IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 3 Ver. IV (May - Jun.2016), PP 92-95
www.iosrjournals.org

Analgesic and Anti-Inflammatory Studies on The Roots *Argemone Mexicana* Linn(FAMILY: PAPAVERACEAE)

[a]H. A. Ibrahim* [b]Mahmud Ali Umar, [c]Bilkisu A Bello, [d]Abdulazeez Aliyu and [e]Abdulazeez Ahmad

*[*a]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences,Ahmadu Bello University,Zaria, Nigeria*
[b]Department of Biology, Faculty of Science, Kano Universty of Science and Technology,Wudil, Kano State
[c]Biology Department, Sa'adatu Rimi College of Education, P.M.B. 3218, Kumbotso, Kano, Nigeria
[d]Department of Pharmacognosy, Faculty of Pharmaceutical Science, Bayero Universty, Kano, Nigeria
[e]Department of Applied Science. Shehu Idris College of Health Sciences and Technology, Makarfi, Zaria
***Correspondence author: ibrokhad203@gmail.com Tel. +234 08036649688**

Abstract: *Analgesic and anti inflammatory studies of the ethanol extract of the roots of A. mexicana were studied in mice and rats respectively. Acetic acid-induced writhing in mice and hind paw oedema in rats were determined. The results of these studies showed the extract to possess anti-nociceptive activity which is dose-dependent at 50mg and 100mg/kg (i.p) in mice and in the same way a slight inflammatory activity at 50 and 100mg/kg were observed. The extract was also found to have an intraperitoneal (i.p) LD_{50} of 368.00mg in mice. These results correlate with the traditional use of the plant by the traditional medical healers and support the use of the plant in the management of inflammation and skin diseases.*

Keywords:*, Analgesic, anti-inflammatory, Argemone.mexicana Linn and roots*

I. Introduction

Plants have long been a valuable source of novel drug compounds (medicines). Phytomedicines which are plant-derived, have shown great promise in the treatment of several diseases [1] [2]. The populations of developing countries worldwide continue to rely heavily on the use of traditional medicines as their primary source of health care. Ethnobotanical studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines [3] [4] [5] [6][7]. With 70-80% of Africa's population relying on traditional medicines, the importance of the role of medicinal plants in the health care delivery is very enormous particularly for the anti-inflammatory diseases.

There is [8] claims that 119 characterized drugs are still obtained commercially from higher plants and that 74% were found from ethnobotanical information. Certain areas of vegetation (Savannah and Guinean forest) are rich in species and types of environments to sbe used to search for natural product compounds, whose choice can be based on ethnobotanical and chemotaxonomic studies, and screen for their ability to inhibit activities. Globally, only a small proportion, out of the several thousand plant species has been investigated both phytochemically and pharmacologically, when one considers that a single plant may contain up to thousand of constituents, the possibilities of making new discoveries become evident [9] .In Northern Nigeria, *A. mexicana* is locally known in Hausa as "Kaki ruwan Allah", "Karanko" or "Kwarkwaro" [10]. The Hausa traditional healers use a ground leaf on swollen area of skin against inflammation of skin (Oral communication with Dan Magori). The plant contains alkaloids, flavonoids, tannins, sterols and terpenes [11]. The dried leaf of this plant was used in the treatment of jaundice [12] and the alkaloid 6-acetonyl dihydrochelerythrine were found to possess an anti-HIV activity[13] . The aim of this study was to determine the possible analgesic and anti-inflammatory effects of the ethanol extract of the of *Argemone mexicana* using the acetic acid- induced writhing and rat hind paw edema model

II. Materials And Methods

2.1 Plant collection and identifications

The roots of *A. mexicana* L were collected at Tudun Wada, Zaria, Kaduna State and were identified by the curator (Mallam S.U. Gallah) of the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria Nigeria. A voucher specimen (number 2439) was deposited for future reference.

2.2 Extraction of Plant material

The powdered roots of *A. mexicana* (80g) was extracted with 95% (v/v) ethanol. The extract was concentrated at reduced pressure using a rotary evaporator at 45°C. The extract was later evaporated to dryness on a water-bath. This yielded a greenish-black mass referred to as the ethanolic extract of the plant material.

2.3 Experimental Animal

Wister rats of either sex weighing between 160- 250g and Swiss albino mice of either sex weighing between 18- 25g were used for the study and were obtained from the Animal house, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria. All animals were kept under well-ventilated conditions fed with food and water ad libitum (Excel feed Plc).

