

% Inhibition of writhes = (Control mean - Test mean / Control mean) x 100.

Statistical analysis

Experimental data from hot plate and acetic acid-induced writhing tests were expressed as mean ± SEM. Differences between given sets of data were considered to be statistically significant when p value was less then 0.05. Results were statistically

evaluated using Kruskal-Wallis and Mann-Whitney U test.

RESULTS

Antinociceptive activity

Hot plate in mice

In the hot-plate test the extract considerably increased the animal's reaction time to the heat stimulus. Table 1 shows the results of the hot plate test. We observed that *Hyoscyamus reticulatus L.* has antinociceptive activity. Values were found to be significant ($P<0.05$) at 60 min after treatment with 50 mg/kg of *Hyoscyamus reticulatus L.* methanol extract. Moreover, in this dose exhibited antinociceptive effect similar to diclophenac sodium. Values measured 30 and 45 minutes after extract was given were statistically insignificant ($p>0.05$).

Writhing test

The methanol extract of *Hyoscyamus reticulatus L.* administered orally at different dose (25, 50 and 100 mg/kg). The administration of extract in dose 100 mg/kg caused a significant reduction in the number of writhing episodes induced by acetic acid compared to the control ($p<0.05$). The results are shown in Table 2. However, the extract did not exhibit antinociceptive effect as potent as diclophenac sodium.

DISCUSSION

The antinociceptive effect of intra peritoneally administered methanol extract of *Hyoscyamus reticulatus L.* leaves was demonstrated in this study by two different test (chemical and thermal nociceptive test). The present study showed that the antinociceptive effect of *Hyoscyamus reticulatus L.* extract in different nociseptive responses generated by a chemical or thermal noxious stimulus. The extract (50 mg/kg) has been effective in the hot-plate test (Table 1). However, tested extract has also been effective in the writhing test (100 mg/kg), (Table 2), which is used to screen for both peripherally and centrally acting agents.[9] Diclophenac sodium, used as a reference drug, also produced a significant antinociceptive effect during all the observation times when compared with control values.

The hot plate test are widely used for assessing central antinociceptive activities.[10-11] The validity of this test has been shown even in the presence of substantial impairment of motor performance.[12] This indicates that this extract is effective against acute phasic pain and antinociceptive effect is mediated centrally at the supraspinal level.[13] The present study findings indicate that the methanol extract of *Hyoscyamus reticulatus L.* may be centrally acting.

The acetic acid-induced writhing model is a sensitive test widely used for the evaluation of peripheral antinociceptive activity.[14-15] However, it can be seen as a general non-selective model for antinociceptive studies, since acetic acid indirectly induces the release of endogenous mediators (like bradykinin, serotonin, histamin, substance P and prostaglandins), stimulating the peripheral nociceptor and sensitive neurons that were sensitive to the inflammatory mediators.[14,16-17] In acetic acid-induced writhing the processor releases arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis plays a role in the nociseptive mechanism.[18] In this study show that 100 mg/kg of the *Hyoscyamus reticulatus L.* extract produced significant analgesic effect and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolite.

CONCLUSION

The present study indicates that the methanol extract of *Hyoscyamus reticulatus L.* exhibited central and peripheral

antinociceptive effects. However, the mechanisms behind the central and peripheral antinociceptive activity of *Hyoscyamus* reticulatus *L.* are not completely understood and may need further studies with different antagonists (such as serotonergic, adrenergic, etc.). Taking findings into account, it seems quite possible that *Hyoscyamus reticulatus L.* contains constituents with antinociceptive activity, which may lead for the development of new natural products having analgesic effect. In further investigations, the different fractions of *Hyoscyamus reticulatus L.* will be evaluated and the structural characterization of responsibl components will be clarified.

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Table 1. Effect of the methanol extract of *H. reticulatus* leaves on the latency time of mice submitted to the hot plate test.

Treatment	Dose (mg/kg/ip)	Latency time (s)		
		30 (min)	45 (min)	60 (min)
Control		8,18±3,65	9,78±1,49	8,15±2,06[a]
Diclofenac sodium	50	12,15±3,06	12,28±2,19	12,58±2,39[b]
H. reticulatus L.	25	7,17±2,96	8,16±2,79	9,67±1,83[a]
	50	9,53±1,37	10,78±3,11	12,85±2,51[b]
	100	8,67±1,09	9,68±2,07	8,25±2,79[a]

Each group represent the mean ± S.E.M. for six animals (n=6).
[b] Statistically different from control group, P<0.05

Table 2. Effect of the methanol extract of H. reticulatus leaves on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg/ip)	Number of writhing	Inhibition (%)
Control		24,83±6,71[a]	
Diclofenac sodium	50	10,67±3,33[b]	57,03
H. reticulatus L. extract	25	21,33±2,94[a]	14,4
	50	19,33±2,16[a]	22,15
	100	16,00±3,29[c]	35,56

Each group represent the mean ± S.E.M. for six animals (n=6)
[b] Statistically different from control group, P<0.05
[c] Statistically different from diclophenac sodium and control groups, P<0.05