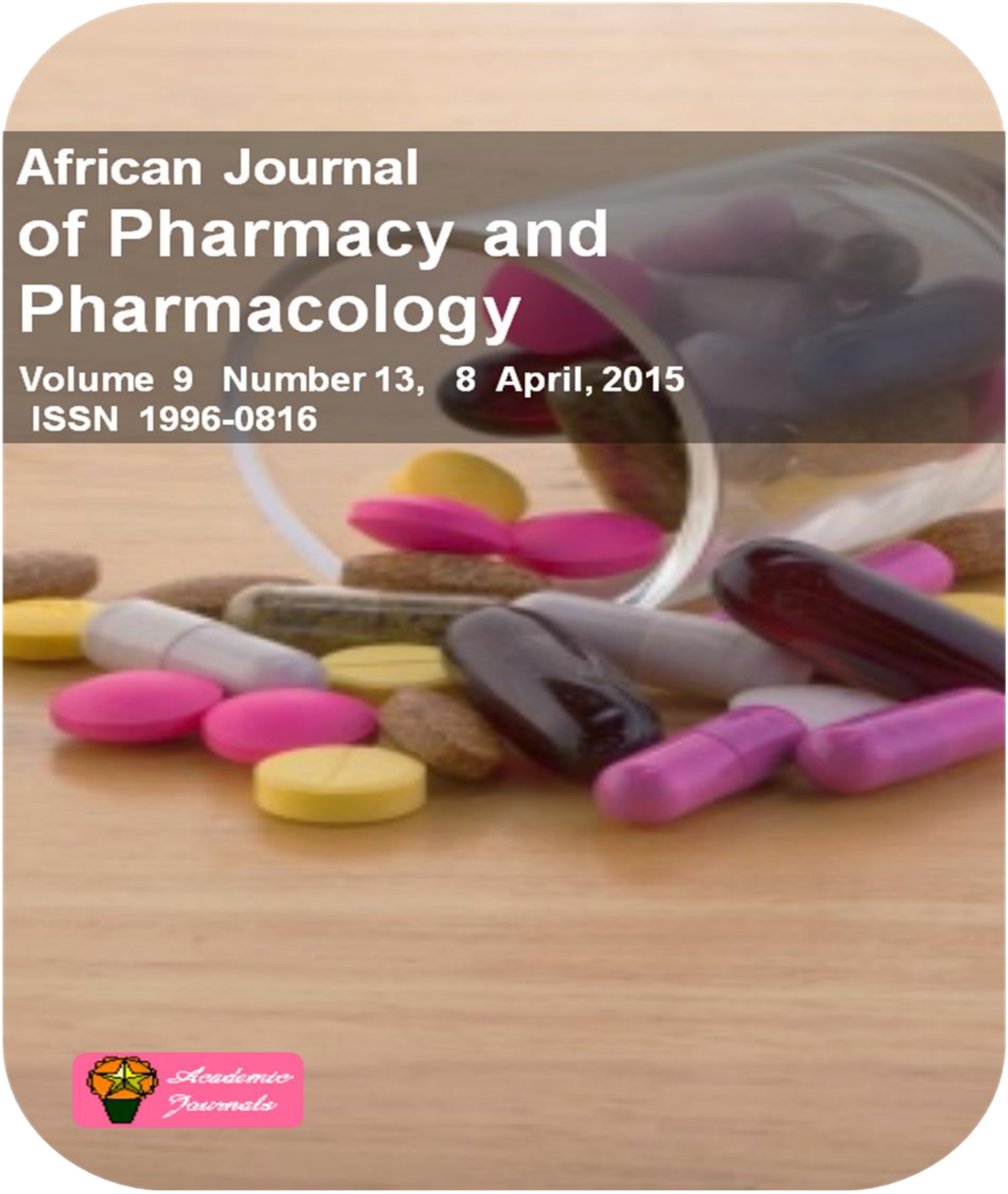

African Journal of Pharmacy and Pharmacology

Volume 9 Number 13, 8 April, 2015
ISSN 1996-0816

Academic Journals

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPP are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: ajpp@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The African Journal of Pharmacy and Pharmacology will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Editorial Board

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 9 Number 13 8 April, 2015

ARTICLES

Research Articles

academicJournals

Vol. 9(13), pp. 451-455, 8 April, 2015
DOI: 10.5897/AJPP2014.4146
Article Number: 60EA9BA52699
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluation of ethanolic extract of *Portulaca tuberosa* Roxb leaves for antipyretic activity

Bhuvnesh Kumar Singh*, Neelanchal Trivedi and Anuj Agarwal

Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad P.O Box: 244001, Uttar Pradesh, India.

Received 23 July, 2014; Accepted 25 March, 2015

***Portulaca tuberosa* (*P. tuberosa*) Roxb belonging to the family Portulacaceae, is traditionally used internally in dysuria as well as applied externally to erysipelas by the natives. The aerial parts of the plant contain diterpenoids, pilosanone A and pilosanone B. Previous studies showed that *P. tuberosa* has good diuretic and analgesic effects when used by the people traditionally, however, *in vivo* antipyretic activity was still lacking for established scientific evidences; hence the present study was carried out to investigate and report the antipyretic activity of ethanolic leaf extract of *P. tuberosa* (ELPT) using aspirin as standard drug against pyrexia induced by subcutaneous injection of 20% w/v aqueous yeast suspension in normal saline at the dose of 10 ml/kg body weight. Swiss albino mice were used for the study and divided into 5 groups containing 6 mice in each group. Three doses of ELPT that is, 200, 400 and 800 mg/kg B/w were used for activity. After 4 h of extract administration, the rectal temperatures were recorded by digital thermometer (Holden medical B.V, Netherlands). The result showed that *P. tuberosa* leaf extract at a dose of 800 mg/kg possessed more potent significant ($p < 0.01$) antipyretic activity in comparison to the other doses that is, 200 and 400 mg/kg when compared to the standard.**

Key words: *Portulaca tuberosa* Roxb, yeast-induced pyrexia, antipyretic, aspirin.

INTRODUCTION

The plant *Portulaca tuberosa* Roxb" commonly known as "Bichhuu-buuti", belongs to the family Portulacaceae. The plant is native to peninsular India, Sind in West Pakistan and to the Srilanka (Ceylon) near sea-coasts. A number of active chemical compounds such as tannins, phenol, terpenoids and saponins have been found in the genus and exhibits antidiabetic, antimicrobial and neuroprotective properties. The plant is an herb perennial, prostrate, diffusely branched, fleshy and glabrous. Branches are straggling, 4 to 15 cm long with 2 to 3 mm long internodes. Roots are tuberous, somewhat fusiform and 5 to 8 cm long. Leaves are alternate, sessile to subsessile, linear or linear-lanceolate, 8 to 14 mm long and 1 to 1.5 mm broad, fleshy, acute; stipular hairs dense, 4 to 8 mm long, somewhat brownish. Inflorescence a small, sessile cluster of 2 to 3 flowers subtended by 6 to 8 leaved involucre. Flowers are 10 mm across, yellow, surrounded by ring of bracteate hairs akin to stipular hairs. In Ayurvedic system of medicine, various parts of plant are traditionally used including analgesic, diuretic

*Corresponding author. E-mail: acakinmoladun@futa.edu.ng, akinmoladun_afolabi@yahoo.co.uk. Tel: +234 803 444 5893

and in fever (Khare, 2007).

MATERIALS AND METHODS

Chemicals

Acetyl salicylic acid (CDH Ltd, New Delhi),Yeast Saccharomyces cerevisiae (CDH Ltd New Delhi), digital thermometer (Dr. Morepen Digital Hard Tip Thermometer (MT 101)), digital weighing balance (Scientech Zeta Analytical Balances), absolute ethanol.

Test animals

The animals used in the study were Swiss albino mice (20 to 30 g), within age 3 to 4 week of either sex. The animals were kept in cages at animal house, and maintained at room temperature of 25 ± 2°C with relative humidity (60 ± 10%) under 12 h night and light cycle. All the animals were kept at overnight fasting before every experiment. The animals used were approved according to animal ethics committee Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, U.P.

Collection and extraction of plan

The whole plant of *P. tuberosa* Roxb was collected from District Gaya Bihar, and was authenticated after washing the leaves, covered with cloth and dried in shade for 20 days at room temperature. The leaves were grinded through mechanical grinder to coarse powder. The powdered plant material (250 g) was extracted through maceration technique using 80% ethanol (1:5) as a solvent for 72 h at room temperature with occasional manual shaking. After maceration, the mixture was then filtered through Whattman filter No.1 paper in a flask and tightly capped. The extract was then concentrated under reduced pressure through rotary evaporator and then air dried (Singh et al., 2013).

Acute toxicity study

Acute toxicity study of the extract was determined on Swiss albino mice of either sex. The dose of extract was increased one to three folds to determine the safety level of the extract. The mice were divided into three groups each containing two mice. The first group received only normal saline and the second and third groups received i.p injection of tested drug at doses of 1000 and 3000 mg/kg. After administration of doses, mice were observed for 72 h for any toxic effect.

