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Artigo

Comparative anti-inflammatory and antinociceptive effects of terpenoids and an aqueous extract obtained from *Croton cajucara* Benth

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RESUMO: "Avaliação comparativa dos efeitos anti-inflamatório e antinociceptivo de terpenóides e de extrato aquoso obtidos de *Croton cajucara* Benth". O 19-*nor*-clerodano *trans*-crotonina (CTN) e o triterpeno ácido acetil aleuritólico (AAA), isolados das cascas do caule de *Croton cajucara* Benth (Euphorbiaceae), uma planta tradicional da Região Amazônica do Brasil, bem como o extrato aquoso (EA) das cascas do caule deste *Croton*, foram submetidos a experimentos farmacológicos utilizando animais, para avaliação das atividades anti-inflamatória e antinociceptiva. A administração oral de AAA (50 mg/kg), CTN (50 mg/kg) ou AE (300 mg/kg) inibiram as contorções em ratos, induzidas por ácido acético. Os terpenóides AAA e CTN, bem como o extrato polar EA, inibiram significativamente o edema de pata em ratos, induzido por carragenina. As inibições foram observadas em todos os intervalos de medições, tendo sido evidenciado melhores inibições para os terpenóides AAA (47,7%, após a primeira hora) e CTN (54,4%, após a segunda hora). Evidenciou-se ainda, após 90 minutos do estímulo, significante inibição no edema induzido por dextrana (31,9% para CTN e 28,5% para AAA) e por histamina (43,2% para CTN e 40,5% para AAA). Estes resultados confirmam o uso popular de *Croton cajucara* na região Amazônica do Brasil, no combate a inflamações.

Unitermos: *Croton cajucara*, ácido acetil aleuritólico, *trans*-crotonina, anti-inflamatório, antinociceptivo.

ABSTRACT: The 19-*nor*-clerodane *trans*-crotonin (CTN) and the triterpene acetyl aleuritolic acid (AAA), isolated from the stem bark of *Croton cajucara* Benth (Euphorbiaceae), a traditional medicinal plant from Amazon region of Brazil, as well as the aqueous extract (AE) from its stem bark, were submitted to pharmacological screening for anti-inflammatory and antinociceptive activities in animal models. The oral administration of AAA (50 mg/kg), CTN (50 mg/kg) or AE (300 mg/kg) inhibited the acetic acid-induced writhing in mice. The AE, CTN and AAA had shown significant inhibition of carrageenin-induced edema in rats, in all time intervals measured after the injection of the stimulus, with the greatest inhibition at the first hour for AAA (47.7%) and the second hour for CTN (54.4%). They have also exhibited significant inhibition in the dextran-induced edema 90 minutes after the stimulus: 31.9% for CTN and 28.5% for AAA. In the histamine-induced edema, the inhibition showed by CTN and AAA were 43.2% and 40.5%, respectively, 90 minutes after the injection of stimulus. This study extends and supports the popular medicine and folkloric uses of *Croton cajucara* in the Amazon region of Brazil.

Keywords: *Croton cajucara*, acetyl aleuritolic acid, *trans*-crotonin, anti-inflammatory, antinociceptive.

INTRODUCTION

Croton cajucara Benth (Euphorbiaceae) has been a very important traditional medicine in Brazil. It occurs widely in the Amazon rainforest region (Brazil) where is popularly known as 'sacaca', which means witchery in Tupi-Guarani indigenous language. The stem bark of this plant is used for primary health care to treat liver and kidney disorders (Di Stasi et al., 1989; Martins, 1989; Farias et al., 1996). In addition, the water extract from the stem bark is also used to treat diabetes, diarrhea, stomachache, fever, jaundice, hepatitis, malaria and also to lower blood cholesterol (Van Den Berg, 1993; Di Stasi et al., 1994; Barbosa-Filho et al., 2005). The

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use of leaves to overcome weigh has been encouraged in the Amazon region of Brazil, however, toxic hepatitis frequently appears as a side effect (Maciel et al., 2000). This observation may be correlated with the chronic use required for losing weigh, since a lack of acute toxicity has been described and this toxicological effect was not noticed (Farias et al., 1996).

