



Evaluation of Methanol Extract of *Argemone mexicana* Aerial Part on Nociception, Inflammation and Gastrointestinal Motility

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ABSTRACT

Argemone mexicana Linn. (Family: Papaveraceae) is traditionally used to relieve inflammatory and pain-related health conditions. It is also used as laxative. The intraperitoneal median lethal dose (LD_{50}) of the methanol extract was determined in mice. The methanol extract of the plant was evaluated for antinociceptive effect using acetic acid-induced writhing test in mice and formalin-induced pain test in rats. Fresh egg albumin-induced oedema model was used to test for its anti-inflammatory effect in rats while the gastrointestinal effect was tested using gastrointestinal motility (transit) model in mice. The intraperitoneal LD_{50} value in mice was calculated to be 894.4 mg/kg. The extract (75, 150 and 300 mg/kg i.p) significantly ($p < 0.05$) and dose-dependently inhibited acetic acid-induced pain with the values of 44.83%, 68.97% and 81.38% respectively. Acetyl salicylic acid (ASA; 150 mg/kg i.p) also significantly ($p < 0.05$) inhibited pain with inhibitory value of 65.52%. The extract (75, 150 and 300 mg/kg i.p) significantly ($p < 0.05$) and dose-dependently inhibited the early phase of formalin-induced pain with values of 56.76%, 75.68% and 78.38% respectively. The extract at the same doses also inhibited the late phase of formalin-induced pain in a dose-dependent manner with the values of 39.51%, 55.56% and 74.08% respectively. The late phase inhibition was only significant ($p < 0.05$) at the doses of 150 and 300 mg/kg i.p. The magnitude of pain inhibition by the extract was higher in the early phase. The extract (75, 150 and 300 mg/kg i.p) produced a dose-dependent inflammatory inhibition with percentages of 15.00 %, 25.00 % and 73.34% respectively. ASA (150 mg/kg i.p) produced 36.67% inhibition. The extract produced gastrointestinal movement inhibition of 54.70%, 55.47% and 73.32% respectively. The inhibition was significant ($p < 0.005$) only at 150 and 300 mg/kg i.p while atropine (0.1 mg/kg i.p) produced a significant ($p < 0.05$) inhibitory effect with percentage of 63.94%. The study provided scientific justification for the traditional use of *A. mexicana* in pain and inflammatory health conditions but did not justify its use as a laxative.

Key words: *Argemone mexicana*, Nociception, Anti-inflammation, Laxative, Acute Toxicity

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INTRODUCTION

Argemone mexicana Linn. (Family: Papaveraceae) is a species of poppy found in Mexico and now widely naturalized in many parts of the world including many tropical and subtropical regions. It is an erect, branched annual to perennial herb with grayish white stem secreting yellow coloured latex. The leaves are exstipulate, deeply lobed with thorny margins. It grows to a height less than one metre. It is an extremely hardy plant tolerant of drought and poor soil [1;2]. It is commonly called 'Mexican poppy', 'Mexican prickly poppy', 'Cardo or Cardosanto' in its native area, the natives of the Western United States and parts of Mexico [3]. In Nigeria, it is called 'kaju' or 'Ahon ekun' by Yorubas and 'kwarko' or 'kadinnia' among the Hausas [4]. Ethnomedicinal reports have shown a very wide usage of *A. mexicana* in folklore medicine. These reports include the use of *A. mexicana* for the relief of inflammatory and pain-related health conditions such as kidney pain, migraine headaches, dental disorders, skin infections, itches, leprosy, inflammations, venereal sores, boils, ulcers, scorpion sting, snake bites, bronchitis. It is used as sedative and analgesic tea. The whole plant is also used as purgative [3;4;5;6;7;8;9;10;11;12].

The use of medicinal plants for treating various ailments ranging from acute to chronic conditions has become an alternative health care option for people in rural communities. This is governed by economic factors, ease of availability and strong belief in plant remedies [13]. However, it is worthy to note that even in urban and technologically advanced societies, the consumers' preference is shifting from pure synthetic to natural based drugs [14; 15]. The medicinal values of these plants lie in bioactive phytochemical constituents that produce definite physiological action in the body [16]. Hence, the need for scientific evaluation of these ethno medicines for the purpose of validating the traditional claims and for the development of safe, effective and globally accepted herbal drugs.

The objective of this study was to establish the scientific justification for the ethnomedicinal use of *A. mexicana* for pain and inflammatory-related health conditions and its use as laxative. The findings would also be valuable for future drug development considering that medicinal plants are important sources of new chemical substances with potential therapeutic effects [17].

MATERIALS AND METHODS

Plant Collection and Extraction

The plant was collected in the month of November from Idu, Abuja situated at 9°06'37"N 7°33'82"E, Nigeria. The plant was identified by Mr. Ibrahim Muazzam, a plant Taxonomist with the Herbarium and Ethnobotany Unit, Department of Medical Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The specimen was given a voucher number: NIPRD/H/6589.

