ORIGINAL ARTICLE

EXPERIMENTAL INVESTIGATIONS ON THE EFFECTS OF *BIDENS TRIPARTITA* EXTRACTS IN NOCICEPTIVE REACTIVITY

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Abstract

Bidens tripartita (B. tripartita), a flowering plant belonging to the *Bidens* genus, *Compositae* family, *Asteroideae* subfamily, has been widely used in traditional medicine for its antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic and sedative effects. The present study investigated the effects of two *B. tripartita* extracts in somatic nociceptive reactivity in rats. The experiment was carried out on 4 groups of 6 Wistar rats each, treated intraperitoneally for 1 month with: group I (control): 0.5 mL/100g body weight saline solution; group II (BT-alcoholic): 200 mg/kg b.w. *B. tripartita* alcoholic extract; group III (BT-aqueous): 250 mg/kg b.w. *B. tripartita* aqueous extract, group IV (KET): 10 mg/kg b.w. ketoprofen. The nociceptive cutaneous testing was performed using the tail flick assay, to determine the latency time response to tail thermal noxious stimulation. The chronic use of BT-alcoholic, but not of BT-aqueous produced a statistically significant increase of the latency time response ($p < 0.05$), compared to control in the tail flick test. Its effects were less intense than those of KET. The administration of BT-alcoholic extract showed analgesic effects in tail flick test in rats.

Rezumat

Bidens tripartita (B. tripartita) (BT) este o plantă erbacee din genul *Bidens,* familia *Compositae,* subfamilia *Asteroideae*, folosită în medicina tradiţională pentru efectele sale: antiseptic, antiinflamator, antioxidant, astringent, diuretic, febrifug, narcotic şi sedativ. Studiul de faţă a urmărit investigarea efectului a două extracte de *B. tripartita* asupra reactivităţii nociceptive somatice la şobolani. Experimentul s-a desfăşurat pe 4 loturi a câte 6 şobolani Wistar, care au fost trataţi intraperitoneal timp de o lună, astfel: lot I (martor): 0.5 mL/100g corp ser fiziologic; lot II (BT-alcoolic): 200 mg/kg corp extract alcoolic de *B. tripartita;* lot III (BT-apos): 250 mg/kg corp extract apos de *B. tripartita*; lot IV (KET): 10 mg/kg corp ketoprofen. Testarea sensibilităţii nociceptive cutanate s-a efectuat cu ajutorul testului *tail flick*, prin determinarea latenţei răspunsului la stimularea termică nociceptivă a cozii. Administrarea cronică de BT-alcoolic, însă nu şi de BT-apos, a indus o creştere semnificativă a latenţei timpului de răspuns ($p < 0.05$), comparativ cu lotul martor. Efectele sale au fost însă mai puţin intense decât ale lotului cu KET. Administrarea extractului BT-alcoolic a evidenţiat efecte analgezice în testul *tail flick* la şobolani.

Keywords: *Bidens tripartite* (BT), tail flick test, analgesic effect

Introduction

Adaptogens are particular herbs and natural remedies which have been used for a long time by traditional medicine for helping the body to adapt to the environment, treating different forms of stress and other various pathological conditions. The adaptogens possess certain characteristics: non-specific effect (increasing the body resistance to a wide range of physical, chemical and biological factors), normalizing effect, regardless of the pathological state, lack of toxicity and safety for administration [2, 3, 15]. Scientific studies have proved that adaptogens make the response to environmental stress less damaging, helping the body to maintain homeostasis in different situations by regulating its adaptive reactions. The exact mechanism of action of the adaptogens is unclear so far, due to the involvement of numerous vegetal active principles. Generally, all adaptogens are potent antioxidants and they are reported to improve body endurance and induce effects associated with stress reduction, such as regulating sleep disturbances and enhancing physical performances [2, 3, 15].

