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ARTICLE

- Analgesic and anti-inflammatory activity of methanolic fraction of total ethereal leaf extract of *Annona senegalensis* Pers. (Annonaceae) 120**
Madièye Sene, Firmin S. Barboza, Abdou Sarr, Dominique Tchemy Outouen, Alassane Wele, Emmanuel Bassene and Guata Yoro S.Y.

Full Length Research Paper

Analgesic and anti-inflammatory activity of methanolic fraction of total ethereal leaf extract of *Annona senegalensis* Pers. (Annonaceae)

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Annona senegalensis leaves are used in African Traditional Medicine as analgesic and anti-inflammatory drugs. This study aimed to investigate the analgesic and anti-inflammatory activities of total ethereal leaf extract (TEE) of *A. senegalensis* and its methanolic fraction (MF). Experiments were performed in acetic acid induced contortions in mice and carrageenan rat paw edema. The preventive effect of TEE (100 mg/kg, per os) and MF (1 mg/kg, 3 mg/kg and 10 mg/kg, per os) was evaluated in carrageenan induced edema at 1, 3 and 5 h. The analgesic effect of MF (1, 3 and 10 mg/kg, per os) was tested in mice contortions. Oral administration of MF (3 mg/kg) showed an analgesic activity less similar than 100 mg/kg of acetylsalicylic acid (ASA) administered in the same conditions (17.60±6.74 vs 26.80±4.66 contortions). The analgesic activity of MF is dose dependent at the lower doses (1 and 3 mg/kg, per os). However, the prevention of mice contortions with MF is less important at a higher dose (30 mg/kg, per os). In rat paw edema, TEE (100 mg/kg, per os) and MF (1 and 3 mg/kg, per os), significantly prevented carrageenan induced edema, as compared to the control and ASA groups. The dose of 3 mg/kg per os of MF induced an edema percentage inhibition similar to 100 mg/kg per os of ASA. Similarly to analgesic activity, the prevention of rat paw edema with MF is less important at a higher dose (10 mg/kg, per os). These results show an analgesic and anti-inflammatory activity of *A. senegalensis* leaf extracts, justifying the use of this plant leaves in African Traditional Medicine to prevent or treat pain and inflammation.

Key words: *Annona senegalensis*, leaves, pain, inflammation.

INTRODUCTION

Inflammation treatment requires glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs). However, they are limited by the several adverse effects such as

peptic ulcer disease and immunosuppression (Wirtha et al., 2006; Henzen, 2003).

Several studies had shown the interest of plant extracts

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on *in-vivo* and *vitro* experimental models of inflammatory and pain processes (Phanse et al., 2012; Hegde et al., 2014). It was reported that the leaves of *A. senegalensis* possess anticonvulsant, central depressant and anxiolytic-like properties attributable to flavonoids (Okoli et al., 2010). An anticonvulsant effect of kaurenoic acid isolated from the root bark of *A. senegalensis* was also described by Okoye et al. (2013). A recent study had shown a toxicity of N-hexane and chloroform fractions of *A. senegalensis* leaf extract on immature stage of mosquito species such as *Anopheles gambiae* and *Culex quinquefasciatus* (Lame et al., 2015).

In African traditional medicine, the leaves of *A. senegalensis* Pers. are used to treat pain and inflammation (Akah and Nwambie, 1994). Yeo et al. (2011) had shown the *in-vitro* anti-inflammatory activity of leaf extract. *A. senegalensis* leaves contain flavonoids, terpenes, glycosides and steroids substances (Ameen et al., 2011; Yisa et al., 2010). Terpene molecules structure isolated from several species belonging to the family Annonaceae showed an analgesic and anti-inflammatory activity.

The aim of the present study was to investigate analgesic and anti-inflammatory activity of the leaf extracts of *A. senegalensis* on contortions-induced acetic acid in mice and carrageenan rat paw edema.

MATERIALS AND METHODS

Study species

A. senegalensis Pers. is an annual shrub or small tree widely distributed in Africa. It is found in semi-arid to subhumid regions of Africa. Leaves are alternate, simple, oblong, ovate or elliptic, green to bluish green, almost without hairs on upper surface but often with brownish hairs on the lower surface. Its aromatic flowers are used to flavour food (Adzu et al., 2005; Ogbadoyi et al., 2007; Orwa et al., 2009).