1.5 kg of the aerial part of the plant material was air-dried on laboratory bench and then pulverized in a mortar. 90 g of the powder was macerated in 1L of methanol for 48h on a shaker (GFLD 3006 mgH Germany). The extract was filtered using Whatman number 1 filter paper. The filtrate was then evaporated with rotary evaporator (KNF RC 900 Neuberger, USA) and crude extract was brought to complete dryness over water bath. The extract was then kept in a refrigerator (4° C) for further studies.

Drugs and Chemicals

Drugs and chemicals used in the studies included Acetyl salicylic acid (Tuyil, Nigeria), Atropine (Sigma, USA), Formalin (M&B, England), Glacial acetic acid (Searle, Essex, England), Tragacanth powder (Sigma-Aldrich, USA), Methanol (Sigma-Aldrich, USA) and Triton X-100 (GE Healthcare, UK).

Animals

Swiss albino mice (15-35 g) and adult Wistar rats (126-250 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja. The animals were housed in appropriately designed cages. They were fed *ad libitum* with water and NIPRD-formulated feed except when starvation was otherwise needed during the investigation. They were maintained under normal environmental temperature and normal 12h day and night illumination cycle.

Acute Toxicity Studies

The method of Lorke [18] was modified and used for the estimation of the median lethal dose (LD_{50}), which is the dose that will kill 50% of the animal population post treatment with the extract. Swiss albino mice (15-29 g) were used for the study. The study was done in two phases. In the first phase, the mice were grouped into three ($n=3$) and were treated intraperitoneally with different doses of the extract (250, 500 and 1000 mg/kg) respectively. The animals were then observed for 72 h post treatment for behavioural signs such as nervousness, excitement, calmness, dullness, incoordination and even death. The second phase of the study was carried out based on the observations made in the first phase. It involved the use of two new groups of mice ($n=3$). They were treated intraperitoneally with the extract at doses of 600 mg/kg and 800 mg/kg respectively and were also observed for 72 h post treatment for the same signs. The LD_{50} was calculated from the geometric mean of the dose that caused no lethality at all and that which caused 100 % mortality as follows:

$$LD_{50} = \sqrt{(D0 \times D100)}$$

Where:

D0 = Highest dose that gave no mortality

D100 = Lowest dose that produced mortality

Acetic Acid-induced Writhing Test

The method used by Chidume *et al* [19] was adopted for the study. Adult Swiss albino mice (23-35 g) of either sex were used for the study. They were grouped into five ($n=5$). Normal

saline (20 ml/kg i.p) was administered to the first group of mice to serve as negative control. Three doses of the extract 75, 150 and 300 mg/kg were administered intraperitoneally to the second, third and fourth groups of mice respectively. Acetyl salicylic acid (ASA) 150 mg/kg i.p was given to the fifth group to serve as the reference standard.

Thirty minutes post treatment, each mouse was given 0.75% aqueous solution of acetic acid (10 ml/kg) intraperitoneally. Five minutes after injection of acetic acid, the number of abdominal constrictions (writhes) made within 10 minutes by each mouse was counted.

The percent writhes for the treated groups were calculated in relation to the control group. The activity was also expressed as percent inhibition of pain.

Formalin-Induced Pain Test

The method used by Jegede *et al* [20] was adopted for this study. Adult Wistar rats (126 – 187 g) of both sexes were used. They were grouped into five ($n=5$). Normal saline (10 ml/kg i.p) was administered to group one rats to serve as negative control. The rats in groups two, three and four were given extract (75, 150 and 300 mg/kg i.p) respectively. Acetyl salicylic acid (150 mg/kg i.p) was given to the fifth group to serve as reference standard. 30 min post treatment, 50 μ l (0.05 ml) of 2.5% formalin was injected into sub-plantar surface of left hind paw of each rat. The severity of pain exhibited by each rat was observed and rated as scores:

0=rat walked or stood firmly on the injected paw

1=rat partially elevated the paw from the floor

2=rat elevated the paw without contact with the floor

3=rat licked, bit or shook the paw

These observations were recorded every 2 min for the first 10 min (early phase) and at every 5 min for the period between the 10th min and 60th min (late phase).

Anti-inflammatory Studies

The extract was tested for its ability to inhibit or suppress inflammation using fresh egg albumin-induced oedema model in Wistar rats. This was in accordance with the technique of

Akah and Nwambie[21]. The rats used for the investigation were deprived of water during the experiment to ensure uniform hydration and to minimize variability in oedematous response. The rats were of both sexes and body weight between 175-250 g. They were grouped into five (n=5). Rats in the first group were treated with normal saline (10 ml/kg i.p) to serve as the negative control. Three doses of the extract (75, 150 and 300 mg/kg) were administered intraperitoneally to the second, third and fourth groups of rats respectively while acetyl salicylic acid (150 mg/kg i.p) was given to the fifth group as the reference standard.

30min post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin into the sub-planta surface of the hind paw of the rats. The measurement of the paw volume (cm^3) was done on the principle and technique of volume displacement using LETICA Digital Plethysmometer (LE 7500) which was earlier calibrated with 0.1% Triton X-100. The readings were taken before injection of egg albumin (zero readings) and at 20 min intervals after the injection of the egg albumin for a period of 2 h (120 min). The oedema at every interval was calculated in relation to the mean paw volume before the injection of the egg albumin. Activity of the treated group was expressed as percent inhibition of inflammation in relation to the control group.