Antipyretic activity

The antipyretic effect of the ethanolic leaf extract of *P. tuberosa* (ELPT) was determined on Swiss albino mice (20 to 30 g). The animals were divided into five groups containing six mice in each. The normal body temperature of each mouse was recorded by using digital thermometer, inserted in rectum at predetermined interval. Fever was induced by subcutaneous injection of yeast 20% w/v in normal saline at the rate of 10 ml/kg body weight. 15 h after the injection of yeast, the rectal temperature of each animal was again recorded by digital thermometer. Only those animals that show a minimum increased of 0.7°C in temperature after injection of yeast were taken for experiments. Aspirin (100 mg/kg, i.p) was used as reference drug. Group 1 received only (10 ml/kg) normal saline i.p, group 2 received Aspirin (100 mg/kg) as a reference drug, while groups 3, 4 and 5 received 200, 400 and 800 mg/kg B/w of ELPT, respectively. After drug administration rectal temperature of each animal were then recorded following 0, 1, 2, 3 and 4 h by digital thermometer. Significant decrease in fever in tested animals was compared to control group (Adams et al., 1968).

RESULTS

The present study was performed to find out the antipyretic effect of the ELPT. The result of the present study showed that the ELPT has significant antipyretic effect with a reasonable safe profile.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The statistical significance between control and treated groups were performed using analysis of variance (ANOVA) test. For multiple comparisons among the groups Bonferroni test was performed. A probability level of $p < 0.05$, $p < 0.01$ and $p < 0.001$ was accepted statistically significant.

Acute toxicity study

The ELPT was tested at two doses, 1000 and 3000 mg/kg for toxicity and then compared with control (normal saline group). No major behavioral changes or mortality were noted post administration of the extract at dose of 1000 mg/kg for 72 h. While mortality was observed at the dose of 3000 mg/kg of extract for 72 h.

Antipyretic effect against yeast induced pyrexia

The antipyretic activity of the ELPT was determined by yeast induced pyrexia in mice. The result showed that tested drug at different doses caused lowering of the body temperature up to 4 h following its administration (Table 1). Seventeen hours after s/c injection of 20% of yeast, a significant increase in rectal temperature was observed in all animals. The effect of ethanolic extract on yeast induced pyrexia shows that rectal temperature was 38.10°C, 15 h after the s/c injection of yeast suspension, which further decreased to 37.31°C within 1 h by the treatment of extract (800 mg/kg), and subside after 4 h showing a sizeable reduction in rectal temperature and was comparable to reference drug Aspirin (Figure 1). The extract at a dose of 200 mg/kg body weight showed reduction of pyrexia induced by yeast but it was not significant statistically $p > 0.05$ up to 3 h and showed significant $p < 0.001$ at 4 h when compared to control. Treatments with ethanolic extract of *P. tuberosa* at a dose of 400 and 800 mg/kg body weight decreased rectal temperature significantly ($p < 0.05$, $p < 0.001$, respectively)

Table 1. Effect of *P. tuberosa* (200, 400 and 800 mg/kg) i.p on yeast induced pyrexia in mice.

Treatment	Dose mg/kg	Normal (X)	After15 h (Y)	Rectal temperature (°C)				
				After administration of drug				
				0 h	1 h	2 h	3 h	4 h
Normal Sali ± Std	10	35.73±0.178	37.88±0.111	37.63±0.148	37.46±0.133	37.36±0.136	37.30±0.132	37.46±0.141
Aspirin ± Std	100	35.70±0.225	37.96±0.133	37.85±0.148	37.35±0.135	36.65±0.177	36.03±0.136	35.40±0.121
	200	35.66±0.254	37.73±0.199	37.66±0.150	36.23±0.176	36.96±0.131	36.37±0.145	36.53±0.145***
Extract± Std	400	35.31±0.248	37.78±0.158	37.70±0.211	37.33±0.165	36.71±0.083 *	36.35±0.131 ***	36.15±0.145 ***
	800	35.80±0.188	38.10±0.086	37.93±0.067	37.31±0.101	36.70±0.093*	35.93±0.117***	35.63±0.0136***

Values are expressed as mean ± SEM, (number of mice N = 6), significant *P < 0.05, ** P< 0.01 and *** P < 0.001, when compared to control.

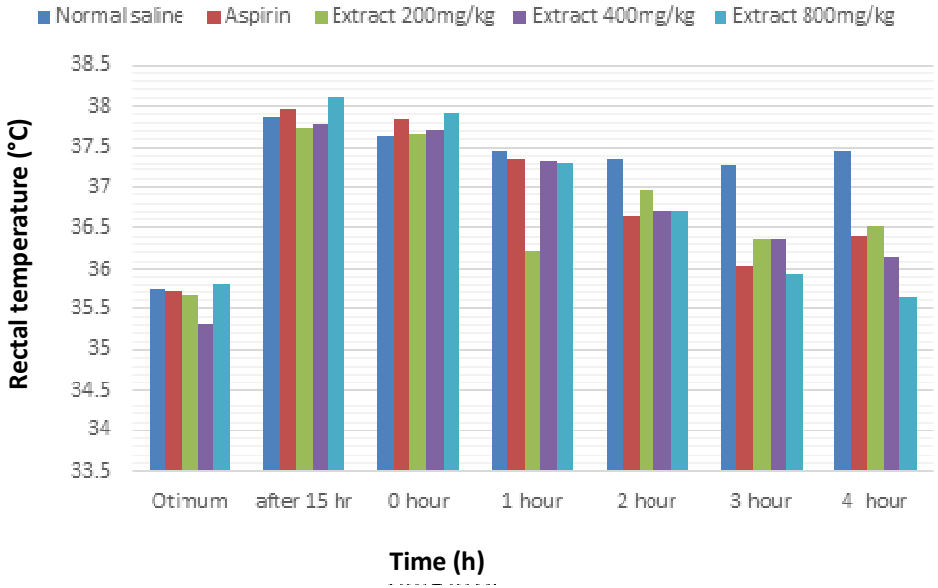

Figure 1. Effect of HEOM on yeast induced pyrexia. The data are expressed as mean ±SEM (N = 6)

at 2, 3 and 4 h after administration compared to normal control. Treatment with Aspirin at a dose of 100 mg/kg significantly p < 0.001 reduced

pyrexia induced by yeast at 3 h after administration. Treatment with ethanolic extract of *P. tuberosa* at dose of 400 and 800 mg/kg was nearly equally potent to reference compound Aspirin (Buffum and Buffum, 2000; Cao and Prescott, 2002; Fadeyi et al., 2005).

DISCUSSION

The ELPT exhibited significant antipyretic activity when compared to standard. The possible mechanism of antipyretic action may be the inhibition of prostaglandin's synthesis. The aim of the present research was to validate the traditional uses of the ELPT for antipyretic action (Olajide et al., 2000; Panthong et al., 2007). Fever or pyrexia is a common medical sign associated primarily with abrupt increase in body temperature above normal and caused by certain illness related behavioral features like fatigue, depression, lethargy, anorexia, hyperalgesia (Fadeyi et al., 2005). The elevation of body temperature occurs as a result of the release of certain chemical substances by immune system (Fung and Kirschenbaum, 1999). Infected or injured tissue enhances the formation of pro-inflammatory mediator that is, cytokines like interleukin-1beta, alpha, beta and TNF- alpha which increase the synthesis of prostaglandin E2 (PGE2) and thereby stimulating hypothalamus to raise body temperature (Fadeyi et al., 2005; Flier et al., 1994).

Fever or pyrexia is a normal response against invading microorganisms to provide defense against infections (Tonks and Watson, 2003). Antipyretic drugs inhibit COX-2 enzyme expression and thus inhibiting biosynthesis of PGE2 to reduce high body temperature (Cao and Prescott, 2002). Cyclooxygenase (COX) also known as prostaglandin (PG) synthetase catalyzes the conversion of arachidonic acid into prostaglandin H2 (Seibert et al., 1995). The common therapy for management and control of fever is nonsteroidal anti-inflammatory drugs (NSAIDs). They are used for relief of inflammation, headache, anti arthritis pain, heart attacks and stroke. NSAIDs drugs inhibit prostaglandin and its derivatives produced through cycloxygenase enzyme that cause inflammation, fever, pain and related diseases (Fung and Kirschenbaum, 1999). However, NSAIDS produces a number of side effects like gastrointestinal bleeding, mucosal erosion, hepatotoxicity, renal toxicity and nephropathy (Greisman and Mackowiak, 2002). Meanwhile, in order to avoid side effect, there are development and introduction of new antipyretic agents that compete with NSAIDs.

The use of natural remedies for the treatment of inflammatory and painful condition has long history starting with Ayurvedic treatment, extends to the Europe. Plant drugs are known to play a vital role in the management of inflammatory diseases. Intraperitonial administrations of the yeast to mice significantly increase rectal temperature and the tested drug significantly reduced rectal temperature. Thus it can be hypothesized that the extract contained pharmacologically compounds that interfere with the release of prostaglandin.

The present results shows that extract possess significant as well as dose dependent antipyretic effect in yeast induced pyrexia which is comparable to that of standard drug. The effect of ethanolic extract on yeast induced pyrexia shows decrease in rectal temperature within 1 h of the extract (800 mg/kg) treatment, and at 4 h a sizeable reduction in rectal temperature which was comparable to reference drug aspirin. The tested drug at a dose of 400 and 800 mg/kg body weight decreased rectal temperature significantly ($p < 0.001$), respectively after administration as compared to negative control. Treatment of the tested drug at a dose of 400 and 800 mg/kg produced a decrease in rectal temperature that was comparable to reference compound aspirin. The evaluated body temperature intensified the process of lipid per oxidation, which indicates that increase of oxidative stress causes pyrexia. The supplementation of antioxidant decreased the lipid per oxidation processes (Sehgal et al., 2011). The flavonoids have antioxidant activity. Thus, antioxidant activity of *P. tuberosa* may be one of the possible mechanisms to reduce the elevated body temperature. The lowering of body temperature observed can be recognized to the presence of flavonoids in *P. tuberosa* that might be responsible for lowered body temperature (Nwafor et al., 2012). The extract may reduce PGE2 by its action on cycloxygenase (COX-2) or by increasing the production of the body's own antipyretic substances like vasopressin and arginine (Olowokudejo et al., 2008). Various studies show that formulation containing tannins, alkaloids, flavonoids and carbohydrates has been reported for their antipyretic potential (Qadrie et al., 2009).