The stem bark of this specie is a rich source of clerodane-type diterpenes (Maciel et al., 1998a,b, 2000, 2003; Souza et al., 2006) and our previously pharmacological studies performed with the isolated terpenoids *trans*-dehydrocrotonin (DCTN), *trans*-crotonin (CTN) and acetyl aleuritolic acid (AAA) (Figure 1) proved striking correlation among these compounds with the folk traditional therapeutic uses of *Croton cajucara*, being DCTN the leading compound (Maciel et al., 2000; Costa et al., 2007). Specifically, DCTN showed antioestrogenic (Luna-Costa, 1999), anti-inflammatory and antinociceptive (Carvalho et al., 1996) effects, and has also demonstrated hypoglycemic (Farias et al., 1997), antiatherogenic and hypolipidaemic activities (Silva et al., 2001). Additionally, an anticancer effect had also been described (Grynberg et al., 1999).

The biological importance of natural and semi-synthetic CTN has been reported (Grynberg et al., 1999; Maciel et al., 2000; Hiruma-Lima et al., 2002; Almeida et al., 2002; Anazetti et al., 2003). Regarding to AAA biological importance, this compound proved to possess antibiotic (Addae-Mensah et al., 1992) and antibacterial (Peres et al., 1997) activities. Gastrointestinal effects in mice were also reported (Maciel et al., 2000). This terpenoid has shown no efficacy on both antitumoral (Grynberg et al., 1999) and gastric mucosa damage in rats, caused by ulcers induced by restraint - cold stress (Maciel et al., 2000).

In the course of our studies on pharmacological active substances from *Croton cajucara*, we describe in this present study the anti-inflammatory and antinociceptive effects related to the triterpene AAA and the diterpene-type 19-*nor*-clerodane CTN, additionally with an aqueous extract obtained from its stem bark.

MATERIAL AND METHODS

Plant extracts, isolation and semi-synthesis

The plant material was collected in Jacundá, PA (Amazon region of Brazil) and identified by Dr. Nelson A. Rosa. A voucher specimen has been deposited in the Herbarium of the Museu Paraense Emílio Goeldi (Belém-PA), under registration # 247.

The compounds used in this study were obtained as previously described (Maciel et al., 1998a,b, 2003). Briefly, the powdered stem bark of *Croton cajucara* was extracted with hexane and then methanol by Soxhlet apparatus. This procedure furnished 471.8 g and 202.0 g of each extract. Isolation procedures yielded 4.5 g of AAA and 37.2 g of DCTN. The hydrogenation of DCTN in 95% ethanol was carried out as previously described (Maciel et al., 2000). The semi-synthetic CTN-derivative was recrystallized twice with hexane-acetone and the identity of this compound was confirmed by comparison with chemical and spectroscopic methods of the isolated CTN (Maciel et al., 2000).

The water extract (AE) was prepared from ground shade-dried stem barks (500 g), extracted with distilled water (70 °C, 30 minutes), concentrated under vacuum and freeze-dried. This process furnished 25 g of water extract (yielding 5%).

Animals

Male rats (*Rattus norvegicus*, albinus, Wistar) and male mice (*Mus musculus*, albinus, Swiss), specific pathogen free, weighing 150 - 200 g and 20 - 25 g, respectively, were acquired from the Central Biotery of Universidade de Alfenas. The animals were kept in polyethylene boxes (n = 6), in a climatic environment (23 ± 2 °C), with air humidity control, in 12 hour/shifts with dark/light control. Food and water were given *ad libitum* at least seven days before the experiments. The animals were provided only with water *ad libitum* during the 12 hours before experimentation. This study was conducted according to internationally accepted principles of laboratory animal use (Porter, 1992; Zimmermann, 1983, 1986).

Drugs

The terpenoids CTN and AAA were administrated in 0.9% saline solution with Tween 80 (5%) or DMSO 0.5%. The doses used (50 mg/kg of CTN and 50 mg/kg of AAA) were chosen according to previous studies (Perazzo et al., 1997; Carvalho et al., 1996). The polar extract AE was diluted in saline solution in a concentration to allow administration of constant volume orally (300 mg/kg, 1.0 mL).

Determinations of aqueous extract ED_{50}

Groups of mice (n = 6) were treated orally with *Croton cajucara* aqueous extract (AE; 100, 300 or 1000 mg/kg) or vehicle (water) 60 minutes before the injection of the stimulus (1.0% acetic acid solution, i.p.). The writhing number was counted for 20 minutes, starting at the 5th minute after the stimulus. ED_{50} was determined from dose-response curve drawn for the percentage of writhing inhibition as a function of the dose (Koster et al., 1959).