2.4 Acute Toxicity Studies

LD_{50} determination was conducted using the classical method of [14]. Male and female mice were divided into three groups of 3 animals each and treated with ethanol extract at 10,100 and 1000mg/kg *i. p*. and were observed for 24 hours after the treatment, 4 mice were divided into 4 groups of one mouse each and administered the second doses of 200, 400, 800, 1000mg/kg *i.p*, and the final LD 50 was calculated.

2.4 Analgesic Activity Studies

A method described by [15] was employed in this study. Four sets of five mice per group were pre-treated with 50 and 100mg/kg; normal saline (negative control); and analgin (positive control). Pre-treatment time for the groups was 30 min, and each group was treated with 0.7% of an aqueous solution of acetic acid ($10cm^3$ /kg). The mice were placed in a transparent observation box and number of writhes was counted for 10 min after treatment. Treatment group were compared with controls (positive and negative) and the percentage inhibition of writhes were calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control mean} - \text{test mean} \times 100\%}{\text{control}}$$

2.5 Anti-inflammatory Studies

A modification of [16] was used. Male and female Wistar rats were divided into four groups of five animals each and treated as follows ; Normal saline (negative control); aspirin (positive control) ; 50 and 100mg/kg of the extract *i.p* after 30 min, each group was administered 0.5 cm^3 raw egg albumin sub planter to the left hind-paw. Measurement of the paw oedema were then taken with a digital Plethysmometer (LE 7150) at 20 min interval for 120 min for all groups, that is 20, 40, 60, 80, and 120 min after albumin treatment

2.6 Statistical analysis

All data were expressed as mean± SEM. Statistical analysis was carried out using student t-test and differences between means were considered significant when $p < 0.05$.

III. Results

3.1Acute Toxicity Studies

The calculated LD_{50} of the root extract was found to be 368.00mg/kg *i.p*

3.2 Analgesic Activity Studies

From the results of this study, it has been observed that 50 and 100mg/kg of the extract decreased significantly ($p< 0.05$) the number of writhes in a dose-dependent fashion (Fig. 1) compared to the control (negative). Highest percentage inhibition of writhes (70.1) was obtained at a dose of 100mg/kg. The activity of the extract at 50 and 100mg/kg was shown to be statistically significant compared to the positive control (Analgin) as given in Table 1.

3.3 Anti-Inflammatory Studies

In this study, the extract was shown to cause an inhibition of albumin-induced oedema over a period of 120 min. This activity, however, did not appear to be dose-related. The highest inhibitory effect was observed at 40 min after treatment. The results were found to be statistically significant ($p< 0.05$) at 40, 60 and 120 min after extract treatment at dose of 50mg/kg appear to be more effective (Table 2). The anti-inflammatory effect exhibited by the different dosages of the extract is not comparable to that of the standard drug that is aspirin used.

IV. Discussion And Conclusion

The results of this study revealed that the aqueous ethanol extract of the roots of *A. mexicana* possess anti-nociceptive properties. The extract significantly reduced the number of acetic acid-induced writhing in mice in a dose-dependant fashion. This fact was correlated with the findings of [17] who reported the plant to possess an analgesic activity in man, when an infusion of powdered aerial part of the plant was taken orally. The extract was also studied for possible role as potential anti-inflammatory drug, using for this purpose the classical model

of rat paw oedema. Egg albumin induces a short lasting oedema of few hours (< 2 hours in Fig. 1) and is therefore suitable for studying the degree of anti-inflammatory actions of drugs. Other irritants used to induce oedema are brewer's yeast, formaldehyde, dextran, sulfated polysaccharides like carrageenan, and paw fluid volume can also be measured by immersion of the paw in mercury [18]. The result of the study indicated the extract to cause a slight inhibition of albumin-induced oedema in rats; in this case, the effect did not appear to be dose dependant. These study also agrees with the results obtained by [19] who showed the plant to be used as a lotion for inflammatory swelling, fever and skin diseases.

