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Research Paper

Analgesic and anti-inflammatory activities of Asena, a herbal preparation for treatment of arthritis, using rodent models

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Abstract. The analgesic and anti-inflammatory activities of Asena, a herbal decoction for the treatment of arthritis, were assessed in various murine models. The analgesic activity was evaluated using the yeast-induced mechanical hyperalgesia, hot plate, acetic acid abdominal constriction and formalin tests while the anti-inflammatory effect was assessed in the carrageenan-induced paw edema test. The possible involvement of the opioidergic system in the analgesic activity was also carried out in the acetic acid writhing test. The results showed that Asena possesses potent ($p < 0.05$) analgesic and anti-inflammatory activities in all the test models employed in the current study. Systemic administration of naloxone, a non-selective opioid receptor antagonist, reversed the analgesic activity of Asena in the abdominal constriction test. It could be concluded that Asena has analgesic and anti-inflammatory effects which may be mediated via both peripheral and central mechanisms. The analgesic activity possibly involves the opioidergic system. These findings thus support its ethnomedicinal use and the anecdotal claims for its use in the treatment of arthritis.

Keywords: Analgesic, anti-inflammatory, Asena, morphine, opioidergic system.

INTRODUCTION

The use of natural products is growing in the world especially in developing countries. In Ghana, the use of plant medicine was hitherto restricted to the rural folks but recently its use is becoming popular among urban dwellers as well. It is estimated that nearly 80% of the world's population and perhaps a greater percentage in rural Africa rely on traditional medicines for their health care (WHO, 2002; Willcox and Bodeker, 2004).

Virtually all known disease conditions both pathogenic and non-pathogenic are accompanied by pain. Inflammation is an intricate pathophysiological condition mediated by several signaling molecules produced by leucocytes, macrophages and mast cells and the activation of complement factors that bring about edema formation as a result of extravasation of fluid and proteins and the accumulation of leucocytes at the inflammatory site (Donkor et al., 2013). Analgesic and anti-inflammatory therapies rely on: non-steroidal anti-inflammatory drugs (NSAIDs), opioids and a group of drugs with diverse pharmacological actions collectively called adjuvant. Most of these drugs are often expensive, inaccessible and cause undesired and serious adverse effects (Warner and Mitchel, 2002; Babu et al., 2009) necessitating the need for the development of novel potent and safer alternatives. It has been argued that natural drug substances provide less toxic and more affordable drug molecules (Olatunji and Atolani, 2009).

Asena is an aqueous decoction prepared by the Centre for Scientific Research into Plant Medicine (CSRPM) for the treatment of arthritis for over two decades. It is made from seven medicinal plants namely *Khaya senegalensis, Kigelia africana, Nauclea latifolia, Clausena anisata,*

Piliostigma thonningii, Trichilia monadelpha and *Strophanthus hispidus.* These plants have various uses in folkloric medicineacross Africa. For instance, in Ghana *K. senegalensis* is used to treat malaria, male sexual dysfunction, dysentery and rheumatism (Abbiw, 1990) while *C. anisata*is used in Nigeria as effective remedies for worms infections, respiratory ailments, hypertension, malaria, fever, rheumatism, arthritis and other inflammatory conditions (Senthikumar and Venkatesalu, 2009). Similarly *P. thonningii* is used to arrest bleeding, as laxatives, to treat fevers, bacterial infection and inflammation while *S. hispidus* is a medicinal plant widely used in the treatment of rheumatic afflictions, ulcer, conjunctivitis, leprosy and skin diseases (Igbe et al., 2012; Ishola et al., 2013). The analgesic and anti-inflammatory activities of all the seven plants used in the preparation of Asena have been well documented in previous studies (Lompo et al., 2007; Owolabi and Omogbai, 2007; Goji et al., 2010; Okokon et al., 2012; Woode et al., 2012; Igbe et al., 2012; Ishola et al., 2013).

Though Asena has been administered for the treatment of arthritis by the Clinical Department of CSRPM for over two decades for the management of arthritis, its analgesic activity has not been authenticated in preclinical studies. Since most agents that possess analgesic activity also have anti-inflammatory activity, we sought to evaluate the analgesic and anti-inflammatory activities of Asena in rodent analgesic and anti-inflammatory models.

MATERIALS AND METHODS

Reagents and Chemical

Diclofenac sodium salt, aspirin, naloxone, yeast from *Saccharomyces cerevisiae,* Type II and Carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Indomethacin was purchased from Cayman Chemical Company (Michigan, USA) and morphine hydrochloride was obtained from TettehQuarshie Memorial Hospital, Mampong-Akwapim. All other chemicals were purchased in their purest form available from British Drug House (BDH) Ltd. (Poole, UK).

Preparation of Asena extract

A total of ten bottles of Asena (330 ml) was obtained from the Production Store of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem in the Eastern Region of Ghana. They were emptied in to a clean vessel making a total volume of 3.30 L. It was lyophilized to produce a dry weight of 22.12 g (0.67%, w/v). The lyophilized Asena extract was kept in a dry place and reconstituted in distilled water before administration.

Phytochemical screening

The Asena extract was screened for the presence or absence of groups of phytochemicals such as saponins, reducing sugars, phenolics, cyanogenic glycosides, polyamides, phytosterols, triterpenes, anthracenosides, flavonoids and alkaloids (Sofowora, 1982; Harborne, 1983).