Writhing test induced by acetic acid

This test was done using the method described by Koster et al. (1959). Groups of mice (n = 6) were

treated orally with CTN (50 mg/kg), AAA (50 mg/kg), AE (300 mg/kg), indomethacin (10 mg/kg) or water (0.5 mL) 30 minutes before an 1.0% acetic acid solution injection (0.25 mL/kg, i.p.). The number of muscular contractions was counted for 20 minutes, starting at the 5th minute after the stimulus. During the experiment, each mouse was kept in individual boxes and results are presented as either cumulative number of writhes or percentage of inhibition of writhes comparatively to the control group.

Carrageenin, dextran and histamine-induced paw edema in rats

Groups of rats (n = 6) were treated orally with CTN (50 mg/kg), AAA (50 mg/kg), AE (300 mg/kg), indomethacin (10 mg/kg), cyproheptadine (10 mg/kg) or 5% Tween 80 [0.9% saline solution (control, 1.0 mL)]. Thirty minutes later, carrageenin (1000 µg, 0.1 mL), dextran (50 µg, 0.1 mL) or histamine (50 µg, 0.1 mL) was injected into the sub-plantar region of the left hind paw of the animals. The right paw was injected with 0.1 mL of 0.9% saline solution. The volume of each paw was determined using a plethysmometer (model 7140, Ugo Basile, Italy) as described by Ferreira (1979). The results were expressed by the difference between the volume of right and left paws.

Statistical analysis

The statistical analyses were done using Analysis of Variance (ANOVA) followed by the Tukey-Kramer multiple comparison test (Sokal; Rohlf, 1995). Results with $p < 0.05$ were considered to be significant. Data are expressed as mean $\pm$ S.D.

RESULTS AND DISCUSSION

The study of ethnopharmacological uses, phytochemistry and pharmacology of *Croton cajucara* represents a successfully combination of efforts to discover new compounds related to some properties of this medicinal specie. As a continuing research of this study, two terpenoids (CTN and AAA; Figure 1) as well as the traditional used tea (comparative to an aqueous extract of the stem bark of *Croton cajucara*) were submitted to a screening in order to investigate its anti-inflammatory activity.

The acetic acid-induced writhing test has been used for pre-clinical evaluation of analgesic potency of novel anti-inflammatory drugs (Loux et al., 1978) and involves the synthesis of prostaglandins, which are the main nociceptive mediators in this model (Deraedt et al., 1980). Oral administration of aqueous extract (AE) produced dose-response reduction of the number of writhes, as showed in Table 1. The maximal inhibition of antinociceptive response was 68.7%, obtained with 1000 mg/kg and the ED_{50} determined as 305 mg/kg. The oral administration of CTN and AAA (50 mg/kg) inhibited the number of muscular contractions in 46.5% and 60.4%, respectively (Figure 2). This effect was not so pronounced with that showed by the group treated with indomethacin (80.2%).

Administration of CTN and AAA significantly inhibited carrageenin-induced edema formation in all time intervals (Figure 3). The great inhibition was at the first hour for AAA (47.7%) and in the second hour for CTN (54.4%). Indomethacin, the standard drug used, inhibited the edema formation by 36.7%, at the first hour after carrageenin injection. Carrageenin-induced edema is one of the first models of inflammation used to investigate new anti-inflammatory drugs and is characterized by an initial phase, mediated by histamine and serotonin, and by a later phase, when the mediators are arachidonic acid metabolites producing edema after mobilization of neutrophils (Vinegar et al., 1987; Ferrándiz; Alcaraz, 1991). CTN and AAA were effective in the two phases of the process and this effect was evidenced by results obtained in other models.

Similar to the results obtained with DCTN (Carvalho et al., 1996), CTN and AAA did not present analgesic activity in either hot-plate and tail-flick tests (data not shown).

Dextran-induced edema is also an acute experimental model of inflammation that induces an anaphylactoid reaction, characterized by a slow developing edema, starting in about 60 - 90 min. after dextran injection. It triggers the release of kinins, lipoxygenase derived products and histamine and serotonin from mast cells (Ankier; Neat, 1972; Nishida; Tomizawa, 1980; Van Wauwe; Goossens, 1989).

The terpenoids CTN and AAA showed a significant inhibition 1.5 and 3.5 hours after dextran injection (Figure 4), with the best inhibition at 1.5 hours (31.9% for CTN and 28.5% for AAA). However, treatment with cyproheptadine (10 mg/kg), a serotonin and histamine H_1 antagonist, was clearly more effective in the two time intervals. Both compounds significantly inhibited the histamine-induced edema (43.2% for CTN and 40.5% for AAA) and this effect was quite similar with that observed with cyproheptadine (Figure 5).

