

Full Length Research Paper

Antinociceptive potential of *Parkia platycephala* Benth. in streptozotocin-induced diabetic rats

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This study aimed to investigate the possible antinociceptive action of the ethanolic extract (E.EtOH) and ethyl acetate fraction (F.AcOEt) from leaves of *Parkia platycephala* in streptozotocin (STZ)-induced diabetic rats. In chronic experiment, STZ-rats were daily treated with E.EtOH (150 or 300 mg/kg, p.o) for three weeks. The mechanical nociceptive threshold (MNT) was determined by application of von Frey filaments to the right hind paw before and 1, 2 and 3 weeks after diabetes induction. The STZ-E.EtOH 300 mg/kg group presented MNT higher than that in the STZ-vehicle group (untreated diabetic rats) at the third week. This effect was similar to the STZ-insulin group (6 U/rat/day), but did not reach the value of the control group (non-diabetic rats). Although, E.EtOH 150 mg/kg had induced a decrease on serum glucose when compared with initial glucose, there was no significant difference between STZ-vehicle and E.EtOH-treated diabetic rats. In acute experiments, untreated diabetic rats received F.AcOEt (6.25, 12.5, 50 and 150 mg/kg, p.o) at the third week of diabetes. F.AcOEt improved the tactile allodynia evaluated by MNT. Moreover, STZ-F.AcOEt 50 mg/kg presented antinociceptive effect in the late phase of formalin test. These findings indicate that E.EtOH and F.AcOEt showed analgesic actions in diabetic rats.

Key words: *Parkia platycephala*, diabetic neuropathy, tactile allodynia, formalin test, analgesic action.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term complications, including retinopathy, nephropathy, peripheral neuropathy with risk of foot ulcers, amputation, and Charcot joints, and autonomic neuropathy leading to sexual dysfunction, gastrointestinal, genitourinary, and

cardiovascular disorders (The Expert Committee, 2002).

For *in vivo* models of diabetes mellitus, the administration of streptozotocin (Rees and Alcolado, 2005) is the most common animal model for the study of acute and chronic diabetic complications, including painful peripheral neuropathy. In this context, the diabetic neuropathy is a recognized complication of diabetes which presents a multifactorial pathogenesis related to many different mechanisms. Glycosylation of myelin characterized by excessive myelin turnover and demyelination (Greene et al., 1999; Harati, 1987), decreased activity of neurotrophic factor (Dobretsov et al., 2007), endothelial dysfunction (Brownlee et al., 1988), increased levels of advanced glycation end-products (AGEs), aldose reductase, and oxidative stress have been postulated to be contributing factors to the development of diabetic neuropathy (Benstead and Sangalang, 1995; Calcutt et al., 1996; Vlassara et al., 1985).

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Abbreviations: STZ, Streptozotocin; MNT, mechanical nociceptive threshold; E.EtOH, ethanolic extract; F.AcOEt, ethyl acetate fraction; TP, total phenols; F.H₂O, aqueous fraction; AGEs, advanced glycation end-products; DMSO, dimethyl sulfoxide; DPN, distal peripheral neuropathy.

Natural compounds have been used for treatment of many pathological processes, including diabetes mellitus. In Brazil, there is a great variety of medicinal plants. In this context, *Parkia platycephala* Benth. (Leguminosae - Mimosoideae), popularly-known as “faveira” is used for nutritional supplementation of ruminant animals, for example, cattle and goat. This is due to the fact that its seeds show elevated nutritional value because of their energy content (Machado et al., 1999). Previous studies showed gastroprotective and antioxidant potential of ethanolic extract (E.EtOH) of *P. platycephala*, accompanied by the absence of acute toxicity in mice and cytotoxicity in rat erythrocytes (Fernandes et al., 2010). Previous analyses of the ethanolic extract from the leaves of *P. platycephala* also revealed the presence of flavonoids, triterpenes and a high level of phenols compounds, which were found in ethyl acetate (F.AcOEt) and aqueous (F. H₂O) fractions (R.D.S. Bezerra, Federal University of Piauí, Brazil, personal communication).