Conclusion

The study concludes the scientific basis in support of its traditional use in fever by the native peoples. The results of the present study showed that plant extract significantly reduced elevated body temperature in dose dependent manners. These results validate the basis for the traditional use of *P. tuberosa* against fever. The preliminarily analysis shows the presence of phenols, flavanoids, alkaloids, saponins, tannins and terpenoids. The pharmacological activity of the plant extract may be due to presence of these phytochemicals. However, further detail studies are necessary for isolation of pure secondary metabolites from the plant to understand the exact mechanism responsible for pharmacological activities of the plant (Singh et al., 2013; Tonks and Watson, 2003; Olajide et al., 2000).

Conflict of interest

Authors have none to declare.

REFERENCES

Khare CP (2007). Indian Medicinal Plants,Springer Science, BusinessMedia, LLC, p. 516.

Adams SS, Hebborn P, Nicholson JS (1968). Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. J. Pharm. Pharmac. 20:305-312.

Singh KB, Trivedi N, Agarwal A, Gupta A, Jha KK (2013). The anti-oxidant potential of Azadirachita indica and Bougainvillea spectabilis, J. Pharm. Biomed. Res. 3(1):358-366.

Buffum M, Buffum JC (2000). Nonsteroidal anti-inflammatory drugs in the elderly. Pain Manage. Nursing 1(2):40-50.

Cao Y, Prescott SM (2002). Many actions of cyclooxygenase2 in cellular dynamics and in cancer. J. Cellular Physiol. 190(3):279-286.

Fadeyi O, Obafem CA, Adewunmi CO (2005). Antipyretic, analgesic, anti-inflammatory and cytotoxic effects of four derivatives of salicylic acid and anthranilic acid in mice and rats. Afr. J. Biotechnol. 3(8):426-431.

Fung HB, Kirschenbaum HL (1999). Selective cyclooxygenase-2 inhibitors for the treatment of arthritis. Clin. Therapeutics 21(7):1131-1157.

Flier JS, Underhill LH, Saper CB (1994). The neurologic basis of fever. New Engl. J. Med. 330(26):1880-1886.

Greisman LA, Mackowiak PA (2002). Fever: beneficial and detrimental effects of antipyretics. Curr. Opinion Infect. Dis. 15(3):241-245.

Nwafor PA, Abia GO, Bankhede HK (2012). Antipyretic and antimalarial activities of crude leaf extract and fractions of Enicostema littorale. Asian Pacific J. Trop. Dis. 2(6):442-447.

Olowokudejo J, Kadiri A, Travih V (2008). An ethnobotanical survey of herbal markets and medicinal plants in Lagos state of Nigeria. Ethnobotanical Leaflets 2008(1):851-865.

Qadrie ZL, Hawisa NT, Samuel M (2009). Antinociceptive and anti-pyretic activity of Benincasa hispida (Thunb.) Cogn. In wistar albino rats. Pak. J. Pharm. Sci. 22(3):287-290.

Sehgal, Momekova D, Pencheva V (2011). Analgesic and Antipyretic activity of Mimusopsele Gil. (Bakul)) Leaves 3:1-6 .

Seibert K, Masferrer J, Zhang Y (1995). Mediation of inflammation by cyclooxygenase-2. Agents and actions. Supplements, pp. 41-46.

Tonks F, Watson R (2003). Pathophysiology, Homeostasis and Nursing (Routledge Essentials for Nurses). November 6, Routledge, pp. 71-80.

Olajide OA, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O, Okpako DT (2000). Studies on the anti-inflammatory, antipyretic and analgesic properties of Alstonia boonei stem bark, J. Ethnopharmacol. 71(1-2):179-86.

Panthong A, Supraditaporn W, Kanjanapothi D, Tae sotikul, T, Reutrakul V (2007). Analgesic, anti-inflammatory and ventonic effects of Cissus quadrangularis Linn. J. Ethnopharmacol. 110(2):264-70.

academicJournals

Vol. 9(13), pp. 456-459, 8 April, 2015
DOI: 10.5897/AJPP2015.4300
Article Number: B23556752701
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Anti-nutrients composition and mineral analysis of *allium cepa* (onion) bulbs

Asanga Edet[1]*, Olorunfemi Eseyin[2] and Ekanem Aniebiet[3]

[1]Department of Biochemistry, College of Medical Sciences, University of Calabar, Cross River State, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, Akwa Ibom State, Nigeria.
[3]Federal School of Medical Laboratory Science, Jos, Plateau State, Nigeria.

Received 24 February, 2015; Accepted 25 March, 2015

Allium cepa **(onion) bulbs are used as spice in the preparation of virtually all meals in Nigeria, and some researchers had reported its medicinal values. This study was designed to estimate the anti-nutrients and mineral composition of this spice. Standard procedures were used for the analysis of antinutrients. Estimation of the various minerals was done using flame photometer and atomic absorption spectrophotometers. The result showed presence of cyanogenic glycosides, total oxalate, soluble oxalate, phytate and tannin and it was found that, all were in low concentration (8.64+0.05, 70.40+0.01, 44.00±0.02, 34.20±0.01 and 51.32±0.03 mg/100 g, respectively) showcasing the safety in consumption of this spice. Mineral analysis of the spice showed presence of Ca^{2+} > Mg^{2+} > Mn^{2+} > K^{+} > P^{-} > Na^{+} > Fe^{2+} in this decreasing order. The determination of Ca^{2+}/P^{-} and Na^{+}/K^{+} indices provided evidence that onion bulb is a good source of ingredients for bone, teeth and muscle growth. It can also serve as a good source of diet for hypertensive patients respectively.**

Key words: *Allium cepa,* anti-nutrients, minerals.

INTRODUCTION

Allium cepa commonly known as onions belongs to the family of Liliaceae. It is a plant found mainly in the temperate region. Its other names are Ayim (Ibibio), Ayo (Igbo), Alubusa (Yoruba), Albasa (Hausa). Michael and Smith (2005), reported the antioxidant abilities of onions in arresting free radicals which cause gastric ulceration. Also, Pamplona and George (1999) reported that onions have hypoglycemic, antihypertensive and anticonvulsant property. Most onions contain vitamin C, vitamin B_6, folic acid and numerous other nutrients in small amounts. They are low in fats and in sodium, and with an energy value of 166 kJ (40 kcal) per 100 g (3.5 oz) serving, they can contribute their flavor to savoury dishes without raising caloric content appreciably (Williamson et al., 1997). Onions contain chemical compounds such as phenolics and flavonoids that basic research shows to have potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties (Michael and Smith, 2005). These include quercetin and its glycosides quercetin 3,4'-diglucoside and quercetin-4'-glucoside. There are considerable differences between different varieties in potential antioxidant content. Shallots have the highest level, six times the amount found in Vidalia onions, the variety with the smallest amount (Yang et al., 2004). Some people suffer from allergic reactions after handling onions, and other symptoms can include contact

*Corresponding author. E-mail: edyasangae1@yahoo.com

dermatitis, intense itching, rhino conjunctivitis, blurred vision, bronchial asthma, sweating and anaphylaxis (Arochena et al., 2012). There may be no allergic reaction in these individuals to the consumption of onions, perhaps because of the denaturing of the proteins involved during the cooking process (Arochena et al., 2012).

While onions and other members of the genus *Allium* are commonly consumed by humans, they can be deadly for dogs, cats, guinea pigs, monkeys and other animals. The toxicity is caused by the sulfoxides present in raw and cooked onions which many animals are unable to digest (Wissman, 1994). Ingestion results in anaemia are caused by the distortion and rupture of red blood cells. Sick pets are sometimes fed with tinned baby foods and any that contain onion should be avoided (Wissman, 1994). Previous studies on onions purchased from a market in Port-Harcourt, Nigeria showed that the following phytochemicals are present tannin, saponin, oxalate and cyanogenic glycosides (Nwinuka et al**.,** 2005)**.**

Antinutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts (Ugwu and Oranye, 2006). Antinutrients in plant foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. However, some antinutrients may exert beneficial health effects at low concentrations. For example, phytic acid, tannins, saponins, and protease inhibitors have been shown to reduce the availability of nutrients and cause growth inhibition.

However, when used at low levels, phytate, tannins, and saponins have also been shown to reduce the blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides. In addition, phytates, tannins, saponins, protease inhibitors, and oxalates have been related to reduce cancer risks. This implies that anti-nutrients might not always be harmful. Despite the result of this, the balance between beneficial and hazardous effects of plant bioactives and anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components. Due to this, they can be considered as anti-nutritional factors with negative effects or non-nutritive compounds with positive effects on health (Habtamu and Negussie, 2014).