CONCLUSION

The limitation of the use of some anti-inflammatory and analgesic agents is the risk to produce gastric irritation. The combination of anti-inflammatory and antiulcerogenic effects in the same compound is very favorable, but unusual. Nevertheless some natural products exhibit both properties (Sertié, 1991). Some of our results in the experimental model of gastric ulcers, used to evaluate gastric ulceration in rodents showed that DCTN and CTN in the doses of 100 mg/kg had protected against ulcers and mucosal damage induced

by cold restraint-induced stress (Maciel et al., 2000). However, AAA was not effective. DCTN and CTN have also demonstrated antiulcerogenic effect in other gastric ulcer models (Hiruma-Lima et al., 2002). Advances in *Croton cajucara* research with DCTN proved that it has not shown nor genotoxicity neither cytotoxicity to bone marrow cells in mice (Agner et al., 1999, 2001), representing that the anti-inflammatory activity of these compounds could be useful in the general bioavailability of these diterpene-type clerodanes.

The results showed in the present study demonstrated that CTN, AAA and AE have antinociceptive activity when assessed in a model of peripheral nociception such as acetic acid-induced abdominal constriction. Furthermore, when investigated in thermal algesiometric test (tail-flick), considered as a selective test for opioid-like analgesic compound (Janssen et al., 1963), AE did not show antinociceptive effect.

Additionally, the present study reinforces the chemical results with the bioactive DCTN and the improvement obtained shows that CTN and AAA together with DCTN, are some of the compounds responsible for anti-inflammatory and antinociceptive activities observed for *Croton cajucara*. Those terpenoids were found to be part of the traditional tea preparation of this specie, showing to have anti-inflammatory and antinociceptive activities. Allied to the fact that the diterpenes DCTN and CTN had shown an antiulcerogenic effect and no gastric damage, the use of such compounds could be advantageous, which extending and supporting the popular medicine and folkloric uses of *Croton cajucara* in the Amazon region of Brazil.

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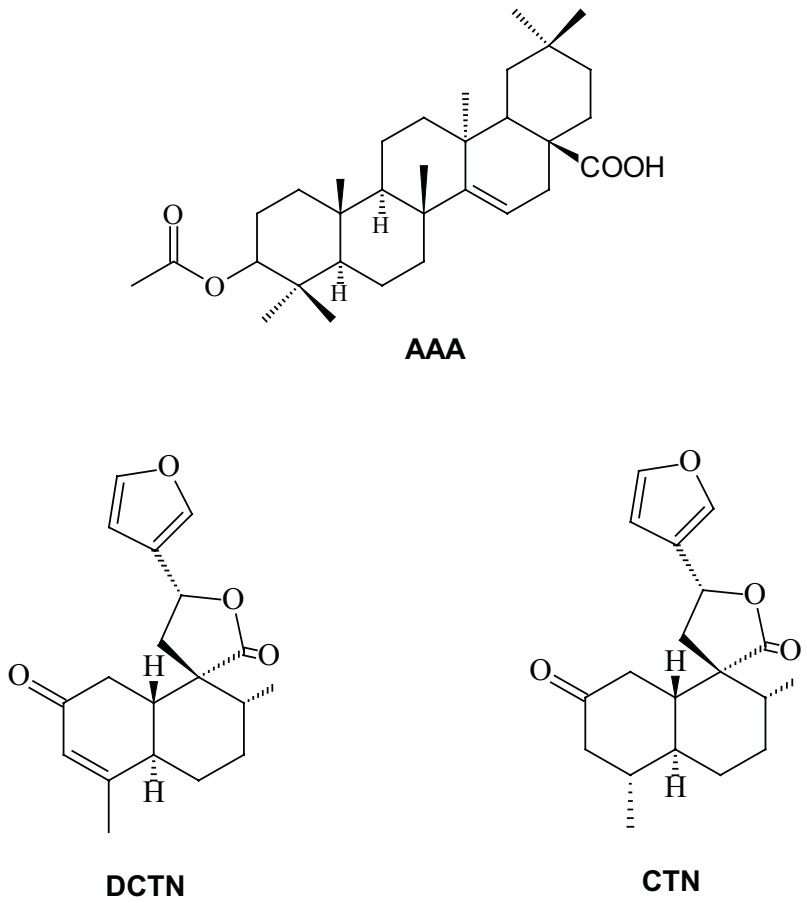

Figure 1. Molecular structure of acetyl aleuritolic acid (AAA), *trans*-crotonin (CTN) and *trans*-dehydrocrotonin (DCTN).