Bidens tripartita (B. tripartita) an adaptogen commonly known as *Three-lobe Beggarticks, Three-part Beggarticks, Trifid Bur-marigold*, belonging to the genus *Bidens*, family *Compositae*, subfamily *Asteroideae* [13]. Literature data show

that *B. tripartita* contains a significant amount of active principles, such as: flavonoids, xanthophylls, volatile oil, acetylene and polyacetylene, sterols, aurones, chalcones, caffeine and tannins [6, 8]. This plant has been widely used in traditional medicine for its antiseptic, anti-inflammatory, antioxidant, astringent, diuretic, febrifuge, narcotic and sedative effects [7, 19]. The aim of this study was to investigate of the effects of two *B. tripartita* extracts in somatic nociception in rats.

Materials and Methods

In our study there were used ethanolic and aqueous extracts from *Bidens tripartita L.*, which was taxonomically identified and authenticated by botanical specialists. The plant was harvested during its flowering stage through July and August of the years 2009, 2010 and 2011 from the Ciric area (Iasi County, Romania), from places where it naturally grows. The flowers of *Bidens tripartita L.* were grinded to fine powder and processed by repeated maceration to obtain the aqueous, respectively ethanolic extract; subsequently, the extraction was followed by evaporation, until the concentrated extracts were obtained [20]. The retained doses of *B. tripartita* extracts administered were 1/20 of the lethal dose 50 (LD50). The experiment was carried out on 4 groups of 6 Wistar rats each (180-200 g), treated intraperitoneally for 1 month: group I (control): 0.5 ml/100g body weight saline solution; group II (BT-alcoholic): 200 mg/kg b.w. *B. tripartita* alcoholic extract; group III (BT-aqueous): 250 mg/kg b.w. *B. tripartita* aqueous extract; group IV (KET): 10 mg/kg b.w. ketoprofen. The propionic acid derivative ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) with a known analgesic effect in this somatic pain model in rats. The animals were housed under standard laboratory conditions (relative humidity 55 - 65%, room temperature 23.0 ± 2.0°C and 12 hours light: dark cycle). The animals were fed with standard diet and water *ad libitum*, except during the time of the experiment. Before the experiment, the rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

Antinociception was assessed using the tail flick test (Panlab Hardvard Apparatus). Rats were positioned on a flat surface and held gently by the operator. Tail withdrawal latencies were recorded in response to heat from a light beam focused on the dorsal surface of the tail (approximately 2 cm from the tip) [1, 14, 18]. When the animal flicks its tail, the light beam activates the photocell, closing a switch, which turns off the heat source. The amount of time taken for the animal to move its tail away from the heat was recorded, representing the latency period response [10, 12]. The baseline latency (before drug injection) in the tail flick test was 4.2 ± 0.2 seconds (mean ± standard error of mean - SEM). The recommended cut-off time of 12 seconds was used to prevent tissue damage. Latency time response was measured 15, 30, 60, 90 minutes after substances administration [4, 5].

Differences between the experimental and baseline latencies are interpreted as an index of analgesia. Increases in the latency of the rat to flick its tail are indicative of analgesia, while decreases in tail flick latency indicate hyperalgesia [9]. Response latency data from tail flick measurements were converted to per cent of maximum possible effect (% MPE) according to the formula:

$$\% \text{ MPE} = [(\text{observed latency} - \text{baseline latency}) / (\text{cut off time} - \text{baseline latency})] \times 100, [18].$$

The results of the tail flick response from each group were calculated as mean ± SD and significance was assessed using the ANOVA test implemented in *SPSS 13 Statistics* software. P-values less than 0.05 were considered statistically significant comparing with those of control group. The experimental protocol was implemented according to recommendations of the "Grigore T. Popa" University of Medicine and Pharmacy Committee for Research and regulations of the International Association for the Study of Pain, which meet the ethical standards of the European Community [21, 22].