Moreover, several pharmacological effects have been verified in some species of the genus *Parkia* such as *P. biglobosa*, whose extracts obtained from the seeds have showed antidiabetic and anti-lipidemic effects (Odetola et al., 2006), inhibition of platelet aggregation (Rendu et al., 1993); while the stem-bark of this plant also presented antinociceptive, anti-inflammatory (Kouadio et al., 2000), and anti-snake venom activities (Asuzu and Harvey, 2003). Antiviral activity was also reported in seeds of *P. pendula* (Favacho et al., 2007) and lectins able to induce histamine release from mast cells were found in *P. platycephala* (Lopes et al., 2005).

Based on pharmacological actions of plants of the genus *Parkia*, we aimed to investigate the antinociceptive activity of ethanolic extract (E.EtOH) and ethyl acetate fraction (F.AcOEt) obtained from leaves of *P. platycephala* in diabetic rats.

MATERIALS AND METHODS

Leaves of *P. platycephala* Benth. were collected in Timon, Maranhão State, Brazil. A voucher specimen (No. 15,553) has been deposited in the Graziela Barroso Herbarium of the Federal University of Piauí, Brazil. The dried and ground leaves of *P. platycephala* (2.265 kg) were submitted to 95% ethanol extraction in 5 consecutive steps at room temperature. The solvent was evaporated, resulting in 392 g of E.EtOH (17.3%). The concentrated ethanol extract (300 g) was suspended in methanol/water (15%) and successively extracted with solvents yielding the aqueous (F.H₂O, 180 g, 60%), ethyl acetate (F.AcOEt, 23 g, 7.7%), ethereal (23 g, 7.7%), and hexane (27 g, 9%) fractions. Considering the high content of total phenols determined by Folin-Ciocalteu method in E.EtOH extract, F.H₂O, and F.AcOEt (R.D.S. Bezerra, Federal University of Piauí, Brazil, personal communication) and, a preliminary evaluation of antinociceptive properties of extract and fractions in mice, E.EtOH and F.AcOEt were chosen for this study.

Chemicals and drugs

Streptozotocin (Sigma, USA), sodium citrate (Vetec, Brazil), citric

acid (Dinamica, Brazil), insulin Novolin (Novo Nordisk, Brazil), sodium thiopental (Cristalia, Brazil), dimethyl sulfoxide (Sigma-Aldrich) and glucose oxidase kit (Labtest, Brazil) were used in this study. All solutions were prepared with substances immediately before each experiment, using as a vehicle a saline (NaCl 0.9%) solution or distilled water. The extracts and fractions concentrations were adjusted for treatment to yield a volume of 10 ml/kg with vehicle solution containing saline (NaCl 0.9%) and dimethyl sulfoxide (DMSO 2%).

Experimental animals

Male Wistar rats (250 to 290 g) from the Animal House of the Federal University of Piauí (Teresina, Brazil) were housed in cages with standard environmental conditions (22 ± 1°C, 12 h light/12 h dark), with free access to a standard commercial diet and water *ad libitum*. All experimental procedures were previously approved by the Animal Ethics Committee at Federal University of Piauí, Teresina, Brazil.

Diabetes induction

Diabetes was induced in rats after 12 h of fasting by a single intravenous injection of freshly prepared streptozotocin (STZ 40 mg/kg) dissolved in 0.01 M citrate buffer, at pH 4.5 (Pepato et al., 2001). The experiments were conducted 2 days after the STZ injection by measuring serum glucose levels after a 12-h overnight fast. Glucose levels were determined spectrophotometrically using an assay kit (Labtest, Brazil) based on the method of glucose oxidase. A fasting serum glucose concentration above or equal to 250 mg/dl was considered to indicate diabetes mellitus.