Table 1. Effect of oral aqueous (AE) extract of *Croton cajucara* on acetic acid-induced abdominal constrictions in mice.

Treatment	Dose mg/kg	Number of abdominal constrictions
Saline solution (control)	-	61.0 ± 5.8
Aqueous extract	100	40.2 ± 3.9 *
	300	33.3 ± 5.3 **
	1000	19.1 ± 6.8 **

Each value represents the mean ± S.D (n = 6). Significantly different from control group (*$p < 0.05$ and **$p < 0.001$, Tukey-Kramer multiple comparison test).

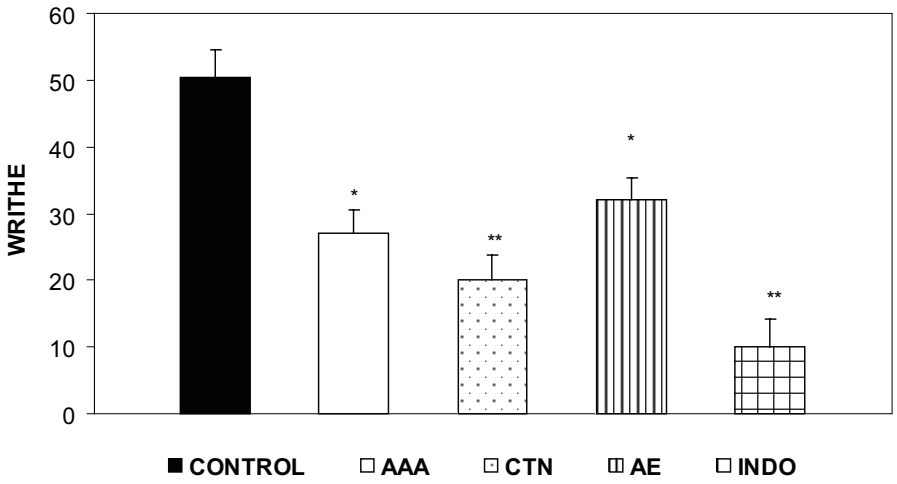

Figure 2. Effect of oral administration of AAA (50 mg/kg), CTN (50 mg/kg), AE (300 mg/kg), and indomethacin (10 mg/kg) on the writhing test induced by intraperitoneal acetic acid injection (1.0%, 0.25 mL/animal) in mice. The results are expressed as mean ± SD obtained (n = 6). Significantly different from the control group (*$p<0.05$ and ** $p <0.01$, Tukey- Kramer multiple comparison test).

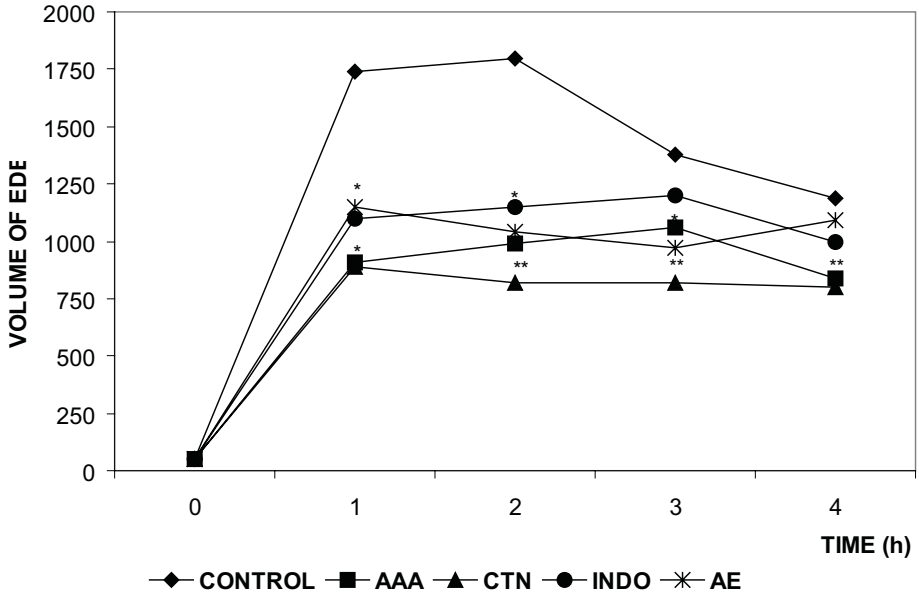

Figure 3. Effect of oral administration of AAA (50 mg/kg), CTN (50 mg/kg), AE (300 mg/kg) and indomethacin (10 mg/kg) on the carrageenin-induced paw edema (1000 mg/paw) over a four-hour period in rats. The results are expressed as mean ± SD (n = 6). Significantly different from the control group (*$p<0.05$ and **$p<0.01$, Tukey- Kramer multiple comparison test).