Animals

Male Sprague-Dawley rats (220 to 240 g) and female C_3H/He mice (24 to 26 g) were obtained from the Animal Unit of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem, in the Eastern Region of Ghana. The animals were fed on animal chow obtained from Ghana Agro Food Company (GAFCO) Tema, Ghana. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH, No. 85-23, revised 1985). Ethical clearance was obtained from the CSRPM's ethics committee and the paper was written in accordance with the ARRIVE guidelines (Kilkenny et al., 2010).

Acute toxicity studies

A single oral dose (OECD, 2001) of the Asena extract was administered at 5000 mg kg^{-1} body weight to six rats and six mice each with an oral gavage needle. Mortality and general behavior of the animals were observed over a 48 hour period. Surviving animals were observed for a further period of 12 days for toxic symptoms of pilo-erection, lachrymatory, locomotor and respiratory activities.

Yeast-induced mechanical hyperalgesia

The mechanical nociceptive thresholds were measured in the rat paw pressure by adopting the Randall and Sellito, (1957) method as modified by Donkor et al. (2013) using an analgesimeter (7200, UgoBasile, Comerio- Varese, Italy). Briefly, rats received three training sessions prior to the testing day and animals that had paw withdrawal thresholds (PWTs) of less than 30 g and more than 250 g were excluded. Selected animals were put in 7 groups of 5 animals each and after baseline measurements were taken,3 groups were administered Asena extract at doses of 3, 10 and 30 mg kg^{-1} p.o. A further 3 groups were administered aspirin similarly as the Asena and a control group received equivalent volume of distilled water. Thirty minutes after the administration of the drugs, 0.1 ml of 20% w/v yeast was injected into the subplanter region of the right hind paw of the rats. The PWTs were measured at 0.5, 1, 2 and 4 h post-yeast administration. The analgesic activity (k) of the drugs was calculated using

previously described formula

$$k = \frac{a + b + c + d}{e \times 4} \times 100\%$$

where e is the baseline PWT and a, b, c and d are the PWTs at 1st, 2nd, 3rd and 4th readings (Donkor et al., 2013).

Hot plate test

The thermal anti-nociceptive model was carried out using a hot/cold plate system (35100, Comerio-Varese, Italy) by following previously described method (Donkor et al., 2013). Prior to the test, animals had three training sessions and those that recorded a reaction time 4 to 9 s on the hot plate (55°C) were used for the experiment. A cut off latency time of 40 s was used to avoid possible tissue damage. Selected mice were divided into groups of 5 animals and 3 groups were administered Asena extract at doses of (3, 10 and 30 mg kg^{-1} p.o.). A further 3 groups were administered morphine similarly as the Asena and a control group received equivalent volume of distilled water. The latency time for each animal was recorded after 30 min and hourly till the 4th hour post drug administration. The analgesic activity calculated as the maximum possible effect (MPE) was carried out using previously described formula:

$$MPE\ (\%) = \frac{L_{t-L_o}}{40 - L_o} \times 100\%$$

L_o and L_t are the latency at baseline and a given time in seconds (Donkor et al., 2013).

Acetic acid induced writhing test

The acetic acid induced writhing test was carried out in mice by adopting a previous method (Donkor et al., 2013). Test animals were put into 7 groups of 5 and 3 groups were administered Asena extract at doses of 3, 10 and 30 mg kg^{-1} p.o. A further 3 groups were administered diclofenac similarly as the Asena and a control group received equivalent volume of distilled water. Thirty minutes after administration of extract/drug, 0.1 ml of acetic acid (0.6% v/v in distilled water) was injected intraperitoneally. The contraction of abdominal muscles together with stretching of the hind limbs was recorded cumulatively over a period of 30 min at 5 min time interval post-acetic acid injection.

Formalin test

The formalin test was carried out in rats using previously described method (Donkor et al., 2013). Each animal was placed in one of 20 test chambers (Perspex chamber, 18 × 18 × 18 cm^3) to acclimatize for 30 min before the experiment. The animals, 5 per group, were then administered orally with the Asena extract at doses of 3, 30 and 30 mg kg^{-1} and three other groups administered with diclofenac (3, 10 and 30 mg kg^{-1}, p.o.). A control group was administered an equivalent volume of distilled water. Thirty minutes after administration of extract/drug each animal was injected intraplantarly with 50 µl of 5% formalin and returned to their respective observation chamber. A mirror was mounted at 45° behind the test chamber to allow for an unobstructed view and recording of the behavior of the animals by a camcorder placed in front of the mirror. Nociceptive response was quantified for 60 min post formalin administration in 5 min time intervals by counting the incidents of spontaneous biting/licking of the injected paw using the public domain software JWatcher, version 1.0 (University of California, LA, USA, and Macquarie University, Sidney, Australia, available at http://www.jwatcher.ucla.edu/). In the present study, the neurogenic phase was defined as 0 to 5 min and the inflammatory phase 10 to 60 min post-formalin administration.

Involvement of the opioidergic system

To evaluate the possible involvement of opioid system in the analgesic effects of Asena, naloxone, (2 mg kg^{-1}, *i.p.*, a non-selective opioid receptor antagonist), was administered to mice (Donkor et al., 2013). After 15 min mice were administered orally with 30 mg kg^{-1} of the extract and a control group given equivalent volume of distilled water. Thirty min after administration of extract, 0.1 ml of acetic acid (0.6% v/v in distilled water) was injected intraperitoneally. The contraction of abdominal muscles together with stretching of the hind limbs was recorded cumulatively over a period of 30 min at 5 min time interval post-acetic acid injection.