Minerals or chemical elements which are inorganic nutrients, are usually required in small amounts from less than 1 to 2500 mg/ day depending on the mineral. The dietary focus on chemical elements derives from an interest in supporting the biochemical reactions of metabolism with the required elemental components (Lippard and Jeremy, 1994). Appropriate intake levels of certain chemical elements have been demonstrated to be required to maintain optimal health. Some of the minerals of much biological importance are Ca^{2+}, Mg^{2+}, Mn^{2+}, K^{+}, P^{-}, Na^{+}, Fe^{2+}. Arising from the use of onions in almost all meals in a typical Nigerian family, the safety, nutritional and anti-nutritional composition of this plant which may be responsible for its reported medicinal abilities must be investigated to unravel new information, authenticate earlier claims or counter same; hence, this study was designed to determine quantitatively the anti-nutrients and mineral composition of onion bulbs.

MATERIALS AND METHODS

Collection and treatment of samples for analysis

100 fresh onion bulbs (15 to 98 g) were bought from Akpan Andem main market, Uyo, Akwa Ibom State, Nigeria from different sellers with no regards to shape. They were identified in the department of Botany and Ecological study, University of Uyo, Nigeria. The fresh onion bulbs were peeled carefully, washed and sliced into many replicates using a sharp knife. It was dried in oven at 30°C in the laboratory. The dried samples were ground using electric blender, the powder of each sample was sieved through mesh 300 µm, and stored in an air – tight cellophane bag as stock sample in a refrigerator until required for analyses (Nwinuka et al., 2005)**.**

Anti-nutrients determination

Standard procedures according to Association of Official Analytical Chemist (1990) were used for the estimation of cyanogenic glycosides, oxalate, tannins and phytic acid.

Mineral analysis

Test for the presence of minerals was carried after acid digestion, the supernatant was decanted and the liquid was analyzed for the levels of Ca^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Fe^{2+}, and P^{-} using standard procedures. Na^{+} and K^{+} were determined using flame photometer, P^{-} level using vanadate/molybdate yellow colour method by Allen and Deitz (1953) whereas Ca^{2+}, Mg^{2+} and Fe^{2+}, were analyzed using Atomic Absorption Spectrophotometer. The concentration of each element in the sample solution was determined by reference to a calibration curve. The results were statistically analyzed and presented as mean $\pm$ SEM at 95 % confidence level.

RESULTS AND DISCUSSION

The cyanogenic glycosides content in *A. cepa* was noted to be about 8.64 mg/100 g, this value was quite low when compared with the toxic level of cyanogenic glycoside level (50 to 200 mg) (NRC, 2001). Implying that the quantity consumed was not harmful to the body. The value was quite small when compared to 30.05 mg/ 100 g earlier reported by Nwinuka et al. (2005) on similar studies. Oxalate present in foods occurs as both soluble

Table 1. Anti-nutrient composition of *A. cepa*.

Antinutrients	Composition
Hydocyanic acid	8.64±0.05
Total oxalate	70.40±0.01
Soluble oxalate	44.00±0.02
Phytic acid	34.20±0.01
Cyanogenic glycoside	51.32±0.03

Values presented in mg/100 g.

Table 2. Mineral analysis of *A. cepa*.

Minerals	Composition
Sodium	16.15±0.05
Potassium	185.05±0.05
Phosphorus	19.24±0.07
Calcium	375.15±0.12
Iron	2.60±0.01
Magnesium	232.05±0.06
Manganese	213.65±0.07

values presented in mg/100 g.

and insoluble components. The soluble component is toxic to the body with a lethal dose of 200 to 500 mg/100 g (Eka, 1997). Values obtained from Table 1 (70.40 mg/ 100 g) showed that onions oxalate level is not toxic to the body because the toxic level (2 to 5 g) is reported by Nwinuka et al. (2005). Therefore, even though oxalates is capable of chelating with bivalent metal ions like Mn^{2+}, Ca^{2+} Mg^{2+} and Fe^{2+} and that some may even precipitates in kidney tubules resulting in oxaluria (Eka, 1997), the level in onions showed it won't cause such effects. The value when compared with that obtained by Nwinuka et al. (2005) as 0.03 g/ 100 g proved to be a good source of adjunct food due to its low level of oxalate.

A. cepa had a lower phytic acid content, therefore, its role in hindering absorption and utilization of certain mineral elements in human and animal bodies (Eka, 1997) will be minimal when consuming a diet rich in onions. The anti-nutrient, tannin is noted for its role in inhibiting the activity of some enzymes such as trypsin, chymotrypsin and amylase by forming complexes with the protein (Eka, 1997). According to Table 2, tannin content in onions was low; hence, the tendency of inhibiting these digestive enzymes will be low on consumption of diets garnished with onions. Moreover, the value was even lower when compared to 0.01 g/ 100 g (Nwinuka et al., 2005). The inorganic mineral analysis of *A. cepa* bulb showed that, it contained minerals in the following order: Calcium > Magnesium > Manganese > Potassium> Phosphorus > Soidum> Iron.

Ca^{2+} is needed for growth and maintenance of bones, teeth and muscles. The Ca^{2+} content of onion bulb (Table 2) was high when compared with the Ca^{2+} content of 3.05 mg/100 g of *Diospyros mesipiliformis* (Hassan et al., 2004) and 313.30 mg/100 g in *Mucuna flagellipes* (Ihedioha and Okoye, 2011). It implies that onion can contribute a meaningful amount to dietary Ca^{2+} to enhance structural function, energy provision, osmotic regulation and, catalytic functions (Hanounes, 2001; Hermanson et al., 2002). Onion bulb provided higher Mg^{2+} level (Table 2) when compared with 2.56 mg/100 g in *D. mespiliformis* (Hassan et al., 2004), 112.73 mg/100 g in *M. flagellipes* (Ihedioha and Okoye, 2011). Magnesium is noted for its roles in chlorophyll formation, germination, amino acid and carbohydrate synthesis in plants as well as forming part of soft tissues, bones and essential components of many enzymes involved in energy transfer in animals. Hence, onion is a good dietary source of Mg^{2+}. Mn^{2+} is a cofactor of enzyme systems involved in energy transfer such as co-enzyme A. It is important for normal functioning of the nervous system, and also plays a part in bone formation (Mc Donald et al., 1995). Table 2 shows that manganese content in onions bulb is higher than 5.82 mg/100 g in *Launeaea taraxacifolia* leaves (Adinortey et al., 2012), 0.3 mg/100 g in lettuce and 0.2 mg/100 g in cabbage (Turan et al., 2003).

K^+ is the principal cation in the intracellular fluid; it functions by influencing acid-base balance, osmotic pressure including water retention, contraction of smooth, skeletal and cardiac muscles (Greeves and Holmes, 1999). The potassium content in this study (Table 2) was found to be higher than 6.42 mg/100 g found in *D. Mespiliformis* and 42.74 mg/100 g in *M. flagellipes* (Ihedioha and Okoye, 2011). Nevertheless, the value was lower when compared with 300 to 600 mg/100 g as the daily requirement of potassium by a healthy adult. Hence, onion bulb has low content of potassium ions. P^- is noted for playing structural roles in building up bones and teeth (Lehninger, 1987). The recommended daily allowance for phosphorus is in the range of 400 to1200 mg/100 g which is very high (NRC, 2001) in comparison to the phosphorus content (Table 2) of the present study. It showed that onion bulb is not rich in phosphorus content. More over, the value in this study was seen to be higher when compared to 1.0 mg/100 g in *D.s mespiliformis* and 5.72 mg/100 g in *M. flagellipes* (Ihedioha and Okoye, 2011). Also, Nieman et al. (1992) considered a food as good source if the Ca/P ratio is above 1 and poor if the ratio is less than 0.5. Onion bulb which had a Ca/P ratio as 19.50 is therefore a good source of Ca and P needed in maintenance of bones, teeth and muscles (Turan et al., 2003).

The Na^+ content of onion bulbs (Table 2) was high when compared to 5.00 mg/100 g in *Tribus terrestris* leaves (Hassan et al., 2005) and 3.29 mg/100 g in *M. flagellipes* (Ihedioha and Okoye, 2011). The recommended dietary allowance (RDA) for Na^+ is 500 mg which means that onion bulb can provide 3.23% of RDA of an adult. Na^+ and K^+ are important in our diets due to

their role in blood pressure regulation (Yoshimura et al., 1991). Na^+/K^+ ratio of less than one in our diet is recommended, hence, onion bulb with Na^+/K^+ ratio as 0.09 is good, and therefore, adequate use of the spice in the diets of hypertensive patients could help in blood pressure control.

The RDA value for Fe^{2+} is 10 to 15 mg (Nieman et al., 1992). From Table 2, Fe^{2+} content of onions bulb was seen to be lower when compared with the RDA value. More over, the value was higher when compared with 1.6 mg/100 mg in spinach, 0.7 mg/100 g in lettuce and 0.3 mg/100 g in cabbage (NRC, 2001). Therefore, onion bulb can provide about 17.3 to 26.0 % of Iron to the RDA thereby helping in boosting the blood level especially in anemic conditions.

Conclusion

The results of this study have revealed that *A. cepa* bulb contain some anti-nutrients which do not pose any toxicity on consumption because of their low concentrations. It also showed the rich mineral composition of this spice which can be recommended for patients in diseased conditions like ricket, osteomalacia and hypertension.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Adinortey MB, Sarfo JK, Quayson ET, Weremfo A, Adinortey CA, Ekloh W, Ocran J (2012). Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. Res. J. Med. Plant 6(2):171-179.

Allen G, Deitz VR (1953). Determination of phosphate by differential spectrophotometer. Analytical Chem. 25:1320-1324.

AOAC (1990). Official methods of analysisl, 15th edition. Association of official analytical chemist, Washington D.C. U.S.A. Pp. 359 - 362.