Chronic treatment

The rats were divided into five groups of six to nine rats. The STZ-E.EtOH groups received ethanolic extract (E.EtOH 150 and 300 mg/kg dissolved in vehicle solution) orally by gavage once a day until the 21st day of the experimental period. The STZ-insulin group was treated with insulin (3 U s.c. per rat, twice at 8:00 am and 6:00 pm). The STZ-vehicle and non-diabetic control groups received a vehicle solution containing saline (NaCl 0.9%) and dimethyl sulfoxide (DMSO 2%) p.o. E.EtOH doses were chosen based on previous toxicity studies (Fernandes et al., 2010) and preliminary experiments in rats. All groups were used to assess the tactile threshold by measuring the mechanical nociceptive threshold (MNT) using the application of von Frey filaments perpendicularly to the plantar surface of the right hind paw. The MNT was tested 0, 1, 2 and 3 weeks after the STZ injection based on the method described previously (Chaplan et al., 1994). At the end of the experimental period, the animals were anesthetized with sodium thiopental 40 mg/kg intraperitoneally (Cherksey and Altszuler, 1974). Blood was collected from the superior vena cava for measuring the fasting serum glucose.

Acute treatments

Mechanical nociception

At the 21st day after the STZ injection, seven groups of 7 to 10 rats were submitted to an acute experimental protocol and received a single dose of F.AcOEt (STZ-F.AcOEt 6.25, 12.5, 50, and 150 mg/kg, p.o) or morphine (STZ-morphine 5 mg/kg, i.p). STZ-vehicle and the control received saline-DMSO 2%, p.o. The MNT was determined in all groups by application of von Frey filaments to the

Table 1. Serum glucose levels and body weight in normal and streptozotocin-induced diabetic rats treated with E.EtOH of *P. Platycephala* for three weeks.

Group	Serum glucose (mg/dl)		Body weight (g)	
	Initial	21st day	Initial	21st day
Control	121.6 ± 14.8	120.1 ± 9.3	244.5 ± 9.4	266.0 ± 8.6 ^d
STZ-vehicle	384.4 ± 18.8 ^a	358.0 ± 46.3 ^a	257.1 ± 13.2	219.3 ± 11.3 ^{acd}
STZ-E.EtOH 150 mg/kg	439.2 ± 46.0 ^a	247.6 ± 31.0 ^d	262.4 ± 3.8	182.0 ± 12.5 ^{acd}
STZ-E.EtOH 300 mg/kg	432.6 ± 24.8 ^a	374.5 ± 46.8 ^{ac}	269.8 ± 6.8	238.2 ± 15.7
STZ-insulin 6 U/rat/day	371.1 ± 43.5 ^a	159.3 ± 28.2 ^{bd}	285.3 ± 5.2	259.7 ± 5.9 ^d

The values represent means ± SEM (n = 6 - 9). ^ap<0.05 vs. control; ^bp<0.05 vs. STZ-Vehicle; ^cp<0.05 vs. STZ-Insulin, and ^dp<0.05 vs. initial time.

right hind paw at the period of 0, 60, 120, and 180 min after treatment.

Formalin test

In these experiments, after 21 days of the STZ injection, five groups of six to eight rats were submitted to formalin test based on Dubuisson and Dennis (1977) and Wheeler-Aceto et al. (1990). In all groups, formalin (1%, 50 µL) was injected subcutaneously into the plantar surface of the right hind paw. To assess the nociceptive behaviour produced by formalin, the number of flinches was counted during the early (0 to 10 min) and late phases (10 to 60 min post-formalin). Diabetic rats were previously treated with a single dose of F.AcOEt (STZ-F.AcOEt 12.5 or 50 mg/kg, p.o.) or morphine (STZ-morphine 5 mg/kg, i.p) 1 h and 30 min before the formalin test, respectively. STZ-vehicle and non-diabetic control rats received saline-DMSO 2% p.o. F.AcOEt doses (12.5 and 50 mg/kg) were chosen based on previous experiments of MNT determination when diabetic rats were treated with F.AcOEt (6.25, 12.5, 50, or 150 mg/kg, p.o) and all doses presented effects in the first 2 h of evaluation.