Carrageenan-induced edema

The anti-inflammatory activity of Asena was determined by the Carrageenan-induced edema test by adopting previously reported method (Donkor et al., 2013) using a plethysmometer (7150 Ugobasile, Comerio- Varese, Italy). Rats were put into 7 groups of 5 and 3 groups were administered Asena extract at doses of (3, 10 and 30 mg kg^{-1} p.o.). A further 3 groups were administered indomethacin similarly as the Asena and a control group received equivalent volume of distilled water. Thirty minutes after administration of drugs, 0.1 ml of 1% w/v carrageenan in normal saline was injected into the subplanter region of the right hind paw of the rats. The paw volume (ml) of the right hind limbs were measured prior to the induction of edema (baseline) and thereafter

Table 1. Phytochemical screening of extracts of Asena.

Groups of phytochemicals	Charaterization tests
Saponins	+
Cyanogenic glycosides	-
Reducing sugars	+
Phenolics	+
Polyuronides	-
Alkaloids	-
Anthracenosides	-
Flavonoids	-
Triterpenes	-
Phytosterols	-
Polyamides	+

+: Present; -: Absent

readings were taken hourly till the 4th h post drug administration. The anti-inflammatory (e) activity was calculated using the formula:

$$e = \frac{E_{t-E_o}}{E_o} \times 100\%$$

where E_o and E_t are paw volume at baseline and at a particular reading time of the right hind paw (Donkor et al., 2013).

Statistical analysis

The one way Analysis of variance (ANOVA) and where applicable followed by Bonferroni post-hoc tests were conducted between control and tests to determine statistical significance. The 5% level of probability was used as criterion of significance in all instances. Graphics andstatistical analysis were done using Graph Pad Prism software version 5.0. Doses for 50% of the maximal effect (ED_{50}) for each drug were determined by using an iterative computer least square method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a - (b - a)}{\left(1 + 10^{(\mathrm{LogED_{50}} - X)}\right)}$$

where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

RESULTS

Phytochemical screening

The results of the phytochemical screening of the Asena extract are shown in Table 1. The preparation showed the presence of saponins, phenolics, reducing sugars and polyamides.

Acute toxicity studies

The effect of a single oral dose of Asena (5000 mg kg^{-1}) administered to six rats and six mice showed no deaths in both animal species suggesting the oral LD_{50} of the extracts is greater than 5000 mg kg^{-1} body weight for both mice and rats. Observations of animals over the next 12 days showed no adverse effects of treatment. There were no physical signs of toxicity as evidenced by normal respiratory, locomotor and lachrymatory activities and the absence of pilo-erection.

Yeast-induced mechanical hyperalgesia

The effects of Asena and Aspirin on the yeast-induced mechanical hyperalgesia are represented in Figure 1. An hour after sub-plantar injection of yeast, the ipsilateral paw showed marked hyperalgesia in all experimental rats which was significantly reduced in both the extract and Aspirin ($p < 0.05$) rats in a dose-related manner compared to controls. Asena (ED_{50} = 7.29 ± 1.98 mg kg^{-1}) was more potent than Aspirin (ED_{50} = 10.25 ± 9.54 mg kg^{-1}) (Table 2).

Hot plate test

The effects of Asena and morphine on the latency times of mice in the hot plate test (calculated as %MPE) are presented in Figure 2. Prior to commencement of experiment, mice showed a baseline reaction time of about 4 to 9 s, which was sustained in control animals throughout the 3 h study period. Asena (3 to 30 mg kg^{-1}, p.o.) and morphine (3 to 30 mg kg^{-1}, p.o.) significantly increased the reaction time of mice ($p < 0.01$) with maximum effect at 30 mg kg^{-1}. From the ED_{50} values obtained (Table 2), Asena (5.89 ± 0.87 mg kg^{-1}) was comparable to morphine (4.75 ± 0.77 mg kg^{-1}) in potency.

Acetic acid induced writhing test

Figure 3 represents the cumulative number of writhes in 5 min intervals for 30 min post-acetic acid injection. The writhing activity (contraction of abdominal muscle and stretching of hind limbs) started about 3 min after acetic acid injection in all experimental mice and increased dramatically in controls to peak around the 20 to 25th min and then receded. Asena (3 to 30 mg kg^{-1}) and diclofenac (3 to 30 mg kg^{-1}) significantly ($p < 0.0001$) reduced the writhing action in a dose-dependent fashion at the tested dose levels compared to controls. The ED_{50} of Asena