Arochena L, Gámez C, del Pozo V, Fernández-Nieto M (2012). Cutaneous allergy at the supermarket. J. Investigational Allergol. Clin. Immunol. 22(6):441–442.

Eka OU (1997). Studies on the levels of oxalic acid, phytic acid in traditional foods of Northern Nigeria. W.A.J. Biol. Chem. 20:24-30.

Greeves MA, Holmes KC (1999). Structural mechanism of muscle contraction. Ann. Rev. Biochem. 68:728.

Habtamu FG, Negussie R (2014). Antinutritional factors in plant foods: potential health benefits and adverse effects. Int. J. Nutr. Food Sci. 3(4):284-289.

Hassan LG, Umar KJ, Usman A (2005). Nutrient content of the leaves of *Tribulus terrestri* (Tsida). J. Trop. Biosci. 5:77-82.

Hassan LG, Abdulrahaman D, Zuru AA (2004). Nutritional and phytochemical investigation of *Diospyros mespiliformis* (L). Nig. J. Basic Appl. Sci. 13:1-8.

Hanounes DN (2001). Regulation and role of adenylcyclase isoforms. Ann. Rev. Pharmacol. Toxicol. 145:45-60.

Hermanson O, Glass CK, Rosenfield MG (2002). Nuclear receptor co-regulation: multiple modes of receptor modification. Trend Endocrinol. Metabol. 13:55-56.

Ihedioha JN, Okoye COB (2011). Nutritional evaluation of *Mucuna flagellipes* leaves: An underutilized legume in Eastern Nigeria. Am. J. Plant Nutr. Fertil. Technol. 1:55-63.

Lehninger A (1987). Principles of Biochemistry. Worth publisher, New York Inc., Pp. 753-759.

Lippard SJ, Jeremy MB (1994). Principles of Bioinorganic Chemistry. Mill Valley, CA: University Science Books. P. 411.

Mc Donald P, Edwards RA, Greenhalgh JFD, Morgan CA (1995). Animal nutrition, 5th edition. Longman Singapore publishers (PVT) limited, Singapore. P. 129-135.

Michael AJ, Smith BA (2005). Antioxidant activities of onions. J. Agric. Food Chem. 21:345-360.

Natural Research Council (NRC) (2001). Recommended daily dietary allowance, U.S.A. Nutritional Rev. 3:374-395.

Nieman DC, Butterworth DE, Nieman CN (1992). Nutrition. WMC Brown publisher, Dubugue. P. 50.

Nwinuka NM, Ibeh GO, Ekeke GI (2005). Proximate composition and levels of some toxicants in four commonly consumed spices. J. Appl. Sci. Environ. Manage. 9(1):150-155.

Pamplona R, George D (1999). Encyclopedia of medicinal plant. Education and Health Library Publishers 1:151-153.

Turan MS, kordali H, zengin A, Dursun A, Sezen Y (2003). Macro and micro mineral content of some wild edible leaves consumed in Eastern Anatolia. Acta Agric. Scand. 53:129-137.

Ugwu FM, Oranye NA (2006). Effects of some processing methods on the toxic components of African breadfruit (Treculia qfricana). Afr. J. Biotechnol. 5:2329-2333.

Williamson G, Plumb GW, Uda Y, Price KR, Rhodes MJ (1997). Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells. Carcinogenesis 17(11):2385–2387

Wissman MA (1994). Anemia caused by onions. Anemia in primates. Retrieved 2013-04-01.

Yang J, Meyers KJ, VanDer HJ, Liu RH (2004). Varietal Differences in phenolic content and antioxidant and antiproliferative activities of onions. J. Agric. Food Chem. 52(22):6787-6793.

Yoshimura M, Takahashi H, Nakanishi T (1991). Role of sodium, potassium, calcium, magnesium on blood pressure regulation: an antihypertensive dietary therapy. Japan J. Nutr. 49:53-62.

academicJournals

Vol. 9(13), pp. 460-467, 8 April, 2015
DOI: 10.5897/AJPP2015.4292
Article Number: 3EE4D2752703
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Effect of *Distichoselinum tenuifolium* (Lag.) Garcia Martin & Silvestre essential oil on analgesic and behavioral assays

Larissa Daniele Machado Goés[1]*, Helisson de Oliveira Carvalho[1], Jonatas Lobato Duarte[1], Lígia Ribeiro Salgueiro[2], Carlos Cavaleiro[2], Fabio Ferreira Perazzo[3] and José Carlos Tavares Carvalho[1]

[1]Laboratório de Pesquisa em Fármacos, Centro de Ciências Biológicas e da Saúde, Curso de Ciências, Farmacêuticas, Universidade Federal do Amapá, Macapá, Amapá 68902-280, Brazil.
[2]Centre of Pharmaceutical Studies, Faculty of Pharmacy, Library of the Health Sciences, University of Coimbra, Portugal.
[3]Laboratório de Insumos Naturais e Sintéticos, Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Rua São Nicolau 210, 2 andar, Diadema, São Paulo, 09913-030, Brazil.

Received 13 February, 2015; Accepted 17 March, 2015

Distichoselinum tenuifolium **(EODT) in Portugal is widespread in the province of Algarve-S Portugal, and it is traditionally used in the treatment of contact dermatitis and skin infections. The acetic acid-induced writhing, hot-plate and formalin tests were utilized for analgesic activity, and the exploratory activity of the rats was observed in an open-field and rota rod tests. The treatment with EODT (p.o) has decreased the writhing induced by acetic acid produced in a dose-dependent manner (r = 0.997). The ED_{50} was determined as 55.0 mg/kg. In the acute toxicity assay, no deaths were observed during the 72h period. In the hot-plate test, when EODT was administered at 55.0 mg/kg (p.o) there was a significant increase ($p < 0.05$) in latency time at all time of observations. The EODT reduced the licking time at both first and second phase ($p < 0.05$) in the formalin test. In the open field test, the EODT inhibited the locomotor activity. The rota-rod assay has shown that the oil has decreased significantly the time spent on the bar in the group treated with EODT after 60 and 90 min. This result has proved that oil has a significant effect on the central nervous system as a depressive agent. Moreover, its activity diminishes the motor activity as well as pain threshold.**

Key words: Essential oil, *Distichoselinum tenuifolium*, apiaceae, analgesic, central nervous system, behavioral assays.

INTRODUCTION

In the last years, the natural products represented an important source of new bioactive compounds. This

*Corresponding author. E-mail: farmacos@unifap.br.Tel:00559640092907.

assertion is supported by Newman and Cragg (2007), which found that 51% of the 983 new small molecule chemical entities introduced as drugs worldwide during 1981 to 2006 were either natural products, natural product derivatives or natural product mimics.

In therapy, various plants containing terpene derivatives have been used as sedative, tranquilizers, anticonvulsants and antinociceptive, given the wide variety of pharmacological activities attributed to the essential oils that have principally terpene compounds such as linalool, limonene and citronellol which have anticonvulsant activity, while menthol and myrcene, analgesic activity. Many monoterpene derivatives have shown activity on the central nervous system (CNS), including sedative, antidepressant and antinociceptive (Pergentino de Souza et al., 2007; Sousa et al., 2007; Perazzo et al., 2007; Leite et al., 2008).

Essential oils are natural products widely used for medicinal purposes. They are complex mixtures of numerous molecules that occur naturally in aromatic plants, which make these species very valuable. Apiaceae (Umbelliferae) is one of the well-known families rich in species bearing essential oils. The medicinal properties of plants from this family are known since immemorial times and nowadays, more than 100 cultivated apiaceae species are registered for several uses, mainly as medicinal plants (41%), particularly due to their essential oils (Khoshbakht et al., 2007; Edris, 2007).

Tavares et al. (2010), demonstrated the antifungal and anti-inflammatory properties of *Distichoselinum tenuifolium* essential oils, justifying and reinforcing the use of this plant on traditional medicine. Continuing the chemical and biological studies on the apiaceae widely used in traditional medicine in Portugal, in this work we report the results obtained with the essential oils of *D. tenuifolium* (Lag.) Garcia Martin & Silvestre on analgesic and behavioral assays.

MATERIALS AND METHODS

Plant material

Ripe umbels of *D. tenuifolium* were collected from field-growing plants in the South of Portugal (Faro-Algarve). Voucher specimens were deposited at the herbarium of the Department of Life Sciences of the University of Coimbra (COI00005906).

Essential oils isolation and analysis

The essential oil (EODT) was isolated by hydrodistillation for 3 h, from ripe umbels with mature seeds, using a *Clevenger*-type apparatus according to the procedure described in the Council of Europe (1997). Analyses of the oil were carried out by both gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). GC was performed on a Hewlett Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame-ionization detectors (FID). A graphpak divider (Agilent Technologies, Part Number 5021-7148) was used for simultaneous sampling in two Supelco (Supelco Inc., Bellefont, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane; 30 m x 0.20 mm i.d., film thickness 0.20 µm) and SupelcoWax 10 (polyethyleneglycol; 30 m x 0.20 mm i.d., film thickness 0.20 µm). Oven temperature program: 70 to 220°C (3°C /min), 220°C (15 min); injector temperature: 250°C; detector carrier gas: He, adjusted to a linear velocity of 30 cm/s; splitting ratio 1:50; detector temperature: 250°C. GC/MS analyses were performed on a Hewlett Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane; 30 m x 0.25 mm i.d., film thickness 0.25 µm), interfaced with a Hewlett Packard Mass Selective Detector 5973 (Agilent Technologies, Palo Alto, CA, USA) operated by HP enhanced ChemStation software, version A.03.00. GC parameters as above; interface temperature: 250°C; MS source temperature: 230°C; MS quadrupole temperature: 150°C; ionization energy: 70 eV; ionization current: 60 µA; scan range: 35 to 350 u; scans/s: 4.51. The volatile compounds were identified by both their retention indices and their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of a series of *n*-alkanes, were compared with those of authenticated samples from the database of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra. Mass spectra were compared with reference spectra from a home-made library or from literature data (Adams, 2004; Joulain and Koning, 1998). Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction. The oils were then preserved in a sealed vial at 4°C for further experiments.