Statistical analyses

Statistical analysis was performed using the GraphPad Prism® 4.0 software (Graphpad Software Inc., USA). Data were expressed as mean ± SEM. Statistical significance of the results was determined using one-way analysis of variance (ANOVA) followed by Tukey's as the post hoc test and Student's *t*-test for paired observations. Data were considered significant at *p*<0.05.

RESULTS AND DISCUSSION

The data in Table 1 show that body weight did not differ between E.EtOH-treated diabetic rats and STZ-vehicle group. At the end of the experiment, body weights in STZ-E.EtOH 150 mg/kg and STZ-vehicle animals were significantly lower than in the control and STZ-insulin groups (*p*<0.05). The destruction of pancreatic beta cells by STZ mimicked the insulin-dependent diabetic status (Pepato et al., 2001), which was characterized by hyperglycemia without significant gain in body weight.

Although, the administration of E.EtOH 150 mg/kg had induced a decrease on serum glucose when compared with the initial value (*p*<0.05), at the end of the

experimental period, serum glucose level did not differ between STZ-vehicle and E.EtOH-treated diabetic rats (STZ-E.EtOH 150 and 300 mg/kg). On the other hand, the daily administration of insulin (6 U per rat/day) produced a significant decrease in glycaemia (*p*<0.05). In cases of diabetes mellitus (rodents and humans), hyperglycaemia contributes to the elevation of glycated proteins, glucose auto-oxidation, oxidative stress, and advanced glycation end-products (AGEs) (Lapolla et al., 2005), which later may be involved in chronic effects of diabetes, including painful peripheral neuropathy. In this context, distal peripheral neuropathy (DPN) has been correlated to many different mechanisms being influenced by direct and indirect effects of hyperglycaemia (Courteix et al., 1993; Sasaki et al., 1998; Zhang et al., 1999) and by loss of trophic support, likely IGF-I, insulin, or C-peptide (Dobretsov et al., 2007). In this work, all rats of the STZ-insulin group were treated with similar doses of insulin to avoid differences in trophic supports that could affect neuropathy progression in this group. Even so, hypoglycemic episodes such as convulsions and/or coma were not observed during the insulin treatment and STZ-insulin animals did not present severe hypoglycaemia at the end of the chronic experiment.

This study evaluated the effects of the E.EtOH obtained from *P. Platycephala* leaves in diabetic rats with high sensitivity to pain. The tactile allodynia was measured in acute and chronic treatments. According to Figure 1, the MNT was reduced in diabetic groups after three weeks of the STZ injection, except for the STZ-insulin group. STZ-diabetic rats showed a marked tactile allodynia when exposed to light touch on the plantar hind paw after three weeks of the STZ injection. MNT decrease is one of the most consistent signs of peripheral neuropathy in diabetic animals (Ahlgren and Levine, 1993; Dobretsov et al., 2003). Some authors have reported that hypersensitivity to mechanical stimulation is detectable one week after the STZ-injection and fully develops between 2 and 8 weeks of diabetes mellitus (Chen and Pan, 1998; Fox et al., 1999). Moreover, in the second and third weeks, STZ-induced diabetic rats treated with insulin showed MNT values lower than those obtained in the initial period