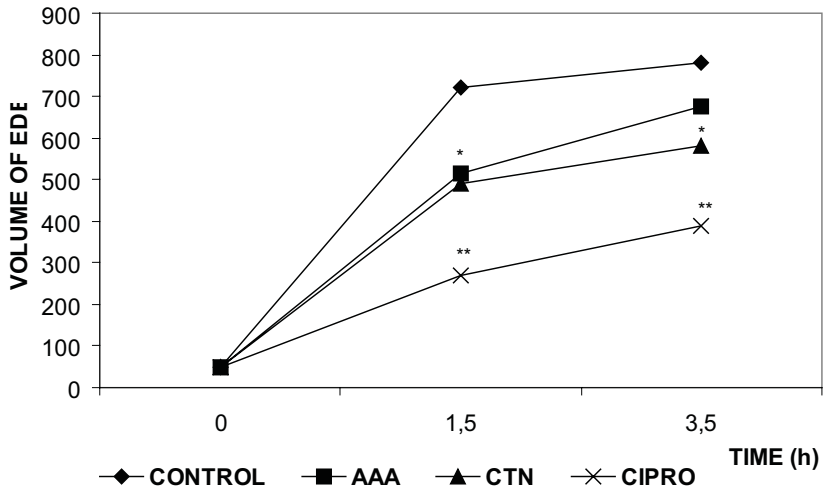

Figure 4. Effect of oral administration of AAA (50 mg/kg), CTN (50 mg/kg) and cyproheptadine (10 mg/kg) on dextran-induced paw edema (50 mg/paw) over a 3.5 hour period in rats. The results are expressed as mean ± SD (n = 6). Significantly different from the control group (*p<0.05 and **p<0.01, Tukey- Kramer multiple comparison test).

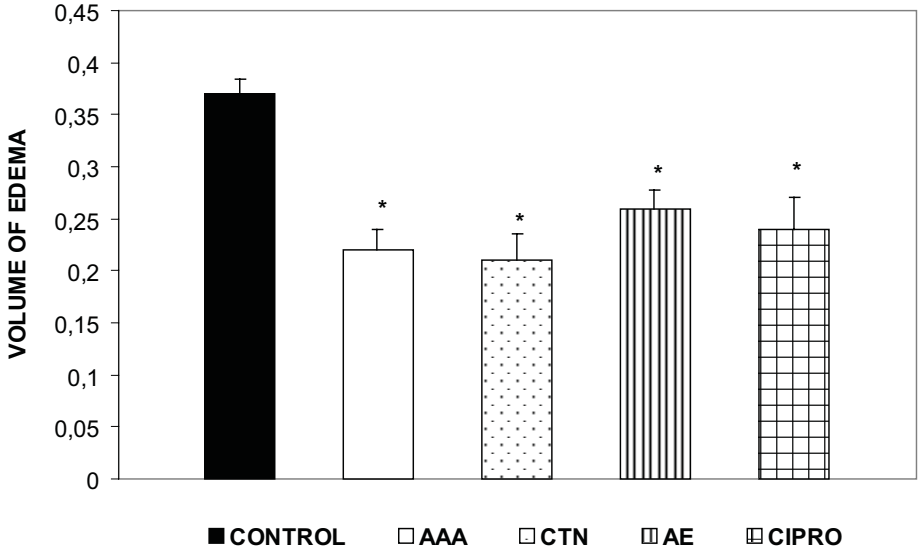

Figure 5. Effect of oral administration of AAA (50 mg/kg) CTN (50 mg/kg), AE (300 mg/kg), and cyproheptadine (10 mg/kg) on histamine-induced paw edema (1000 mg/paw) over a 90 minutes period in rats. The results are expressed as mean ± SD (n = 6). Significantly different from the control group (*p<0.05 and Tukey- Kramer multiple comparison test).

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