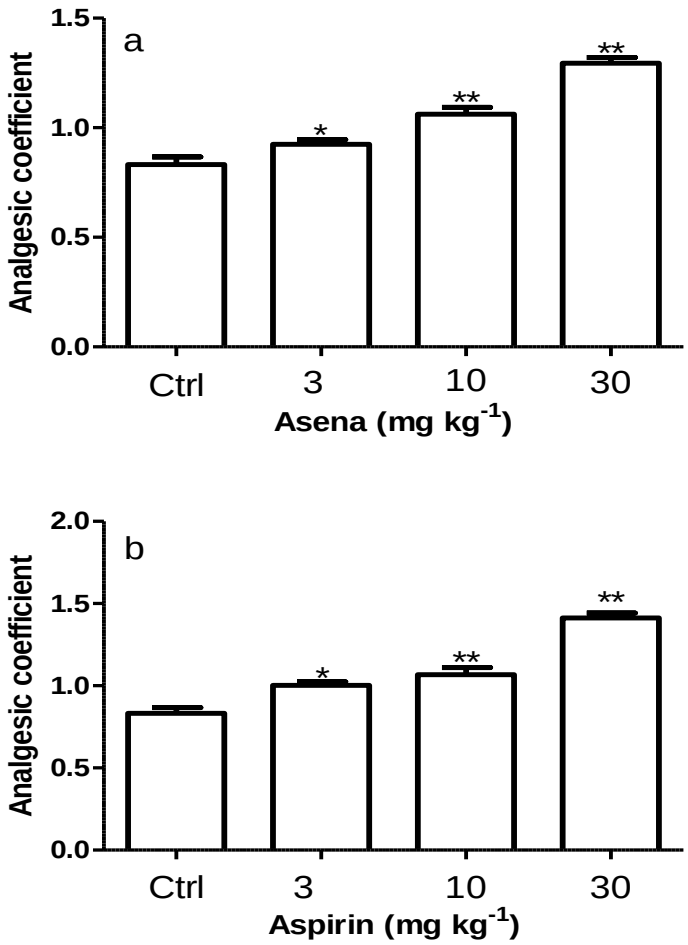

Figure 1. (a) Effect of Asena (3 to 30 mg kg^{-1}, p.o.) and (b) aspirin (3 to 30 mg kg^{-1}, p.o.) on yeast-induced hyperalgesia in rats. Values indicates Mean ± SEM (n = 5). *p < 0.05, **p < 0.01, compared to untreated controls.

Table 2. ED_{50} values of Asena and various standard drugs used in the various assays.

Test	ED_{50} values (mg kg^{-1})				
	Asena	Indomethacin	Diclofenac	Aspirin	Morphine
Anti-inflammatory	13.66 ± 5.56	11.93 ± 2.35			
Formalin	4.20 ± 0.65[a]		8.65 ± 1.54[a]		
	13.38 ± 2.51[b]		27.28 ± 3.21[b]		
Mechanical Hyperalgesia	7.29 ± 1.98			10.25 ± 9.54	
Hot plate	5.89 ± 0.87				4.75 ± 0.77
Writhing	7.35 ± 2.01		5.79 ± 0.59		

[a]First Phase of the formalin test; [b]Second Phase of the formalin test

(7.35 ± 2.01 mg kg^{-1}) is comparable to that of diclofenac (5.79 ± 0.59 mg kg^{-1}) (Table 2).

Formalin test

The analgesic effects of Asena and diclofenac in the formalin-induced nociceptive test are shown in Figure 4. Sub-plantar administration of formalin evoked biphasic nociceptive behavior (flinching, lifting, shaking and licking of injected paw) in test animals. Asena and diclofenac produced significant ($p < 0.01$)] dose-dependent inhibition of the nociception caused by the injection of formalin in mice in both the first and second phase compared to