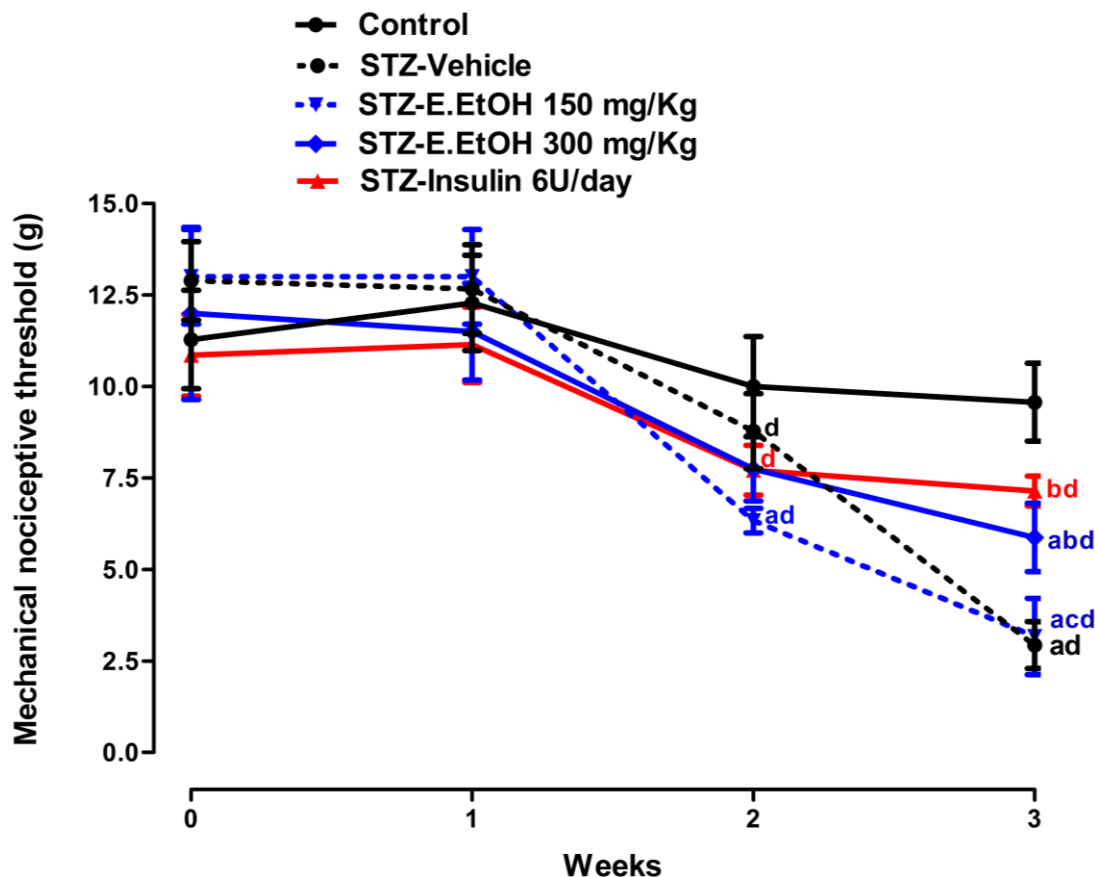


Figure 1. Mechanical nociceptive threshold in control and diabetic rats treated with E.EtOH of *P. platycephala* for three weeks. The values represent means $\pm$ SEM ($n = 6 - 9$). ^a $p < 0.05$ vs. control, ^b $p < 0.05$ vs. STZ-vehicle, ^c $p < 0.05$ vs. STZ-insulin, and ^d $p < 0.05$ vs. initial time.

($p < 0.05$). However, in the third week, MNT values were significantly higher than in the STZ-vehicle ($p < 0.05$). Diabetic rats that received chronic treatment with E.EtOH 300 mg/kg of *P. platycephala* increased pain threshold similarly to the STZ-insulin group in the third week, but did not reach the value of the control group ($p < 0.05$).

Several studies have suggested that insulinopenia induces neuropathic effects as well as hyperglycaemia. In fact, for short term diabetes, the effects of insulin treatment on regulation of axon-glia relationships, vascular permeability, and function of nociceptive primary afferent neurons may protect against early diabetic neuropathy (Sugimoto et al., 2000, 2002; Dobretsov et al., 2007). Thus, benefits of insulin therapy were described; peripheral nerve fibre regeneration, regulation of inner mitochondrial membrane potentials, suppression of NADPH oxidase (Dandona et al., 2005), control of expression of $\text{Nf-}\kappa\text{B}$ and inflammatory reactions (Nedrebo et al., 2004; Dandona et al., 2005), regulation of Na, K-ATPase (Davel et al., 2000; Sweeney and Klip, 1998), and endothelial nitric oxide (NO) production (Davel et al., 2000; Steinberg et al., 1994). On the other hand, peripheral nerve regeneration is suppressed in long-term

DPN (Dobretsov et al., 2007). Singh et al. (2012) had proposed a neuronal nuclear targeting of insulin and evidence for insulin-induced resistance to its trophic properties.