Drugs and reagents

The animals were treated with 25, 50 or 75 mg/kg of EODT by oral route for the ED_{50} determination. Afterwards, the animals were treated with the ED_{50} (55 mg/kg). An emulsion of EODT was prepared using Tween 80, subsequently diluted in 0.9% saline solution. Control groups were treated with solution of 3% Tween 80 in 0.9% saline solution. Indomethacin and amfepramone were obtained from Prodome Co.

Animals

Wistar male rats (*Rattus norvegicus* – albinus), weighing 180 to 200 g and Swiss Albino mice (*Mus* musculus), weighing 20 to 25 g, were acquired from the "Centro Multidisciplinar para Investigação Biológica" (CEMIB - Multidisciplinary Center for Biological Investigation) of the Universidade Estadual de Campinas - Unicamp. The animals were kept in polyethylene boxes (n = 5), in a controlled environment (23 ± 2°C), and air humidity (53%), in 12 h/shifts with dark/light control (7 a.m. to 7 p.m.). Food and water were "ad libitum". Animals were fasted overnight prior to each experiment. The project was approved by the Ethics Commission of the Universidade Federal do Amapá, Amapá, Brazil, under Protocol Number 03A/2010.

Determination of the median lethal dose (LD_{50})

Mice (n = 10) were given single doses (by oral gavage) at different concentrations of *D. tenuifolium* essential oil were given to mice to determine the median lethal dose (LD_{50}). These animals were observed during a 48 h period. The number of animals that died

during this period was expressed as a percentile, and the LD_{50} was determined by probit test using death percent versus dose's log (Sokal and Rohlf, 1995). Each group was constituted by five males and five females, separated according to the gender. The animals had been observed for behavioral alterations or toxic effects.

Acetic acid-induced writhing

Different groups of mice (n = 10/group) were treated orally with 5% Tween solution (0.2 mL), indomethacin (MSD Co.,10 mg/kg) and EODT (25, 50 and 75 mg/kg) 30 min before the experiments. The writhes were stimulated using 0.6% v/v acetic acid (10 mL/kg, i.p.). The writhes were observed during 20 min and the results were given as mean ± S.E.M. during the interval (Koster et al., 1959). The writhing inhibition was calculated by with the control group. ED_{50} was determined from the curve drawn for the percentage of writhing inhibition as a function of the dose (Bianchetti et al., 2010).

Hot-plate test

The hot plate test was performed following the method of Jacob and Ramabadran (1978), with few modifications (Perazzo et al., 2008), which involved exposing mice to a hot surface. Groups of mice (n = 5) received 55 mg/kg EODT p.o.; 0.2 mL of 5% Tween solution p.o. (negative control) or 4.0 mg/kg morphine i.p. (positive control). The hot plate apparatus (Ugo Basile DS37, Italy) was maintained at 55 ± 0.5°C. Animals were individually exposed, and the time they spent licking the footpad or any paw (latency time) was recorded. The cut-off time was set at 20 seconds. The measurements were performed at 0, 30, 60, 90 and 120 min, respectively after treatment.

Formalin test

This test was performed with mice (n=5). The animals received 20 μL of a 2.5% formalin solution in their right footpad, according to Tjolsen et al. (1992). After formalin administration, the animals were isolated and observed for the first 5 min (early phase – neurogenic pain) and between 20 to 25 min (late phase – inflammatory pain). Treatments were undertaken by oral administration of 55 mg/kg EODT; 0.2 ml 5% Tween solution (control group) and 10 mg/kg of indomethacin (positive control).

Open field test

The exploratory activity of the rats was also observed in an open-field. The EODT (55 mg/kg), 0.5 ml of 5% Tween solution (control group), 10 mg/kg diazepam (positive group) and 10 mg/kg of amfepramone were given 30 min before the rat has been placed into an unknown openfield (100 x 100 x 50 cm). The ambulation, exploration, rearing and grooming of the rat in the open-field were recorded for 5 min. This test was used to evaluate the EODT effects on rats' locomotor activity (Borsini et al., 1986). This experiment was also recorded for further analysis.

Rota rod test

This test consists of placing the animals on a platform with a rotating axis, divided by circular plates into four compartments (Acceler rota rod - Jones and Roberts for mice, Ugo Basile mod. DS 37), according to the method described by Dunham and Miya (1957). The animals were selected 24 h before the experiment, and those who could not keep themselves on the device for 200 seconds at 32 rpm without falling down were rejected. The animals were treated orally with 55 mg/kg EODT; 0.5 ml of 5% Tween solution (control group); and 10 mg/kg of amfepramone for 60 min before the assay. After this period, the animals were placed in the equipment, and the latency time was determined in 30, 60 and 90 min after drugs administration. The time until the fall was measured with a chronometer, considering a cut-off time of 60 s.

Statistical analysis

Results are presented as mean ± S.E.M. (Standard Error of the Mean). The level of statistic significance was determined using ANOVA followed by Newman–Keuls' test or Student "t" test when appropriated. Levels of 95% of interval confidence or higher were considered significant. The ED_{50} was determined by linear regression using Prisma 5.0 for Windows. Statistical significance was shown as $*p<0.05$, $**p < 0.01$ or $***p < 0.001$.

RESULTS AND DISCUSSION

The essential oil was obtained in yield of 1.1 % (v/w). The identified compounds and their percentages are listed in order of their elution on a polydimethylsiloxane column (Table 1). The main constituent was myrcene, that account for 85% of the composition of the oil. Our previous studies (Tavares et al., 2010) also showed myrcene as the major compound of the oils from other origins. This fact points to a very high homogeneity in the composition of the essential oils of *D. tenuifolium* from Portugal.

In the lethal dose assay, no deaths were observed during the 72h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhea or increased diuresis. The median lethal dose (LD_{50}) was determined to be higher than highest dose tested, 2.0 g/kg. Previous report on safety of essential oils have been published before (Sousa at al., 2009; Perazzo et al., 2007), showing the beneficial effects of this chemical class. Previously, it was demonstrated that *D. tenuifolium* essential oils had fungicidal activity and significantly inhibited nitric oxide production induced by LPS in macrophages, at concentrations that did not affect mammalian cells viability (Tavares et al., 2010).

The model of writhing induced by acetic acid is described as a typical model of inflammatory nociception visceral and is widely used as a tool for detection and evaluation of new agents with analgesic and anti-inflammatory properties (Reichert et al., 2001). Intraperitoneal administration of acetic acid induces the activation of non-selective of cation channels, and promotes the release of pro-inflammatory mediators such as prostaglandins and cytokines (Julius and Basbaum, 2001; Ikeda et al., 2001). Due to these different mechanisms, this model has good sensitivity, but low

Tabela 1. Composition of the essential oil of *Distichoselinum tenuifolium*

RI^a	RI^p	Compounds*	Percentage (%)
922	1030	α-Thujene	0.1
930	1030	α-Pinene	0.7
943	1073	Camphene	0.1
964	1128	Sabinene	0.2
970	1118	β-Pinene	0.5
980	1161	Myrcene	85.0
997	1171	α-Phellandrene	0.2
1010	1187	α-Terpinene	0.1
1011	1275	*p*-Cymene	0.1
1020	1206	Limonene	2.5
1020	1215	β-Phellandrene	0.1
1046	1249	γ-Terpinene	0.1
1050	1458	*trans*-Sabinene hydrate	0.1
1076	1288	Terpinolene	0.5
1081	1543	Linalool	0.1
1081	1542	*cis*-Sabinene hydrate	0.1
1145	1666	Criptone	v
1157	1839	*p*-Cimene-8-ol	v
1158	1597	Terpinene-4-ol	0.1
1169	1692	α-Terpineol	v
1194	1824	*trans*-Carveol	v
1203	1467	Fenchyl acetate	0.2
1210	1724	Carvone	v
1328	1688	α-Terpinyl acetate	0.1
1369	1487	α-Copaene	v
1466	1699	Germacrene D	0.4
1508	1751	δ-Cadinene	v
1542	-	*trans*-Nerolidol	0.1
1618	2174	T- Muurolol	0.1
1622	2212	β-Eudesmol	0.1
1630	2216	α-Cadinol	0.2
1639	2197	Bulnesol	0.1
-	-	Monoterpene hydrocarbons	90.2
-	-	Oxygen containing monoterpenes	0.9
-	-	Sesquiterpene hydrocarbons	0.5
-	-	Oxygen containing sesquiterpenes	0.6
88	-	Total identified	92.2

* Compounds listed in order to their elution on the SPB-1 column. t = traces (≤0.05%), RI^a = Retention indices on the SPB-1 column relative to C_8 to C_{24} *n*-alkanes, RI^p = Retention indices on the SupelcoWax-10 column relative to C_8 to C_{24} *n*-alkanes.