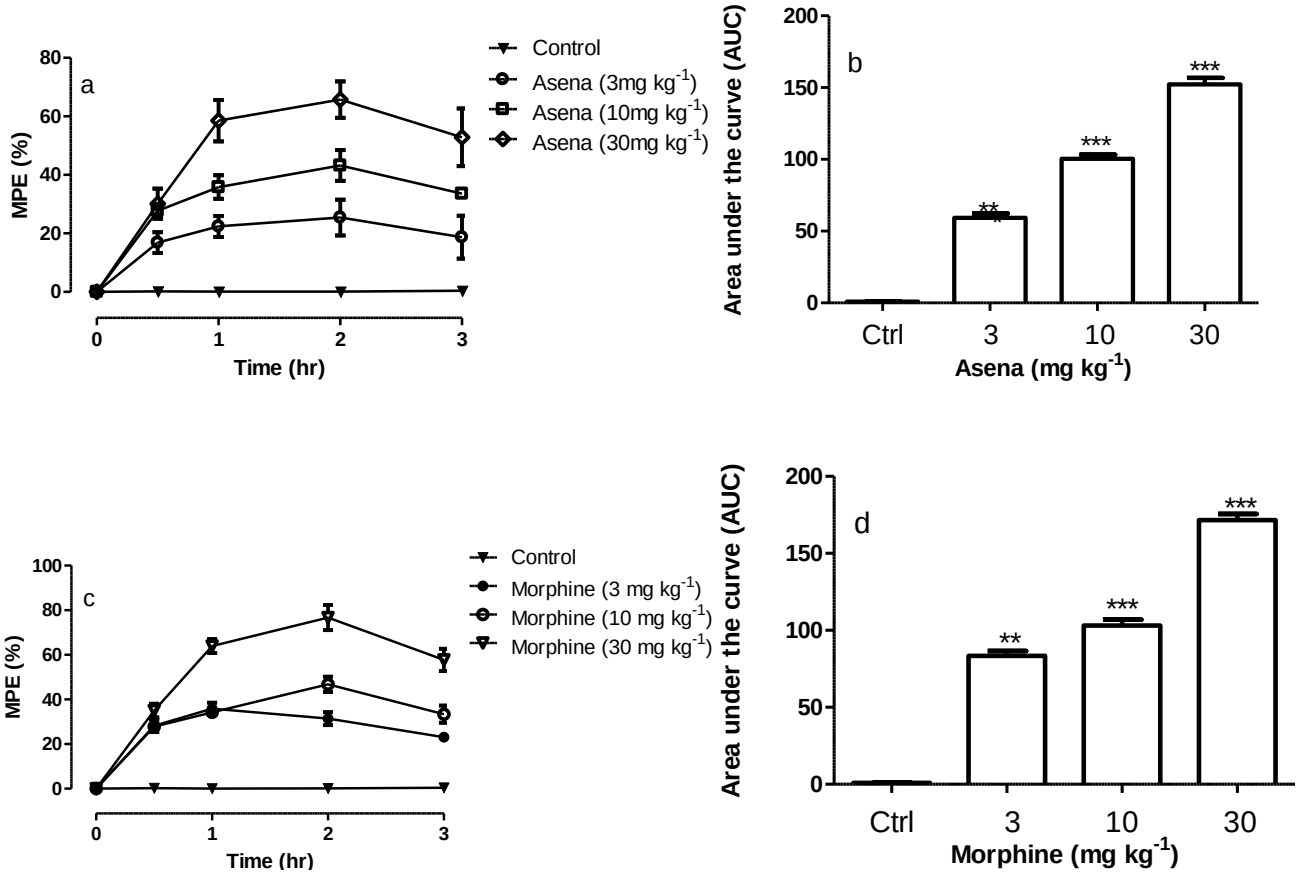

Figure 2. (a) Effect of Asena (3 to 30 mg kg^{-1}, *p.o.*) and (c) morphine (3 to 30 mg kg^{-1}, *p.o.*) on the time course of maximum possible effect (MPE %)of mice in hot plate test; (b) and (d) are AUC's determined from (a) and (c) respectively. Each point indicates Mean ± SEM (n = 5). **p < 0.01, ***p < 0.001 compared to untreated controls.

controls. Asena (3 to 30 mg kg^{-1}, *p.o.*) is more potent in both the neurogenic phase (ED_{50} = 4.20 ± 0.65 mg kg^{-1}) and the inflammatory phase (ED_{50} = 13.38 ± 2.51 mg kg^{-1}) than the diclofenac (3 to 30 mg kg^{-1}, *p.o.*) in the neurogenic phase (ED_{50} = 8.65 ± 1.54 mg kg^{-1}) and the inflammatory phase (ED_{50} = 27.28 ± 3.21 mg kg^{-1}) (Table 2).

Involvement of opioidergic system

Results presented in Figure 5 show that pre-treatment of mice with naloxone (2 mg kg^{-1}, *i.p.*) almost completely reversed the anti-nociceptive action of the Asena extract.

Carrageenan-induced edema

The effects of Asena and indomethacin on carrageenan-induced paw edema are shown in Figure 6. The sub-plantar injection of carrageenan caused an increase in paw volume of all experimental rats with controls experiencing a sustained increase in paw volume over the 3 h study period which peaked after an hour post-edema induction (Figure 6a and 1c). Both the Asena extract and indomethacin caused significant (p < 0.05) dose-dependent reduction in the percentage edema formation compared to the controls. The potency of Asena (ED_{50} = 13.66 ± 5.56 mg kg^{-1}) and indomethacin (ED_{50} = 11.93±2.35 mg kg^{-1}) were also comparable (Table 2)

DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of these were based on the uses of the agents in traditional medicine (Cordell, 2000). Asena is an herbal decoction used in our clinic with anecdotal evidence for its use for the treatment of arthritis. The present study shows that the preparationhas potent analgesic and anti-inflammatory activities in rodent analgesic and anti-inflammatory models.

The Randall and Sellito, pressure test is a pain model which is often used to distinguish between central and peripheral analgesic actions. Inflammation is known to