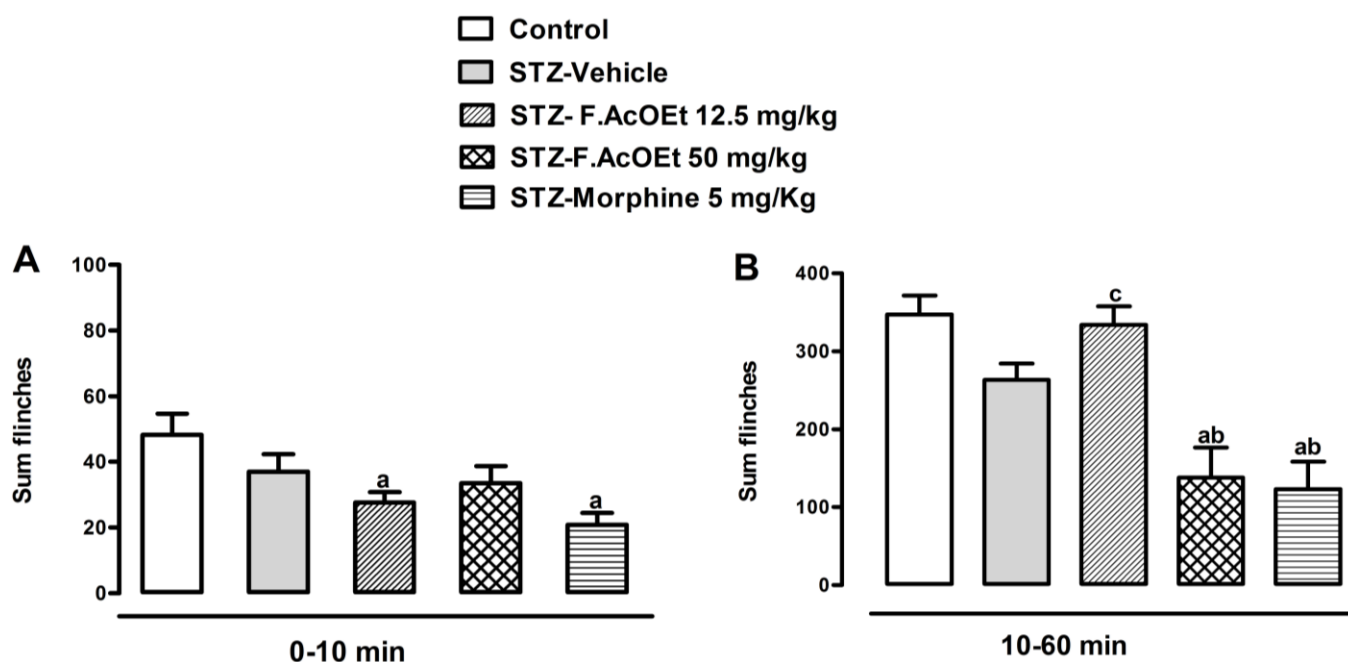
Moreover, in order to clarify the effects of E.EtOH of *P. platycephala* leaves, acute experiments were carried out by treating diabetic rats with ethyl acetate fraction (F.AcOEt) obtained from E.EtOH partition at the 21st day after the STZ injection when the MNT decreased in diabetic rats. According to Table 2, F.AcOEt promoted a similar increase on the MNT of the diabetic rats treated orally with different doses of fraction (6.25, 12.5, 50, and 150 mg/kg) in the first hour when compared with the STZ-vehicle group. These effects of fraction were similar to morphine. Meanwhile, in the STZ-F.AcOEt 150 mg/kg group, the antinociceptive effect lasted for 180 min. These data are suggestive of an analgesic property of F.AcOEt.