Gastrointestinal Motility Test

Adult Swiss albino mice (18-32 g) of both sexes were used. The test was done according to the method of Akah *et al*[22]. The mice were starved for 18 h prior to the experiment but were allowed free access to water throughout the duration of the experiment. They were divided into five groups (n=5). The first group was given normal saline (20 ml/kg, i.p) to serve as the negative control while the mice in groups two, three and four received the extract (75, 150 and 300 mg/kg, i.p) respectively. The fifth group received atropine (0.1 mg/kg i.p) to serve as the reference standard. Thirty minutes after drug administration, each mouse was given 0.5 ml of charcoal meal orally (5% deactivated charcoal in 10% tragacanth powder). The mice were sacrificed 30min later and their abdomen opened. The intestine of each mouse was dissected out and the total distance travelled by the charcoal plug along the small intestine (from the pylorus to the caecum) was measured

for both the control and treated groups. The results were expressed as percentage of distance travelled from the pylorus to the caecum [23].

Statistical Analysis

The results of the studies were expressed as mean $\pm$ SEM. The significance difference between the control and treated means were determined using one-way or two-way analysis of variance (ANOVA) and student t-test as appropriate. P-values < 0.05 were considered to be statistically significant.

RESULTS

Plant Extract

The methanol extract of *A. mexicana* was a brownish-black powder and the yield was 5.8g (6.4 % w/w).

Acute Toxicity Study

In the first phase of the study (which involved the administration of 250, 500 and 1000 mg/kg.i.p doses of the extract), all the mice became dull and calm 10-15 min post extract administration up to the first 3 h of the study. Those treated with the extract (500 mg/kg i.p) were observed sleeping at the 30th min and up to 2 h into the experiment. However, only mice treated with the dose of 1000 mg/kg i.p died within 24 h of treatment with 100% motility rate.

In the second phase of the study in which 600 mg/kg and 800 mg/kg i.p doses of the extract were administered, all the mice were calm post treatment. However, no lethality was observed in this phase (Table 1).

The calculation of the geometric mean of the dose that caused 100% mortality and that which caused 0% mortality in the acute toxicity study revealed that the intraperitoneal median lethal dose (LD_{50}) of methanol extract of *Argemone mexicana* aerial part is 894.4 mg/kg in mice.

Table 1: Intraperitoneal acute toxicity study of methanol extract of *A. mexicana* aerial part in mice.

Treatment	Number of deaths	% Lethality
Phase 1		
250 mg/kg i.p	0/3	0 %
500 mg/kg i.p	0/3	0 %
1000 mg/kg i.p	3/3	100 %
Phase 2		
600 mg/kg i.p	0/3	0 %
800 mg/kg i.p	0/3	0 %

Acetic Acid-induced Writhing Test

The extract of *Argemone mexicana* (75, 150, 300 mg/kg i.p) significantly ($p < 0.05$) reduced the degree of acetic acid-induced writhing in mice at all doses with percentage abdominal writhes (indication of pain) of 55.17%, 31.03%, and 18.62% respectively. This is equivalent to the percent pain inhibition effect of the extract calculated to be 44.83 %, 68.97 %, and 81.38% for the 75, 150 and 300 mg/kg i.p treated groups

respectively. This shows a dose-dependent reduction of acetic acid-induced writhes. The study revealed that the anti-nociceptive effect of the extract was significant at the 30th minute which was the time the effect of the extract was tested. The effect was comparable to that of ASA (150mg/kg i.p.) which showed percent writhes of 34.48%. This is equivalent to 65.52% pain inhibition (Table 2).

Table 2: Effect of methanol extract of *A. mexicana* aerial part (75, 150, 300mg/kg i.p) on acetic acid-induced writhes in mice.

Treatment	Time (Min)	Mean Writhe $\pm$ SEM	% Writhe (In relation to control)	% Pain Inhibition
Normal saline (20 ml/kg i.p)	30	29.0 $\pm$ 4.75	100.00	0.00
<i>A. mexicana</i> (mg/kg ip)				
75	30	16.0 $\pm$ 2.76*	55.17	44.83
150	30	9.0 $\pm$ 1.16*	31.03	68.97
300	30	5.4 $\pm$ 2.11*	18.62	81.38
ASA (mg/kg i.p)				
150	30	10.0 $\pm$ 3.01*	34.48	65.52