but low specificity for the induced nociception, which can be prevented by anti-inflammatory agents, analgesics, muscle relaxants and sedatives (Reichert et al., 2001; Feng et al., 2003). The treatment with EODT has decreased the writhing induced by acetic acid produced in a dose-dependent manner (correlation coefficient r = 0.997 and linear regression $y = 10.94x + 2.45$). ED_{50} was determined as 55.0 mg/kg (Figure 1). Studies with the

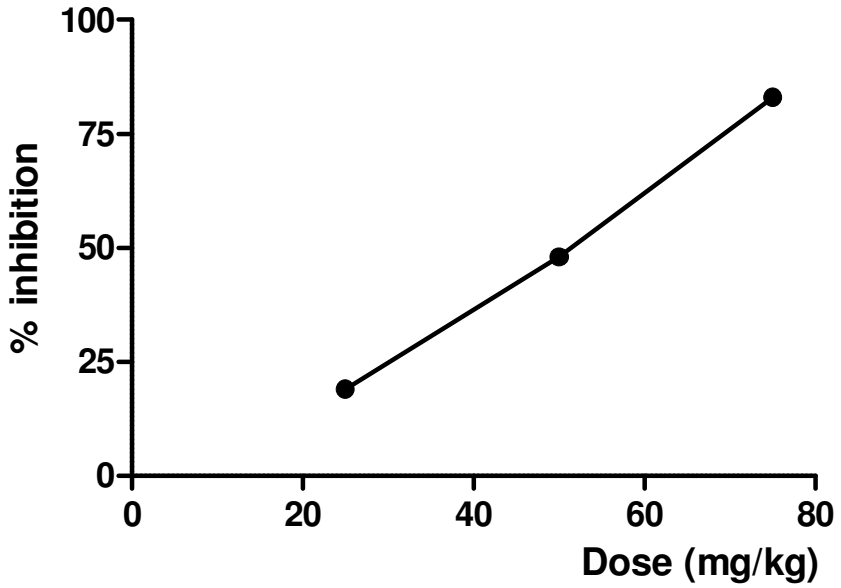

Figure 1. Effect of oral administration of EODT (25, 50 or 75 mg/kg) on the number of writhes in mice induced by the intraperitoneal injection of 0.6% acetic acid. Each point represents the mean of each test group. P = 0.0344, r = 0.997, Y = 10.94x + 2.45 and ANOVA (F = 341.3), ED_{50} = 55 mg/kg.

Table 2. Effect of the oral administration of EODT (55 mg/kg) and morphine (4 mg/kg, ip) on latency (s) time in the hot plate test in mice.

Groups (n = 5)	Dose (mg/kg)	Latency (s)		
		0 min	30 min	60 min
Control	-	10.40±1.05	9.02±1.08	9.24±0.82
EODT	55	9.76±0.82	18.4±1.02*	19.2±1.25*
Morphine	4	10.18±1.12	20.0±1.18*	20.0±1.23*

The results are expressed as mean ± S.E.M. n = 5/group. *p < 0.05, when compared to control; ANOVA followed by Newman-Kuels test.

majority compound of EODT (myrcene) showed nociceptive activity (Rao et al., 1990; Perazzo et al., 2003).

The hot-plate test is commonly used for assaying narcotic like analgesics. However, other drugs such as sedatives, muscle relaxants or psychomimetic drugs may show positive activity (Vaz et al., 1996). The hot-plate test is characterized by supra-spinal organized answers that involve brain functions to pain stimulus (Gardmark et al., 1998). When EODT was administered at 55 mg/kg, there was a significant increase ($p < 0.05$) in latency time at all observations. When EODT was administered at 55 mg/kg, it was observed a significant activity at 30 ($p < 0.05$), and 60 ($p < 0.05$) min. In this dose, EODT has increased the latency time significantly ($p < 0.05$). The animals treated with morphine shown a latency period longer than 20 seconds, since it was established as the cut-off time for the protocol (Table 2). This effect can be associated with the high contents of myrcene in this oil, presenting analgesic activity (Rao et al., 1990).

The formalin test is considered a valid model for clinical pain (Tjolsen et al., 1992). In this test, the first phase or acute phase (0 to 5 min) has been thought to result from direct activation of nociceptive afferent fibres. The second or tonic phase (20 to 25 min) is an inflammatory peripheral process (Coderre and Melzack, 1992; Abbadie et al., 1997). Drugs which mainly act centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripheral analgesics only inhibit the second phase (Santos et al., 1997). The EODT administration reduced

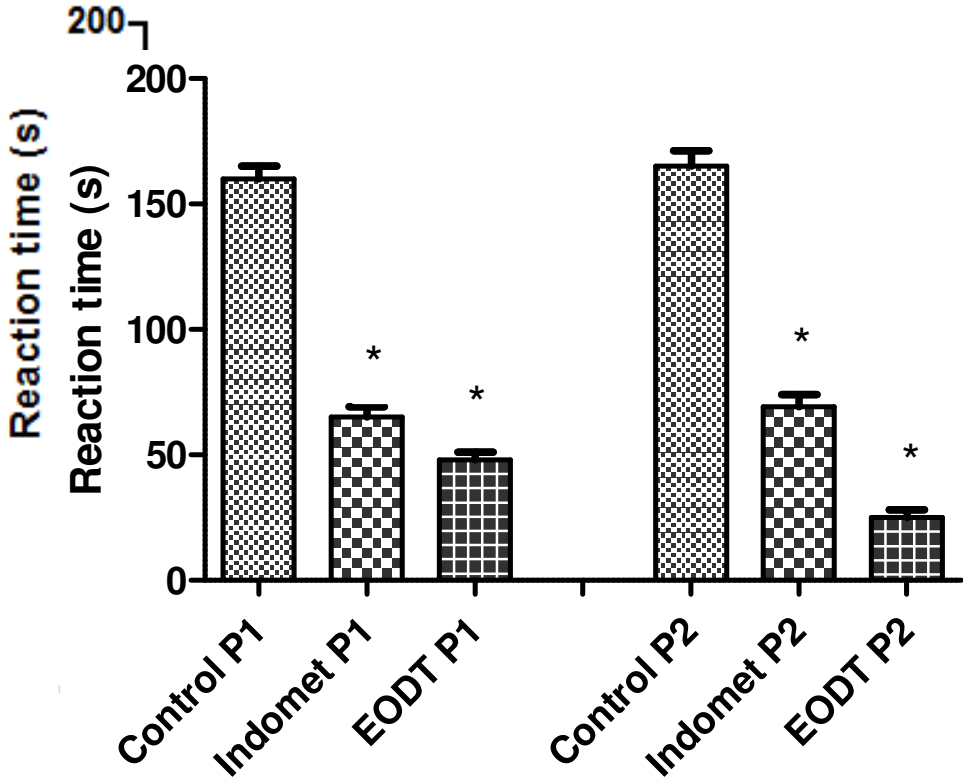

Figure 2. Effect of the oral administration of EODT (55 mg/kg), indomethacin (10 mg/kg) and 5% Tween solution (0.2 mL) on the formalin test in mice (P1 first phase, P2 second phase). Each bar represents the mean ± SEM of each group (n = 5/group). *$p < 0.05$ (Student's t test).

Table 3. Effect of the oral administration of EODT (55 mg/kg), amfepramone (10 mg/kg) and 5% tween 80 solution (0.2 mL) in the open field test in rats.

Group	Dose	Crossing	Rearing (no. of times)
Control	0.2 ml, p.o	58.6±10.34	22.2±2.39
EODT	55 mg/kg, p.o	22.4±3.28**	8.14±2.1**
Amfepramone	10 mg/kg,p.o	136±12.84*	48.3±6.37*

The results are expressed as mean ± S.E.M. n = 5/group. *p < 0.05, when compared to control; ANOVA followed by Newman-Keuls test.

the licking time at both first and second phase ($p < 0.05$) of the test (Figure 2). Moreover, the EDOT has shown to be more effective to decrease both neurogenic and inflammatory phases than indomethacin. The inhibition of the two phases of formalin-induced pain test suggests that the EODT shows direct activation of the fibers nociceptive afferents and acts on the peripheral nerve endings, by inhibiting cyclooxygenases (Bispo et al., 2001; Asongalem et al., 2004).

In the open field test, the EODT inhibited the locomotor activity (22.4 ± 3.28), while both amfepramone (136 ± 12.84) and the control (58.6 ± 10.34) did not show this effect. In the rearing activity, all groups were statistically different, but the one treated with EODT (8.14 ± 2.1) presented a weak exploratory activity when compared to the control (22.2 ± 2.39) and the amfepramone group (48.3 ± 6.37). Results are presented in Table 3. The reduction of the locomotion time is suggestive of depressant activity been more specific on the central nervous system, suggesting anxiolytic action (Oliveira et al., 2008).