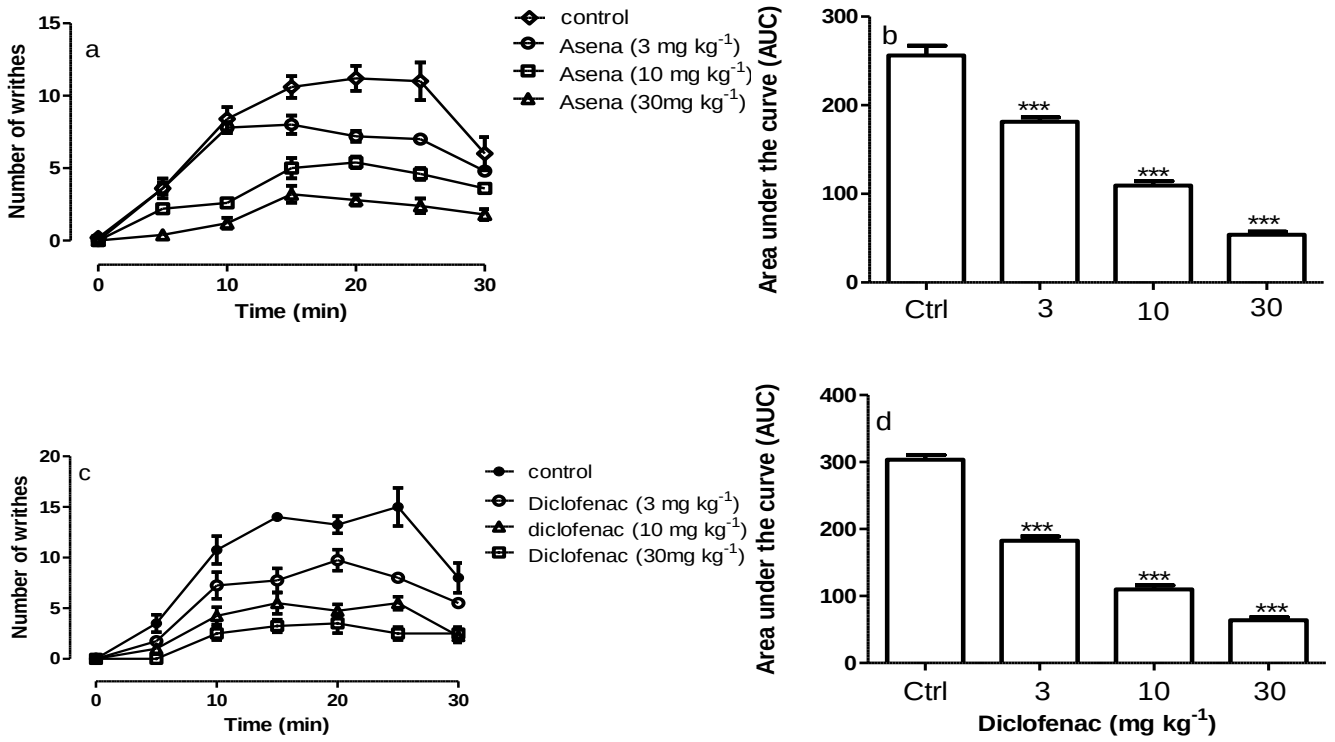

Figure 3. (a) Effect of Asena (3 to 30 mg kg^{-1}, p.o.) and (c) diclofenac (3 to 30 mg kg^{-1}, *p.o.*) on the time course of acetic acid writhing test in mice; (b) and (d) are AUC's determined from (a) and (c) respectively. Each point indicates Mean ± SEM (n = 5). ***p < 0.001 compared to untreated controls.

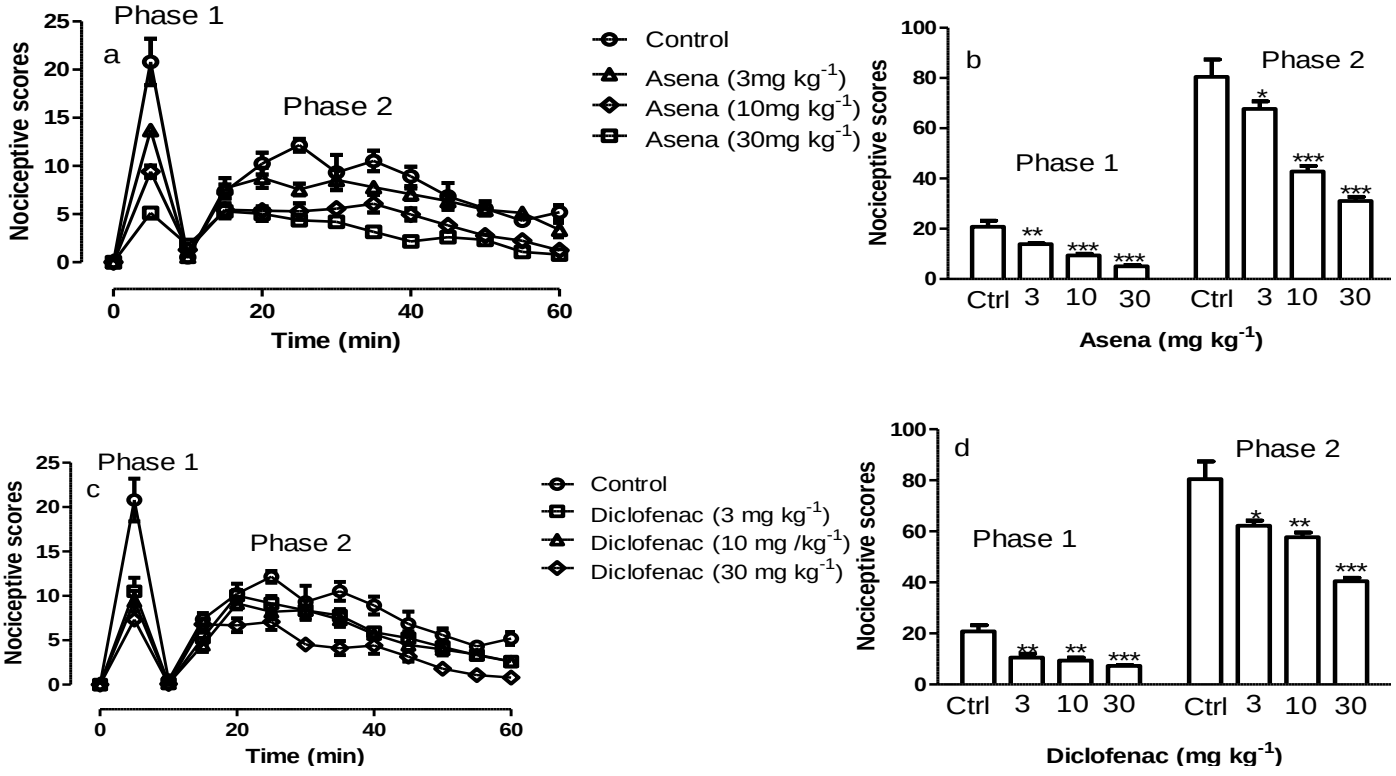

Figure 4. (a) Effect of Asena (3 to 30 mg kg^{-1}, *p.o.*) and (c) diclofenac (3 to 30 mg kg^{-1}, *p.o.*) on the time course of formalin induced nociception in rats; (b) and (d) are total cumulative nociceptive responses determined from (a) and (c) respectively. Each point indicates Mean ± SEM (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 compared to untreated controls.