* $P < 0.05$ = significant reduction from the control; One-way ANOVA

Formalin-Induced Pain Test

The study revealed that the normal saline-treated rats (negative control group) had mean pain severity of 7.4 (100.00 %) which is equivalent to 0.00 % pain inhibition in the early phase (0-10 min) of formalin test while the extract of *A. mexicana* (75, 150 and 300 mg/kg i.p) showed a mean severity of pain of 3.2 (43.24%), 1.8 (24.32%) and 1.6 (21.62%) respectively, which translates to percent pain inhibition of 56.76%, 75.68% and 78.38% respectively. The effect of the extract was dose-dependent and significant ($P < 0.05$) at all the tested doses in the early phase. In the late phase (10-60 min) of formalin test, the normal saline-treated rats (negative control group) showed mean pain severity of 16.2 (100.00 %) equivalent to 0.00 % pain inhibition while the

extract (75, 150 and 300 mg/kg i.p) showed mean pain severity of 9.8 (60.49%), 7.2 (44.44%) and 4.2 (25.92%) respectively. This is equivalent to percent pain inhibition of 39.51%, 55.56% and 74.08% for the respective doses. The effect of the extract was dose-dependent at the late phase (10-60 min) of the formalin test. However, the extract pain inhibitory effect at the late phase was significant ($P < 0.05$) only at doses of 150 and 300 mg/kg i.p. The percent inhibitory effects of the extract were higher in the early phase than in the late phase. ASA (150 mg/kg i.p) showed mean severity of pain of 4.2 ± 1.21 (56.75%) equivalent to percent pain inhibition of 43.25% in the early phase and mean severity of pain of 4.4 ± 2.68 (27.16%) equivalent to 72.84 % pain inhibition in the late phase. (Table 3).

Table 3: Effect of methanol extract of *A. mexicana* aerial part (75, 150, 300 mg/kg i.p) on early and late phases of formalin-induced pain in rats.

Treatment	Early Phase		Late Phase	
	Score of Pain	% Pain Inhibition	Score of Pain	% Pain Inhibition
Normal saline (10 ml/kg i.p)	7.4 ± 1.12	0.00	16.2 ± 2.32	0.00
<i>A. mexicana</i> (mg/kg i.p)				
75	$3.2 \pm 1.53^*$	56.76	9.8 ± 2.70	39.51
150	$1.8 \pm 0.99^*$	75.68	$7.2 \pm 1.42^*$	55.56
300	$1.6 \pm 0.53^*$	78.38	$4.2 \pm 1.70^*$	74.08
ASA (150 mg/kg i.p)	$4.2 \pm 1.21^*$	43.25	4.4 ± 2.68	72.84
	$4.2 \pm 1.21^*$	43.25	$4.4 \pm 2.68^*$	72.84

* $P < 0.05$ = statistically different from the control; Early phase (0– 10 min) and Late phase (15 – 60 min) post formalin administration.

Anti-inflammatory studies

The extract of *A. mexicana* demonstrated a reduction of egg albumin-induced inflammation at doses of 75, 150 and 300 mg/kg i.p in rats. The extract (300 mg/kg i.p) caused a significant ($p < 0.05$) inhibition of inflammation at every study interval within 2 h (120 min) observation period. The extract (150 mg/kg i.p) produced significant ($p < 0.05$) inhibitory effect up to 1 h (60 min) while the inhibition produced by the extract at the

dose of 75 mg/kg i.p had no significant effect at any of the observation intervals. ASA (150 mg/kg i.p) also caused anti-inflammatory response throughout the observation period of 2 h (120 min) and its effect was significant ($p < 0.05$) up to the 40th min observation interval. The maximum inhibition of oedema occurred at the 20th minute for ASA (150 mg/kg i.p) and extract (75, 150 and 300 mg/kg i.p; Figure 1).

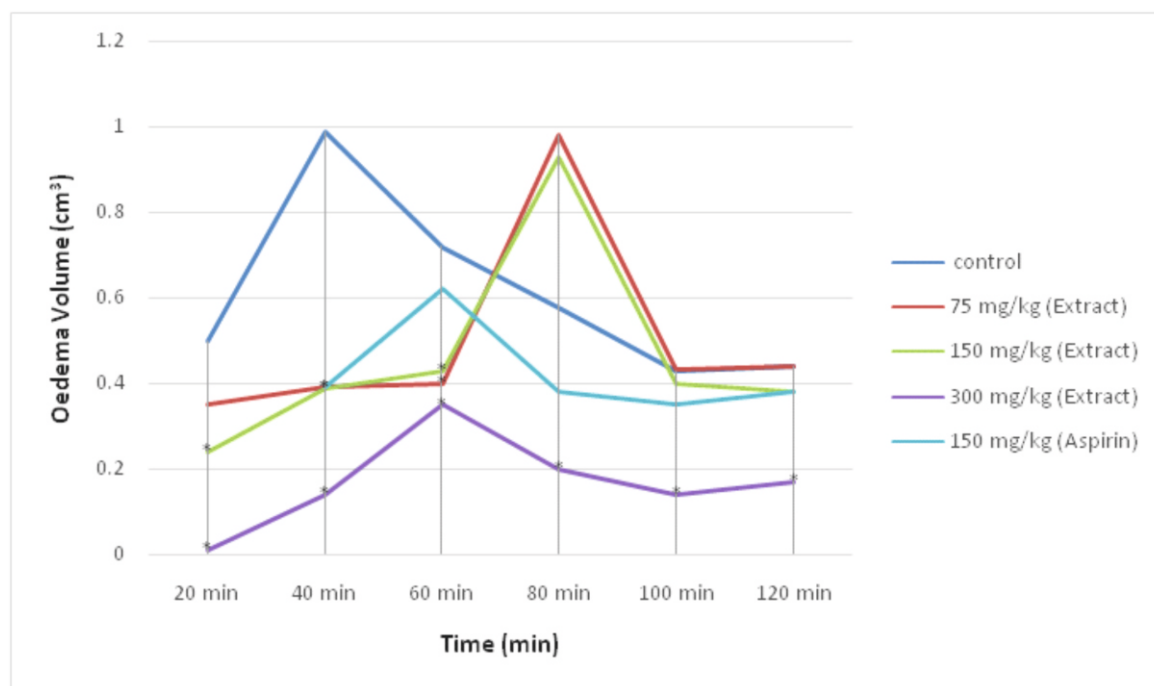


Figure 1: Effect of methanol extract *A. mexicana* aerial part on egg albumin-induced paw oedema in rats. * $P < 0.05$ = statistically different from the control