Plant material and extraction

A. senegalensis leaves were collected from Pout (Senegal). Botanical samples were identified at the Department of Botany and Pharmacognosy, Faculty of Medicine and Pharmacy, University of Dakar, where the voucher specimen (DPB-15-03) was deposited. *A. senegalensis* powder (300 g) was mixed with petroleum ether (2 L), boiled for 2 h and filtered. The ethereal leaf extract filtrate was removed with methanol. The ethereal and methanolic phases were recovered and evaporated to dryness.

Animals

Adult Wistar rats (110 - 160 g) and mice (19 – 26 g) were used for the experiments. The animals had free access to food and water.

Experimental procedures

Anti-inflammatory activity

The rat paw edema was induced with carrageenan 1% (100 μ l)

(Winter et al., 1962). The rats were distributed in groups of 5 and fasted 12 h before the experiment, with free access to water. The initial volume of right hind paw was measured using a plethysmometre.

The total ethereal extract (TEE, 100 mg/kg), methanolic fraction (MF; 1, 3, 10 mg/kg), acetyl salicylic acid (ASA, 100 mg/kg) and vehicle (1 mL/100 g) were given orally to different groups, one hour prior to the local injection of carrageenan 1% into the plantar aponeurosis. The volume of rat hind paw was measured during 5 h, the percentages of increase (INC%) of rat hind paw were determined.

$$\text{INC (\%)} = (V_{T\text{minutes}} - V_o) \times 100/V_o$$

$V_{T\text{minutes}}$: rat hind paw volume at Tminutes; V_o : initial volume of rat hind paw. The anti-inflammatory activity is evaluated by calculating the mean percentage inhibition (%INH) of edema.

$$\text{INH (\%)} = (\% \text{INC}_{\text{control}} - \% \text{INC}_{\text{test compound}}) \times 100/\% \text{INC}_{\text{control}}$$

Analgesic activity

The writhing test in mice was used (Koster et al., 1959). Contortions were induced by intraperitoneal injection of acetic acid 3%. The animals were divided into groups of 5 mice each. Different doses (1, 3 and 10 mg/kg) of methanolic fraction (MF) of *A. senegalensis* total ethereal leaf extract, acetic salicylic acid (ASA) (1, 100 mg/kg) and physiological water (Control, 1 mL/100 g) were administered orally to groups, one hour prior to acetic acid 3% (20 μ L) injection. The total number of contortions was counted at observation time of 30 min.

Statistical analysis

The means of contortions in treated groups were compared to control with Student t-test. A value of $p < 0.05$ had been considered as significant and $n = 5$ represent the number of mice in each group. The means of rat hind paw volumes were compared by an analysis of variance (ANOVA), in order to prove homogeneity between groups. The means of percentages of rat hind paw oedema variations at the 1 and 5 h were also compared to control group with t-test. A value of $p < 0.05$ had been considered as significant and $n = 5$ represent the number of rats in each group. Statistical analysis was done using a GraphPad Prism 5 software.

RESULTS

Effect of methanolic fraction of total ethereal leaf extract of *A. senegalensis* on contortions induced with acetic acid 1% in mice

Intraperitoneal acetic acid 3% induced contortions (72.60 \pm 6.64) in mice which were treated with vehicle *per os*. Pre-treatment with acetylsalicylic acid (1 and 100 mg/kg, *per os*) prevented significantly and dose dependent manner the writhes induced with acetic acid, as compared to the control group. In fact, contortions were respectively 47.80 \pm 8.30 and 26.80 \pm 4.66 at 1 and 100 mg/kg *per os* in acetylsalicylic groups. Prior administration of *A. senegalensis* methanolic fraction (MF) of total ethereal leaf extract (TEE), dose