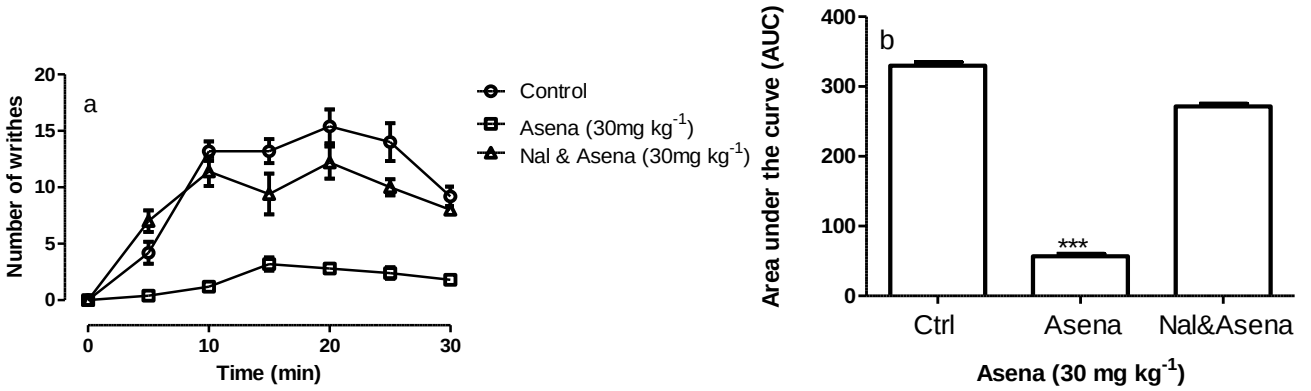

Figure 5. (a) Effect of naloxone, (2 mg kg^{-1}, *i.p.*) on the anti-nociceptive activity of Asena (30 mg kg^{-1}, *p.o.*) in acetic acid writhing test in mice. Each point indicates Mean ± SEM (n = 5). ***p < 0.001 compared to untreated controls.

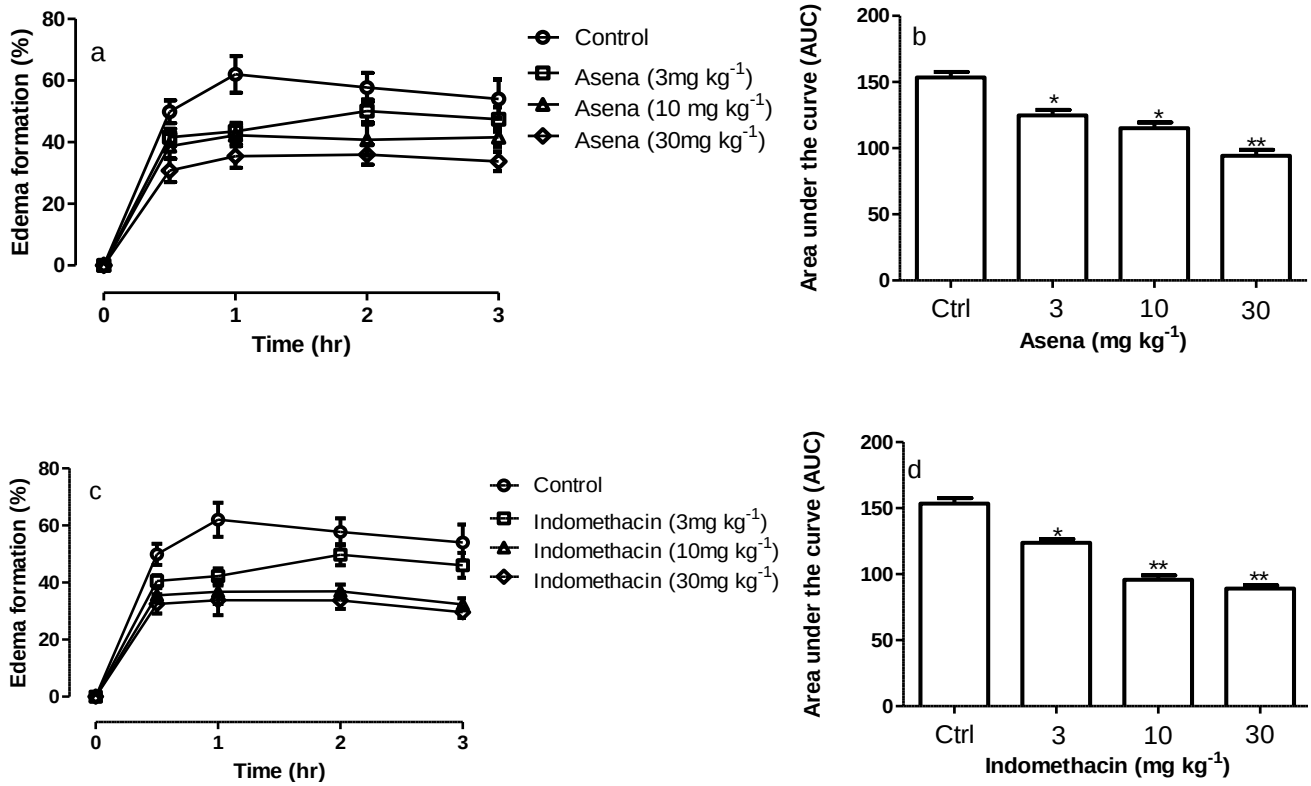

Figure 6. (a) Effect of Asena (3 to 30 mg kg^{-1}) and (c) indomethacin (3 to 30 mg kg^{-1}, *i.p.*) on the time course of carrageenan induced edema in rat paw; (b) and (d) are AUC determined from (a) and (c). Each point represents Mean ± SEM (n = 5). *p < 0.05, **p < 0.01 compared to untreated controls.