Gastrointestinal Motility Test

The extract of *A. mexicana* (75, 150 and 300 mg/kg i.p) decreased the propulsive movement of charcoal meal through the gastrointestinal tract (GIT) in a dose-dependent manner with the percent charcoal movement of 45.30%, 44.53% and 26.68%. This is equivalent to the inhibitory percentages of 54.70%, 55.47% and

73.32% for 75, 150 and 300 mg/kg i.p of the extract respectively. The decrease was however, significantly ($p < 0.05$) different only at doses of 150 and 300 mg/kg i.p. Atropine (0.1mg/kg i.p) also significantly ($p < 0.05$) decreased the intestinal propulsion with charcoal movement of 36.06% and inhibitory percent of 63.94%. (Table 4).

Table 4: Effect of methanol extract *A. mexicana* aerial part on intestinal motility in mice.

Treatment	Mean intestinal length (cm)	Meandistance travelledby charcoal (cm)	Movement of charcoal as % of intestinal length	Movement inhibition (%)
Normal saline 20 ml/kg i.p	40.37 ± 0.9	30.75 ± 1.5	76.17	23.83
<i>A. mexicana</i> 75 mg/kg i.p	41.87 ± 1.6	18.97 ± 2.2	45.30	54.70
	42.62 ± 1.2	18.98 ± 1.8	44.53*	55.47
	43.10 ± 1.8	11.50 ± 2.9	26.68*	73.32
150 mg/kg i.p				
300 mg/kg i.p	44.20 ± 1.1	15.94 ± 3.0	36.06 *	63.94
Atropine 0.1 mg/kg i.p				

Values are expressed as mean ± SEM (n = 5); one decrease from the control

-way ANOVA; * $P < 0.05$ = significant

DISCUSSION

The World Health Organisation [WHO; 24], in recognition of the immense value of herbal medicine to primary health care, advocated for proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies. *Argemone mexicana* which is a plant reported to have been widely used ethnomedicinally was evaluated in this regard.

Acute toxicity study was carried out to estimate the median lethal dose (LD_{50}) which is the dose that would kill 50 % of *A. mexicana* extract-treated population. The study revealed an intraperitoneal LD_{50} value of 894.4 mg/kg in mice. According to Lorke [18], LD_{50} value > 1 g are generally considered safe for all practical purposes in the laboratory.

These suggest that *A. mexicana* aerial part extract may not be relatively safe considering that the intraperitoneal LD_{50} value is < 1,000 mg/kg. This also suggests that it may not have a wide therapeutic window or wide safety margin. This may explain why poisoning occurred in the populace that ingested *Brassica nigra* (Mustard) seeds adulterated by *Argemone* seeds. 1 % adulteration of mustard oil by argemone oil has been shown to cause clinical disease [26]. *A. mexicana* is also reported to be poisonous to grazing animals and it is rarely eaten. It has been listed as a noxious weed in South Africa and in states in Australia. It is included in the prohibited plants that must be controlled as they possess characteristics that are harmful to humans, animals or the environment. An epidemic occurred in South Africa following the contamination of wheat flour with *A. mexicana* [27, 28, 29]

Ultimately therefore, conscious effort should be made to ensure that care is taken while choosing efficacious dose and frequency of administration of *A. mexicana* aerial part extract in order not to compromise the safety of the patients. However, sub-acute and chronic

toxicity studies are required for detailed toxicity profiling of *Argemone mexicana* plant extract considering that cumulative toxic effect may occur if the extract is taken over time.

The acute toxicity study also revealed that the mice treated with *A. mexicana* at doses < 1000 mg/kg became dull, calm, sleepy. This suggests that the extract possibly has central nervous system (CNS) activity and this indicates potential for therapeutic use of *A. mexicana* extract as sedative, hypnotic, anxiolytic and other possible CNS effects. The nervous signs observed in mice treated with the dose of 1000 mg/kg i.p before their death further suggests possible CNS effects. Further research will clarify these.

Acetic acid-induced writhing study is a test on chemical pain. It involves the use of abdominal constrictions (writhes) for detection of anti-nociceptive activity. The writhing response partly involves local peritoneal receptors [30, 31, 32]. The present study revealed that *A. mexicana* extract (75, 150 and 300 mg/kg i.p) significantly ($P < 0.05$) and dose-dependently reduced the number of acetic acid-induced abdominal pain. The percent inhibition of pain was 44.83 %, 68.97 % and 81.38 % for the respective doses. The pain inhibitory effect of the extract was comparable to that of acetyl salicylic acid (ASA; 150 mg/kg i.p) with percent pain inhibitory effect of 65.52 %. This possibly indicates that *A. mexicana* plant extract has the ability to reduce the receptor sensitivity to the chemically-induced pain in a dose-dependent manner and therefore has the potential to be developed into analgesics.