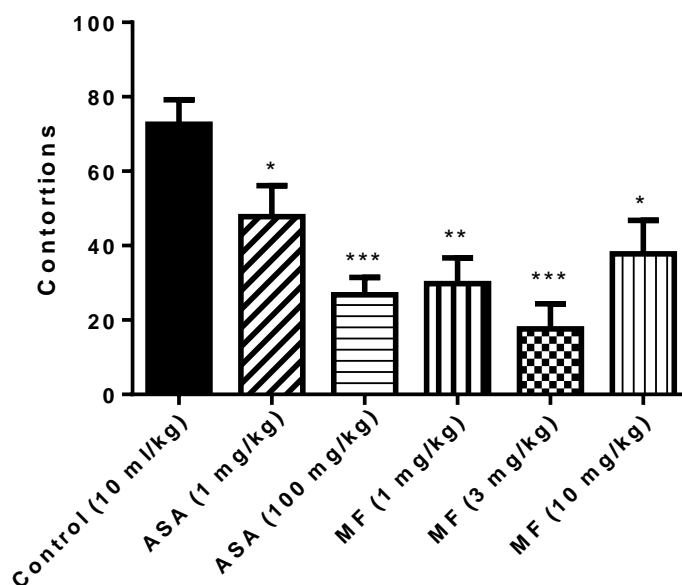


Figure 1. Analgesic activity of methanolic fraction (MF) of total ethereal leaf extract of *A. senegalensis* on contortions induced with acetic acid 3% in mice *P<0.05; ** P<0.01; *** P<0.001 vs. control group. (n=5). ASA = Acetylsalicylic acid.

independently prevented the number of contortions at 1 (29.80 ± 6.92) and 3 mg/kg *per os* (17.60 ± 6.74). However, the prevention of contortions was less important with 10 mg/kg of methanolic fraction (37.80 ± 9.00) (Figure 1).

Effect of total ethereal leaf extract of *A. senegalensis* (TEE) on carrageenan induced rat paw edema

The oral administration of the TEE (100 mg/kg, *per os*) significantly prevented the increase of rat paw edema induced with carrageenan, as compared to group control treated with normal saline (10 mL/kg, *per os*) (Table 1).

Effect of methanolic fraction (MF) of *A. senegalensis* total ethereal leaf extract on carrageenan induced rat paw edema

Oral administration of MF (1 mg/kg, *per os*) did not prevent the inflammatory edema. MF (3 mg/kg, *per os*), significantly prevented the rat paw edema. However, the prevention of inflammatory edema with 10 mg/kg of MF is less important than the prior dose of 3 mg/kg previously tested (Table 2).

The percentage inhibition evaluation shows that MF relatively low dose (3 mg/kg) induced anti-inflammatory activity. The percentages of inhibition were 87.62 ± 3.16 and 74.12 ± 8.38 , respectively at 1 and 5 h, showing a

similar edema inhibition than ASA (100 mg/kg, *per os*) reference group (91.88 ± 1.79 , 69.25 ± 5.48) (Figure 2).

DISCUSSION

The aim of the present study was to evaluate the analgesic and anti-inflammatory extracts of *A. senegalensis* leaves, an African traditional medicine plant. Preliminary experiments had shown a similar anti-inflammatory activity between *A. senegalensis* total ethereal leaf extract and acetylsalicylic acid used in the same conditions.

The analgesic and anti-inflammatory activity of methanolic fraction of total ethereal leaf extract was evaluated on contortions-induced acetic acid in mice and carrageenan-induced paw edema in rats. The methanolic fraction is analgesic and anti-inflammatory at dose dependent manner (1 and 3 mg/kg, *per os*). However, at a higher dose (10 mg/kg, *per os*), both decrease of analgesic and anti-inflammatory activity of methanolic fraction was noted. These results suggest the existence of identical mechanism of action of the methanolic fraction in the prevention of pain and inflammatory edema. This biphasic effect on pain and inflammation observed with the methanolic fraction relative to dose, suggest the probable existence in the extract, compounds which probably act by a functional antagonism mechanism.