cause a lowering of the thresholds of various mechanoreceptors and mechanotransduction pathways (Park et al., 2008). In the yeast-induced hyperalgesia test which detects the time to movement of an inflamed hind paw to noxious stimuli revealed hyperalgesia in all yeast-treated animals. The Asena and Aspirin showed a significant ($p < 0.05$) dose-dependent analgesic activity when compared to untreated control. However, the Asena had a higher potency (ED_{50} = 7.29 ± 1.98 mg kg^{-1}) than Aspirin (ED_{50} = 10.25 ± 9.54 mg kg^{-1} (Table 2) suggesting the preparation may be a more effective analgesic in managing acute hyperalgesia than Aspirin. In a recent study, *T. monadelpha,* one of the plants used in the preparation of Asena showed analgesic activity in the yeast-induced hyperalgesia test (Woode et al., 2012).

The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used to specifically study central

nociceptive activity. The Asena and morphine caused a significant ($p < 0.01$) dose-dependent increase in the latency time of test mice compared to untreated controls. The opioid agents such as morphine exert their analgesic effects via supra spinal and spinal receptors (Nemirovsky et al., 2001). The present result suggests that the extracts may act via a similar mechanism (Le Bars et al., 2001). In previous studies, *C. anisata* and *K. senegalensis* caused a dose-dependent increase in reaction time in the tail-flick test (Lompo et al., 2007; Okokon et al., 2013).

The intraperitoneal injection of acetic acid causes the release of inflammatory mediator which excites nociceptors and it is an assay used to screen for both peripheral and central acting agents. It has been shown that the abdominal constriction is as a result of sensitization of nociceptors to prostaglandins (Bose et al., 2007). The Asena extract and diclofenac showed significant ($p < 0.01$) dose-related reduction in the number of writhes of test animals compared to controls. The NSAIDs including diclofenac reduce the number of writhes in this test by inhibiting cyclooxygenase in peripheral tissues by blocking the release and/or synthesis of inflammatory mediators (Panthong et al., 2007) suggesting the extract may be acting via a similar mechanism. Previous studies show that *P. thonningii, T. monadelpha* and *S. hispidus*all have demonstrable analgesic activities in the abdominal constriction test (Igbe et al., 2012; Woode et al., 2012; Ishola et al., 2013).

Oral administration of the Asena extract and diclofenac significantly and dose-dependently inhibited ($p < 0.05$) both the neurogenic (first) and inflammatory (second) phases of formalin-induced nociception in rats. Centrally acting drugs, such as opioids, inhibit both phases of the nociceptive response equally (Shibata et al., 1989) while many NSAIDs and corticosteroids inhibit only the inflammatory phase suggesting Asena may be acting via both peripheral and central mechanisms to produce analgesic effect in this test. The inhibitory effect of Asena in the second phase also suggests its anti-inflammatory action.

In order to assess possible involvement of the opioidergic system in the analgesic effects of Asena, naloxone was administered prior to testing. Naloxone, a non-selective opioid antagonist almost reversed the anti-nociceptive effect (Figure 5) of the extract suggesting activation of opioid receptors and/or an increase in endogenous opioids (Bjorkman et al., 1990) centrally and/or peripherally may be involved in eliciting the analgesic effect of the preparation. This observation together with the potent analgesic activity in the thermal assay confirms the involvement of central mechanism in the analgesic activity.

Carrageenan induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs since the relative potency estimates obtained from most drugs tend to reflect clinical experience (Winter et al., 1962). Induction of inflammation by carrageenan involves three distinct phases of mediator release. The first phase involves the release of histamine and serotonin, and lasts between the first to the second hour; the second phase is the release of kinins lasting from the second to the third hour; while the third phase involves the release of prostaglandins and lasts from the third to the fifth hour (DiRosa et al., 1971; Surender and Mafumdar, 1995). Histamine and serotonin are implicated in the increase of vascular permeability and prostaglandins mediate maximum vascular responses during the third phase of inflammation (Vinegar et al., 1969). The Asena extracts and indomethacin showed significant ($p < 0.05$) dose-dependent edema inhibition when compared to untreated controls. It has been shown that the ethanolic extracts of the stem bark of *K. africana,* one of the plants used in the preparation of Asena, has anti-inflammatory activity (Owolabi and Omogbai, 2007). This data together with the potent analgesic activity in the inflammatory phase of the formalin test confirms the anti-inflammatory activity of Asena.

In all the assays, the Asena extract had comparable or lower ED_{50} than all the standard drugs used in the various tests (Table 2). Asena, thus, appears to be a good potential analgesic and anti-inflammatory drug candidate.

The 5000 mg kg^{-1} used in the acute toxicity studies did not result in the death of test animals or any sign of toxicity suggesting the LD_{50} is greater than 5000 mg kg^{-1}. The result of the phytochemical screening revealed that Asena contains saponins, phenolics, reducing sugars and polyamides.It has been reported that such phytochemicals as phenolics and saponins possess analgesic and anti-inflammatory activities (Lee et al., 2006; Zhao et al., 2013). Thus the analgesic and anti-inflammatory effects observed in the current studies may be attributable to one or more of the groups of phytochemicals.

In conclusion, it may be said that Asena has analgesic and anti-inflammatory effects which may be mediated via both peripheral and central mechanisms. The analgesic activity possibly involves the opioidergic system. These findings thus support its ethnomedicinal use and the anecdotal claims for its use in the treatment of arthritis.

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