This result is similar to the studies of Sourabie *et al* [33] which showed that lyophilized extract of *A. mexicana* at doses of 250 and 500 mg/kg p.o presented reduced number of writhes at the two dose levels, which were found to be significant ($p < 0.05$; $p < 0.001$) compared to the control group. The revelation also justifies the traditional use of *A. mexicana*

plant extract as sedative and analgesic tea for pain-related health conditions such as kidney pain, migraine headaches, dental pain, boils, scorpion stings, snake bites among others [4, 5,6,7,8, 9, 10, 11, 12].

In the present study, formalin-induced pain test was used to further evaluate *A. mexicana* for antinociceptive effect and for possible elucidation of the site(s) of activity (central, peripheral or both). The method is useful for elucidating the mechanism of pain and analgesia [34]. The study revealed that *A. mexicana* aerial part extract (75, 150 and 300 mg/kg i.p) had a significant ($p < 0.05$) and dose-dependent, percent pain inhibition of 56.76 %, 75.68 % and 78.38 % respectively in the early phase (0 – 10 min) while the extract showed a dose-dependent percent pain inhibition of 39.51 %, 55.56 % and 74.08 % for the respective doses in the late phase (10 – 60 min). Although the percent pain inhibition at the late phase was only significant at doses of 150 mg/kg and 300 mg/kg i.p. These findings have further buttressed the pain inhibitory effects previously revealed by the acetic acid-induced writhing test in mice.

According to Dubuisson and Dennis [35] and Tjolsen *et al* [34], nociception induced by formalin occurs in two distinct phases. The first (early) phase represents the phasic pain and starts immediately after formalin injection and continues for 5 min after which nociception appears to diminish. In the second (late) phase which represents the tonic pain, a high level of nociception returns within 15 – 20 min after formalin injection and continues for 60 min. The first phase is probably due to direct stimulation of nociceptors in the paw while the second phase may reflect the process of inflammation and at least to some degree, the sensitization of central nociceptive neurons [36, 37].

The present study showed that the aerial part extract of *A. mexicana* (75, 150 and 300 mg/kg i.p) appreciably inhibited both the early phase

(with percent pain inhibition of 56.76 %, 75.68 % and 78.38 % respectively) and the late phase (with percent pain inhibition of 39.51 %, 55.56 % and 74.08 % respectively). However, the pain inhibitory effect was higher in the early phase. This did not only support the analgesic activity of the extract but also depicted its possible mechanisms of action. Drugs that act mainly centrally such as narcotics inhibit both early and late phases of formalin-induced pain while drugs such as hydrocortisone and dexamethasone which are primarily peripherally acting only inhibit the late phase [38, 39]. The activity of the extract on both early and late phases of formalin-induced pain therefore suggests that *A. mexicana* plant extract produces anti-nociceptive effect through central mechanism.

Anti-inflammatory study of *A. mexicana* aerial part extract (75, 150 and 300 mg/kg i.p) revealed inhibition of egg albumin-induced oedema. It shows that the extract possibly attenuated mediators of inflammatory process in a dose-dependent manner, although, the effect was more remarkable at the dose of 300 mg/kg i.p. This observation is in line with results in the late phase (10 – 60 min) of formalin-induced pain which is a phase that involves peripheral anti-inflammatory process. Since the extract was able to inhibit this late phase involving inflammation, it might mean an involvement of the peripheral mechanism in anti-nociceptive effect. The later assumption of involvement of the peripheral mechanism may be true since anti-inflammatory study carried out showed that the extract at the tested doses of 150 and 300 mg/kg i.p reduced egg albumin-induced inflammation in rats. This is comparable to the study carried out by Jegede *et al* [40] on *Ipomoea asarifolia* leaves.

In the present study, gastrointestinal motility test showed that the aerial part extract of *A. mexicana* (75, 150 and 300 mg/kg i.p) decreased the propulsive movement of charcoal meal through the gastrointestinal tract

in a dose-dependent manner. This activity was revealed by the charcoal meal study which allows for the *in vivo* evaluation of the effects of drugs on gastrointestinal motility [41]. The doses produced inhibitory percentages of 54.70 %, 55.47 % and 73.32 % respectively. The decrease was significant ($p < 0.05$) at doses of 150 and 300 mg/kg i.p. The result was comparable to the effect of atropine (0.1 mg/kg i.p) which also decreased the intestinal propulsion with an inhibitory percent of 63.94 %. However, the extract (300 mg/kg i.p) exerted greater anti-motility effect than atropine (0.1 mg/kg i.p) which is an anti-muscarinic drug.