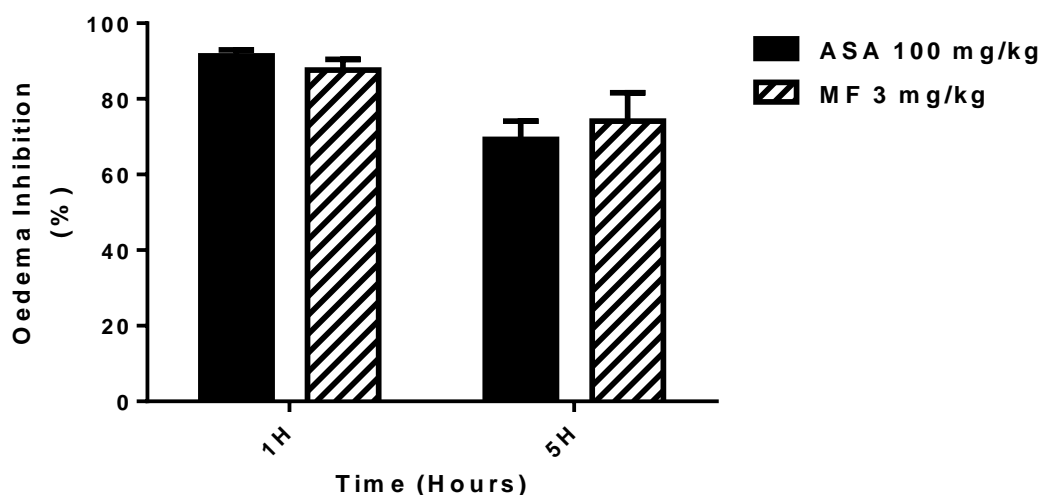


Figure 2. Inhibition of carrageenan induced rat paw edema by oral administration of methanolic fraction (MF) of total ethereal leaf extract of *A. senegalensis*. (n=5). ASA = Acetylsalicylic acid.

Table 1. Effect of total ethereal leaf extract of *A. senegalensis* on carrageenan induced rat paw edema. (n=5). **P<0.01; *** P<0.001 vs. control group. p.o. (*per os*). ASA = acetylsalicylic acid.

Groups	Doses (mL/kg p.o.)	Increased edema (%)		
		1 h	3 h	5 h
Control	10	45.23±11.99	81.13±14.34	103.46±10.01
ASA	100	3.39±0.91**	16.26±6.29***	31.91±6.20***
<i>A. senegalensis</i> (TEE)	100	21.92±4.57	44.00±6.04**	43.57±5.75***

Table 2. Effect of methanolic fraction (MF) of total ethereal leaf extract of *A. senegalensis* on carrageenan induced rat paw edema. (n=5) *P<0.05; ** P<0.01; *** P<0.001 vs. control group; p.o. (*per os*). ASA = acetylsalicylic acid.

Groups	Doses (mL/kg p.o.)	Increased edema (%)		
		1 h	3 h	5 h
Control	10	45.23±11.99	81.13±14.34	103.46±10.01
ASA	100	3.39±0.91	16.26±6.29	31.91±6.20
<i>A. senegalensis</i> (MF)	1	40.00±13.30	81.14±24.51	88.95±22.70
<i>A. senegalensis</i> (MF)	3	4.97±1.51*	20.11±3.52**	24.76±6.96***
<i>A. senegalensis</i> (MF)	10	22.31±3.75	49.66±15.09	52.10±11.20**

At phytochemical level, *A. senegalensis* leaves contain flavonoids and terpenics substances (Ameen et al., 2011). Terpenics molecules isolated from several species of Annonaceae have analgesic and anti-inflammatory activity. This is the example of 18-acetoxy-ent-kaur-16-ene, an isolated molecule of *A. squamosa* bark which is analgesic on model induced by intraperitoneal administration of acetic acid in the albino mice and anti-

inflammatory in the prevention of acute paw edema carrageenan-induced rats (Chavan et al., 2011). The berenjenol triterpenic molecules were isolated from *Oxandra xylopioides*, a species of the family Annonaceae which is anti-inflammatory on the acute and sub-chronic inflammation models (Aquila et al., 2009). Works performed by Chavan et al. (2011) showed that terpenic compounds are extractables in the Soxhlet with

petroleum ether at temperatures of 40 to 60°C.

In this study, the total ethereal leaf extract and its methanolic fraction could probably contain terpenic compounds of *A. senegalensis* leaves. The presence of these molecules may explain the origin of analgesic and anti-inflammatory activity of the total ethereal leaf extract of *A. senegalensis* and its methanolic fraction.

Conclusion

The leaf extracts of *A. senegalensis* prevented pain and inflammation in experimental acetic acid induced contortions in mice and carrageenan rat paw edema. These results justify the use of this plant leaves in African traditional medicine to prevent or treat pain and inflammatory processes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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