In another experiment, behavioural responses to noxious chemical stimuli were measured using the formalin test 21 days after diabetes induction (Figure 2). Untreated diabetic rats did not show hyperalgesia during early and late phases of the formalin test compared to

Table 2. Mechanical nociceptive threshold (g) of normal and diabetic rats treated with F.AcOEt of *P. platycephala* at the 21st day after diabetes induction.

Group	Time after treatment (min)			
	0	60	120	180
Control	12.1 ± 1.0	11.1 ± 0.9	10.1 ± 0.6	11.6 ± 1.0
STZ-vehicle	2.7 ± 0.5 ^a	3.0 ± 0.5 ^a	3.3 ± 0.5 ^a	3.8 ± 0.5 ^a
STZ-F.AcOEt 6.25 mg/kg	2.8 ± 0.5 ^a	7.6 ± 1.3 ^{bc}	6.4 ± 1.4 ^{ac}	3.8 ± 0.5 ^a
STZ-F.AcOEt 12.5 mg/kg	3.1 ± 0.6 ^a	8.6 ± 0.6 ^{bc}	6.9 ± 1.1 ^{bc}	6.6 ± 1.0 ^{ac}
STZ-F.AcOEt 50 mg/kg	2.4 ± 0.4 ^a	8.1 ± 1.1 ^{bc}	7.0 ± 1.1 ^{bc}	6.0 ± 0.7 ^{ac}
STZ-F.AcOEt 150 mg/kg	2.7 ± 0.4 ^a	7.6 ± 1.8 ^{bc}	7.0 ± 0.7 ^{bc}	7.0 ± 0.8 ^{abc}
STZ-Morphine 5 mg/kg	3.7 ± 0.5 ^a	11.3 ± 1.5 ^{bc}	7.3 ± 0.8 ^b	6.5 ± 1.0 ^a

The values represent means ± SEM (n = 7 - 10). ^ap<0.05 vs. control, ^bp<0.05 vs. STZ-vehicle, and ^cp<0.05 vs. initial time (0').

**Figure 2.** Flinch responses during the early and late phases of the formalin test in control and diabetic rats treated with F.AcOEt of *P. platycephala* at the 21st day after diabetes induction. The values represent means ± SEM (n = 6 - 8). ^ap<0.05 vs. control, ^bp<0.05 vs. STZ-vehicle, and ^cp<0.05 vs. STZ-morphine.

controls (both $p > 0.05$). During the early phase (0 to 10 min), the STZ-group treated with F.AcOEt 12.5 mg/kg and morphine-injected diabetic rats had responses to formalin that were significantly different from the controls ($p < 0.05$). In the late phase, STZ-groups treated with F.AcOEt 50 mg/kg or morphine presented antinociceptive effects when compared with controls and STZ-vehicle groups (both $p < 0.05$). In rat and mouse, intraplantar injections of formalin produce a biphasic behavioural reaction. The early phase is caused predominantly by C-fibre activation due to the peripheral stimulus, while the late phase is characterized as an inflammatory response with pain that can be inhibited by anti-inflammatory drugs (Hunskar and Hole, 1985; Hunskar et al., 1987;

Tjølsen et al., 1992; Calcutt et al., 1996). According to Tjølsen et al. (1992), opioid analgesics seem to be antinociceptive for both phases, although the latter is more sensitive to these substances. These data are suggestive of antinociceptive effects of F.AcOEt in a model of inflammatory pain.

Conclusion

The present data indicates that E.EtOH and F.AcOEt obtained from leaves of *P. platycephala* showed analgesic properties in STZ-diabetic rats. Nevertheless, further investigation is necessary to elucidate the

possible mechanism of action and long-term toxicological profile.

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