This result is indicative of reduction in peristaltic activity with eventual reduction in gastrointestinal motility and rate of gastric emptying. The reduction in motility could also mean a decrease in gastrointestinal secretions. This finding contradicts the ethnomedicinal use of *A. mexicana* extract as laxative. Further studies involving other gastrointestinal models would be required for more elucidation of the effect of *A. mexicana* extract on gastrointestinal tract and for elucidation of its possible mechanism(s) of action. However, the significant ($p < 0.05$) delay in gastrointestinal transit caused by *A. mexicana* extract may be considered to be a beneficial property in ulcer patients since Dash and Murthy [42] demonstrated that extract of *A. mexicana* accelerated wound healing in rats. It is also generally known that delayed gastric emptying increases the absorption of orally administered anti-ulcer agents, thus promoting ulcer healing [43].

Conclusion

The results corroborate the ethnomedicinal use of *A. mexicana* plant extract for the relief of pain and inflammatory-related health conditions. The findings suggest that *A. mexicana* plant extract has the potential to be developed into analgesic and anti-inflammatory agents. On the other hand, the

results for the gastrointestinal motility test did not justify the traditional use of *A. mexicana* as laxative although therapeutic advantage can still be taken of its inhibitory effects on the propulsive movement of the intestine.

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REFERENCES

1. Pullaiah, T. (1995): Medicinal Plants in Andhra Pradesh, India, Regency Publications, New Delhi. India.
2. Siddiqui, I.A., Shaukat, S.S., Khan, G.H. and Zaki, M.J. (2002): Evaluation of *Argemone mexicana* for control of root-infecting fungi in potato. *Journal of Phytopathology*. 150: 321–329.
3. Felger, R.S. and Moser, M.B. (1985): *People of the Desert and Sea*. Tucson, AZ, University of Arizona Press.
4. Osho, A. and Adetunji, T. (2010): Antimicrobial activity of the essential oil of *Argemone mexicana* Linn. *Journal of Medicinal Plant Research*. 4: 19–22.
5. Chaudhari Rai, H.N., Pal, D.C. and Tarafdar, C.R. (1985): Less known uses of some plants from the tribal areas of Orissa. *Bull Botanical Survey of India*. 17: 132–136.
6. Chopra, R.N, Nayer, S.L. and Chopra, I.C. (1986): Glossary of Indian Medicinal Plants (including the supplement). Council of Scientific and Industrial Research (CSIR), New Delhi, India.

7. Moore, M. (1990): *Los Remedios. Traditional Herbal Remedies of the Southwest*. Santa Fe, NM: Museum of New Mexico Press, Pp 1 – 120.
8. Kala, C.P. (2005): Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. *Journal of Ethnobiology and Ethnomedicine*. 1(11): 1 – 8.
9. Wilcox, M.L., Graz, B and Falquet, J. (2007): *Argenome mexicana* decoction for the treatment of uncomplicated falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101: 1190 – 1198.
10. Ganesan, G. (2008): Traditional oral care medicinal plants survey of Tamil Nadu. *Natural Product Radiance*. 7: 166 – 172.
- 11 Alagesaboopathi, C. (2009): Ethnomedicinal Plants and their utilization by villagers in Kumaragiri hills of Salem District of Tamil Nadu, India. *Africa Journal of Traditional Complementary and Alternative Medicines*. 6: 222 – 227.
12. Jyothi, A.C., Santosh, J.P. and Ganes, P.B. (2011): Screening of aqueous plant extracts against *Beauveria bassiana* infection to 5th instar larvae of *Bombyx mori* L. *Journal of Medicinal Plants Research*. 5: 3936 – 3939.
13. Sofowora, A. Ogunbodede, E. and Onayade, A. (2013): The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*. 10 (5): 210 – 229.
14. Wambebe, C (1998): Development of standardized phytomedicines in Africa (Review). *Journal of Pharmaceutical Research and Development*, 3(1):1-11.
15. UNESCO (1998): FIT/504 – RAF – 48 Terminal Report: Promotion of Ethnobotany and the Sustainable use of Plant Resources in Africa, Paris. P 60.
16. Akinmoladun, A.C., Ibukun, E.O., and Afor, E. (2007): Chemical constituents and antioxidant activity of *Alstonia boonei*. *African Journal of Biotechnology*. 6(10): 1197 – 1201.
17. Chandrakant, K., Arun, G., Satyajyoti, K. and Shefali, K. (2012): Drug discovery from plant sources: An Integrated approach. *An International Quarterly Journal of Research in Ayurveda*. 33(1): 10 – 19.
18. Lorke, D. (1983): A new approach to acute toxicity testing. *Archives of Toxicology*, 54: 275 – 287.
19. Chidume, F.C., Kwanashie, H.O., Adekeye, J.O., Wambebe, C. and Gamaniel, K. S. (2002): Antinociceptive and smooth muscle contracting activities of the methanolic extract of *Cassia tora* leaf. *Journal of Ethnopharmacology*, 81: 205 – 209.
20. Jegede, I.A., Nwinyi, F.C., Ibrahim, J., Ugbabe, G., Dzarma, S. and Kunle, O.F. (2009): Investigation of phytochemical, anti-inflammatory and antinociceptive properties of *Ipomoea asarifolia* leaves. *Journal of Medicinal Plants Research* 3(3): 160 – 165.
21. Akah, P.A and Nwambie, A.I (1994): Evaluation of Nigeria Traditional Medicine. Plants useful for rheumatic disorders. *Journal of Ethnopharmacology* 42:179-182.
22. Akah, P.A., Orisakwe, O.E., Gamaniel, K.S. and Shittu, A. (1998): Evaluation of Nigeria Traditional Medicines II; Effects of some Nigerian folk remedies on peptic ulcer. *Journal of Ethnopharmacology*. 62: 123 – 127.