The rota-rod test allows to evaluate the specificity of

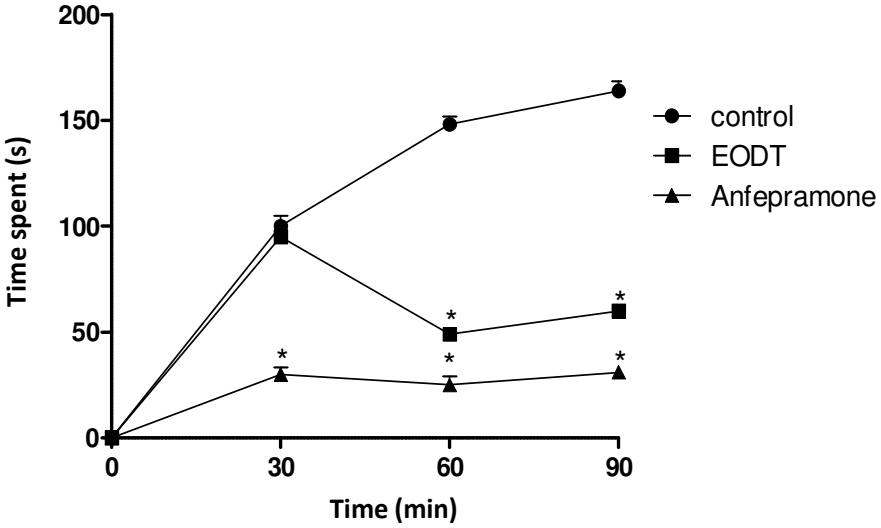

Figure 3. Effect of the oral administration of EODT (55 mg/kg), amfepramone (10 mg/kg) and 5% Tween solution (0.2 mL) on time spent in the rota rod test. The points represent the mean ± SED of each group (n=5) in the measurement times. *$p < 0.05$ ANOVA followed by Newman Keuls post-hoc test.

the drugs' nociceptive action, checking if they promote motor incoordination, or sedation and/or muscle relaxation (Rosland et al., 1990). The rota-rod assay has shown that the oil has decreased significantly the time spent on the bar in the group treated with EODT after 60 and 90 min. The animals treated with EODT have spent 51 ± 2.4 sec and 64 ± 1.9 s on the bar after 60 and 90 minutes, respectively. Amfepramone reduced this time after 30 (21 ± 3.4 s) and 60 min (20 ± 4.1 s). The control group has presented the best performance after 60 (148 ± 3.7) and 90 min (164 ± 4.3) (Figure 3). The results of rota-rod test demonstrated that the EODT presents effect on the central nervous system, reducing the permanence time of the animals in the rota-rod with possible influence motor (Pultrini et al., 2006).

Conclusion

Tests of nociception added to the results of the open field have proved that this oil has a significant effect on the central nervous system as a depressive agent. Moreover, its activity diminishes the motor activity as well as pain threshold.

ACKNOWLEDGEMENTS

The authors are grateful to Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support, and also to Fundação de Amparo a Pesquisa do Estado do Amapá – FAPEAP, Guyamazon Project. Thanks are also due to Dr. Ana Cristina Tavares (CEF/Faculty of Pharmacy, Univ. of Coimbra) for collecting the plant material.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Abbadie C, Taylor BK, Peterson M A, Basbaum A I (1997). Differential contribution of the two phases of the formalin test to pattern of *c-fos* expression in the rat spinal cord: studies with remifentanil and lidocaine. Pain 69:101-110.

Adams RP (2004). Identification of essential oil components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation Stream, IL, USA.

Asongalem EA, Foyet HS, Ngogang J, Folefoc GN, Dimo T, Kamtchouing P (2004). Analgesic and antiinflammatory activities of Erigeron floribundus. J. Ethnopharmacol. 91:301-308.

Bianchetti ES, Santos KC, Perazzo FF, Carvalho JCT (2010). Preclinical Study of the Interference of Different Nutritional Diets on the Anti-Inflammatory and Analgesic Actions of Etoricoxib. Lat. Am. J. Pharm. 29:213-218.

Bispo MD, Mourão RHV, Franzotti EM, Bomfi m KBR, Arrigoni-Blank MF, Moreno MPN, Marchioro M, Antoniolli AR (2001). Antinociceptive and antiedematogenic effects of the aqueous extract of Hyptis pectinata leaves in experimental animals. J. Ethnopharmacol. 76:81-86.

Borsini F, Evangelista S, Meli A (1986). Effect of GABAergic drugs in the behavioral despair test in rats. Eur. J. Pharmacol. 121:265-268.

Coderre TJ, Melzack R (1992). The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J. Neurosci. 12:3665-3670.
Council of Europe (1997). European Pharmacopeia, 3rd ed., Strasbourg p 1817.
Dunham NW, Miya TS (1957). A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. Sci. 46:208-209.
Edris AE (2007). Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: A review. Phytother. Res. 21:308-323.
Feng Y, Cui M, Willis W (2003). Gabapentin markedly reduces acetic acid–induced visceral nociception. Anesthesiology 98:729-733.
Gardmark M, Höglund AU, Hammarlund-Udenaes M (1998). Aspects of tail-flick, hot-plate and electrical stimulation tests for morphine antinociception. Pharmacol. Toxicol. 83:252-258.
Ikeda Y, Ueno A, Naraba H, Oh-Ishi S (2001). Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. Life Sci. 69:2911-2919.
Jacob JJC, Ramabadran K (1978). Enhancement of a nociceptive reaction by opiate antagonist in mice. Br. J. Pharmacol. 64:91-98.
Joulain D, Koning WA (1998). The Atlas of Spectral Data of Sesquiterpene Hydrocarbon.B. Verlag, Hamburg.
Julius D, Basbaum AI (2001). Molecular mechanisms of nociception. Nature 413:203-210.
Khoshbakht K, Hammer K, Pistrick K (2007). *Eryngium caucasicum* Trautv. Cultivated as a vegetable in the Elburz Mountains (Northern Iran). Genet. Resour. Crop Evol. 54:445-448.
Koster R, Anderson M, Beer EJ (1959). Acetic acid for analgesic screening. Fed. Proc. 18:412.
Leite MP, Fassin Jr. J, Baziloni EMF, Almeida RN, Mattei R, Leite JR (2008). Behavioral effects of essential oil of *Citrus aurantium* L. inhalation in rats. Rev. Bras. Farmacogn. 18:661-666.
Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70:461-477.
Oliveira RB, Nascimento MVM, Valadares MC, Realino de Paula J, Costa EA, Cunha LC (2008). Avaliação dos efeitos depressores centrais do extrato etanólico das folhas de *Synadenium umbellatum* Pax. e de suas frações em camundongos albinos. Rev. Bras. Cienc. Farm. 44:485-491.
Perazzo FF, Carvalho JCT, Carvalho JE, Rehder VLG (2003). Central properties of the essential oil and the crude ethanol extract from aerial parts of *Artemisia annua* L. Pharmacol. Res. 48:497-502.
Perazzo FF, Carvalho JCT, Rodrigues M, Morais EKL, Maciel MAM (2007). Comparative anti-inflammatory and antinociceptive effects of terpenoids and an aqueous extract obtained from *Croton cajucara* Benth. Rev. Bras. Farmacogn. 17:521-528.
Perazzo FF, Lima LM, Padilha MM, Rocha LM, Sousa PJC, Carvalho JCT (2008). Anti-inflammatory and analgesic activities of *Hypericum brasiliense* (Willd) standardized extract. Rev. Bras. Farmacogn. 18:320-325.
Pergentino de Souza D, Farias Nóbrega FF, Almeida RN (2007). Influence of the chirality of (R)-(-)- and (S)-(+)- carvone in the central nervous system: a comparative tudy. Chirality 19:264-268.
Pultrini AM, Galindo LA, Costa M (2006). Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. Life Sci. 78:1720-1725.
Rao VS, Menezes AM, Viana GS (1990). Effect of myrcene on nociception in mice. J. Pharm. Pharmacol. 42:877-8.
Reichert JA, Daughters RS, Rivard R, Simone DA (2001). Peripheral and preemptive apioid antinociception in a mouse visceral pain model. Pain 89:221-227.
Rosland JH, Hunskaar S, Hole K (1990). Diazepam attenuates morphine antinociception test-dependently in mice. Pharmacol. Toxicol. 66:382-386.
Santos FA, Rao VSN, Silveira ER (1997). Anti-inflammatory and analgesic activities of *Psidium guianense.* Fitoterapia 68:65-68.
Sokal RR, Rohlf FJ (1995). Biometry. W.H. Freeman eds.,, San Francisco pp. 175-205, 404-486.
Sousa PJC, Araujo JS, Pereira LLS, Modro MNR, Maia JGS, Araujo MTF, Carvalho JCT, Perazzo FF (2009). Phytochemical and Toxicological Evaluations of the Essential Oil From the Bark of *Aniba canellila* (H.B.K.) Mez. J. Essent. Oil Res. 21:381-384.
Sousa DP, Nóbrega FFF, Claudino FS, Almeida RN, Leite JR, Mattei R (2007). Pharmacological effects of the monoterpene α,β-epoxy-carvone in mice. Rev. Bras. Farmacogn. 17:170-175.
Tavares AC, Gonçalves MJ, Cruz MT, Cavaleiro C, Lopes C, Canhoto J, Salgueiro L (2010). Essential oils from *Distichoselinum tenuifolium*: Chemical composition, cytotoxicity, antifungal and anti-inflammatory properties. J. Ethnopharmacol.130:593-598.
Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K (1992). The formalin test: An evaluation of the method. Pain 51:5-17.
Vaz ZR, Cechinel Filho V, Yunes RA, Calixto JB (1996). Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4, 6-dimethoxybenzofuran, a novel xanthoxyline derivative, on chemical and thermal models of nociception in mice. J. Pharmacol. Exp. Ther. 278:304-312.


African Journal
of Pharmacy and
Pharmacology
Related Journals Published by Academic Journals
Journal of Medicinal Plant Research
African Journal of Pharmacy and Pharmacology
Journal of Dentistry and Oral Hygiene
International Journal of Nursing and Midwifery
Journal of Parasitology and Vector Biology
Journal of Pharmacognosy and Phytotherapy
Journal of Toxicology and Environmental Health Sciences
academicJournals