Results and Discussion

Statistical analysis of the results obtained in the tail flick test showed that the administration of KET 10 mg/kg b.w. determined a rapid and statistically significant increase of the latency time period of the response in tail flick test ($p < 0.01$) (Figure 1).

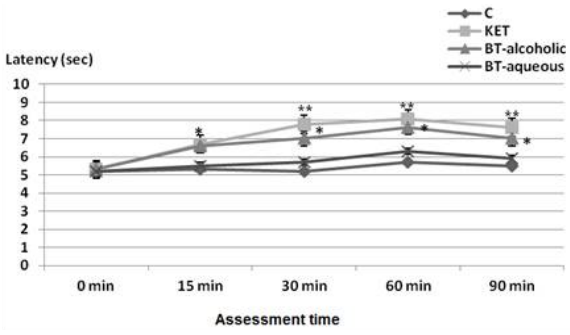

Figure 1.
The latency time response of KET, BT-alcoholic, BT-aqueous to thermal noxious stimulation in tail flick test
(n = 6, *p < 0.05, **p < 0.01 *vs.* Control group)

The administration of BT-alcoholic determined a prolongation of the pain reaction latency time, beginning after 15 minutes ($p < 0.05$), with a peak effect after 60 minutes ($p < 0.01$), and maintained for 90 minutes ($p < 0.01$) in the experiment. The effects were less intense than those of KET in this somatic pain model in rats (Figure 1). The chronic treatment with BT-aqueous extract showed a slight increase of the latency period, statistically non-significant compared to control in the performed experiment (Figure 1).

Additionally, we used the percentage of maximum possible effect (%MPE) to quantify the intensity of antinociception, which is an important tool to corroborate and confirm the previous results. KET 10mg/kg b.w. determined an increase in % MPE in the tail flick test, statistically significant within the time interval 15-90 minutes.

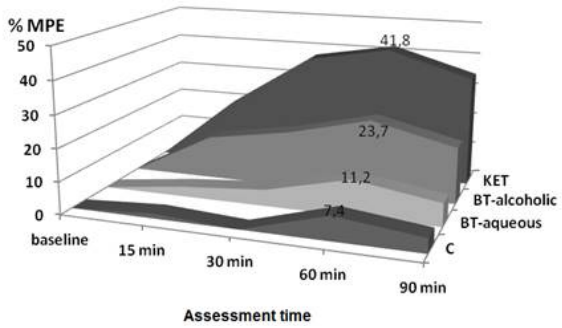

Figure 2.
Time course of the maximum possible effect (% MPE) of KET, BT-alcoholic, BT-aqueous in tail flick test (n = 6)

The maximum antinociception was registered after 60 minutes (% MPE_{60} = 41.8 ± 0.9%), statistically significant compared to the % MPE_{60} (7.4 ± 1.2%) of the control group (Figure 2). Intraperitoneal chronic administration of BT-alcoholic extract produced an increase in % MPE statistically significant after 15 minutes, with a maximum antinociception observed after 60 minutes (% MPE_{60} = 23.7 ± 1.4%) in the experiment. The BT-aqueous extract maximum antinociception was less intense compared to the % MPE of BT-alcoholic extract at all assessed intervals of time in this somatic pain model in rats (Figure 2).

In our previous investigations we demonstrated that these *B. tripartita* extracts are relatively toxicologically safe, when they were administered intraperitoneally in rodents, also displaying good *in vivo* biocompatibility [16, 17].

Conclusions

The chronic administration of BT-alcoholic extract, but not of BT-aqueous extract showed significant antinociceptive effects that started after 15 minutes, with a maximum intensity after 60 minutes and prolonged for 90 minutes in the tail flick assay. The analgesic effects of BT alcoholic extract were less intense than those of ketoprofen, used as a reference drug in this somatic pain model in rats. BT-aqueous extract did not display antinociceptive effects in the same experimental model. We can conclude that the alcoholic extract of *Bidens tripartita* proved an analgesic effect in the tail flick test in rats.

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