23. Gamaniel, K.S. and Akah (1996): Analysis of the gastrointestinal relaxing effect of the stem extract of *Gongronema latifolium*. *Phytomedicine*. 2 (4): 293–296.
24. WHO (2008): Herbal Medicine Research and Global Health: an ethical analysis (Jon. C. Tilburt, Ted. J. Kaptchuk). *Bulletin of the World Health Organization*. 86 (8): 577–656.
25. Walum, E. (1998): Acute oral toxicity. *Environmental Health Perspectives*. 106: 497–503.
26. Sharma, B.D., Malhotra, S., Bhatia, V., Rathee and Mandeep (1999): Epidemic dropsy in India. *Postgraduate Medical Journal*. 75 (889): 657–661.
27. Andrew, C., Graham, J.K., John, H.P., Shailendra, A. and Lei, L (2018): Assessing the risk of epidemic dropsy from black salve use. *Journal of Applied Toxicology*. 38 (10): 1274 – 1281.
28. Henderson, L. (2001): Alien weeds and invasive plants. A complete guide to declared weeds and invaders in South Africa. Plant Protection Research Institute Handbook No 12, 300 pp, PPR, ARC South Africa.
29. Macdonald, A.W., Reaser, J.K., Bright, C., Neville, L.E., Howard, G.W., Murphy, S.J. and Preston, G. (2003): Invasive alien species in South Africa: National Reports and Directory Resources. GISP, Cape Town, SA.
30. Bentley, G.A., Newton, S.H. and Starr, J.B. (1983): Studies on the anti-nociceptive action of alpha antagonist drugs and their interaction with opioid mechanisms. *British Journal of Pharmacology*, 79: 125- 134.
31. Mat Jais, A.M., Dambisya, Y.M. and Lee, T.J (1997): Anti-nociceptive activity of *Channa striatus* (haruan) extracts in mice. *Journal of Ethnopharmacology*, 57: 125–130.
32. Atta, A.H. and Alkofahi, A. (1998): Anti-nociceptive and anti-inflammatory effects of some Jordian Medicinal extracts. *Journal of Ethnopharmacology* 60: 117–124.
33. Sourabie, T.S., Ouedraogo, N., Sawadogo, W.R., Nikiema, J.B., Guissou, I.P. and Nacoulma, O.G. (2012): Biological evaluation of anti-inflammatory and analgesic activities of *Argemone mexicana* Linn. (Papaveraceae) aqueous leaf extract. *International Journal of Pharma Sciences and Research*. 3: 451–458.
34. Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H. and Hole, K (1992): The formalin test: An evaluation of the method. *Pain*, 51: 5–17.
35. Dubuisson, D. and Dennis, S.G. (1977): The formalin test: A quantitative study of the analgesic effect of morphine, mepetidin and brain stem stimulation in rats and cats. *Pain*, 4: 161–174.
- 36.Coderre, T.J., Vacarino, A.L. and Melzack, R. (1990): Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. *Brain Research*, 535: 155 – 158.
37. Coderre, T.J. and Melzack, R. (1992): The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. *Journal of Neuroscience*, 12: 3665–3670.
38. Chen, Y.F., Tsai, H.Y. and Wu, T.S. (1995): Anti-inflammatory and analgesic activity from roots of *Angelica pubescens*. *Planta Medica*. 61: 2–8.
39. Masoume, R., Mandana, S., Vahid, N., Sattar, O. and Azam, B (2014): The study of analgesic effects of *Leonurus cardiaca* L. in mice by formalin, tail flick and hot plate tests. *International Scholarly Research Notices*. Vol 2014/Article ID 687697/5

40. Jegede I.A., Nwinyi F.C., Ibrahim. J., Ugbabe, G., Dzarma, S and Kunle, O.F. (2009): Investigation of phytochemical, anti-inflammatory and anti-nociceptive properties of *Ipomoea asarifolia* leaves. *Journal of Medicinal Plants Research*. 3(3): 160–165.
41. Chidume, F.C., Gamaniel, K., Amos, S., Akah, P., Obodozie, O. and Wambebe, C. (2001): Pharmacological activity of the methanolic extract of *Cassia nigricans* leaves. *Indian Journal of Pharmacology*. 33: 350–356.
42. Dash, G.K. and Murthy, P.N (2011): Evaluation of *Argemone mexicana* Linn, leaves for wound healing activity. *Journal of Natural Product Plant Resources*. 1: 46–56.
43. Bertaccini, G., Castiglione, R. and Scapignato, C. (1981): Effect of substances and its natural analogues on gastric emptying in the conscious rat. *British Journal of Pharmacology*. 72: 221–223.