Resolution of inflammation occurs by different mechanisms in different tissues and these mechanisms which serve to terminate inflammation include [20] , among other factors, short half-life of inflammatory mediators *in vivo*, production and release of transforming growth factor (TGF) beta from macrophages[21] [22] [23], production and release of interleukin-10 (IL-10) [24], production of anti-inflammatory lipoxins [25], down regulation of pro-inflammatory molecules, such as leukotrienes, up regulation of anti-inflammatory molecules such as the Interleukin 1 receptor antagonist or the soluble tumor necrosis factor receptor (TNFR), apoptosis of pro-inflammatory cells [26], increased survival of cells in regions of inflammation due to their interaction with the extracellular matrix (ECM) [27] [28], and cleavage of chemokines by matrix metalloproteinases (MMPs) might lead to production of anti-inflammatory factors [29]. Due to the presence of pure compounds identified as protopine and allocryptopine which are isoquinoline alkaloids [17], the plant may be used as a potential agent in the treatment of drug abuse.

References

[1]. Cowan, M. M. (1999). Plant Products as Antimicrobial Agents.*Clin.Microbiol. Rev.*, 12(4): 564-582.

[2]. Mitscher, L. A. and Baker, W. R. (1998).A search for novel Chemotherapy against tuberculosis amongst natural products.*Pure Appl. Chem.*, 70(2): 365-271.

[3]. [3] Adjanohoun E., Ahyi, M.R.A., Ake-Assi, L., Elewude, J.A., Dramane, K., Fadoju, S.O., Gbile, Z.O., Goudote, E., Johnson, C.L.A., Keita, A., Morakinyo, O., Ojewole, J.A.O., Olatunji, A.O., and Sofowora, E.A. (1991). Traditional medicine and Pharmacopoeia*:* Contribution to ethnobotanical floristic studies in Western Nigeria. Organization of African Unity, Scientific Technical and Research Commission, Lagos, Nigeria.

[4]. Hedberg, I., Hedberg, O., Madati, P., Mshigeni, K.E., Mshiu, E.N. and Samuelsson, G. (1983). Inventory of plants used in traditional medicine in Tanzania. II. Plants of the families Acanthaceae and Cucurbitaceae. *J. Ethnopharmcol*., 9: 105-128.

[5]. Kokwaro, J. O. (1976). Medicinal Plants of East Africa.*East African Literature Bureau*, Nairobi, Kenya.

[6]. Mann, A., Mohammed, G. and Abdulkadir, N.A. (2003).*Medicinal and Economic plantsof Nupeland*.Jube-Evans Books and Publications, Minna, Nigeria.P. 19.

[7]. Oliver-Bever, B. (1986). *Medicinal plants in tropical West Africa*. Cambridge University Press, Cambridge.

[8]. Farnsworth, N.R. (1990). *Bioactive Compounds from Plants*. In: Chadwick, D. J., and Marsh J, editors. John Wiley, Chichester, P. 2

[9]. Hostettmann, K., Marton, A., Wolfender, J.L. (1995). *Phytochemistry of Plants used in Traditional Medicine*. In: Hostettmann, K., Marton, A., Mailard, M., Hamburger, M, editors. Clarendon Press, Oxford, P. 17.

[10]. Mann, A. (1998). Identification of Some Ethnomedicinal and Grain Protectant Plants in Nupeland of Niger State, Nigeria.*NJTE.*,15(1): 158-166.

[11]. Quinn-Beatie, M.L. (2002). *Natural Product Alert (NAPRALERT) database report*. The University of Illinois, Chicago.Pp.17-20.

[12]. Bhat, R.B., Eterjere, E.O. and Oladipo, V.T. (1990) Ethno botanical Studies from Central Nigeria. *Economic Botany*.44 3: 382 - 390.

[13]. Chang, Y.C., Hsieh, P.W., Wu, R.R., Law, C.C., Lee, K.H. and Wu, Y.C.(2003).Two new protopines Argemaxicaines A and B and the anti HIV alkaloid 6-Acetonyl Dihydrochlcrythrine from Formosan Argemone mexicana.*PlantaMedica*68 (2) : 148-152.

[14]. Lorke, D. (1983). A New Approach to Acute Toxicity Testing.*Achieves of Toxicology;* 54:275-287

[15]. Koster, R., Anderson, M., De Beer, E. J. (1959).Acetic acid for analgesic screening.*Federation Proceedings,* 18:412.

[16]. Winter, E. A., Risley, E. A., and Nuss, G. V. (1963).Antinflammatory and anti- pyretic activities of indomethacine.*Journal of Pharmacology and Experimental Therapeutics*, 141:369-376.

[17]. Capasso, A., Piacente, S., Pizza, C., Tommasi, N., Jativa, C., and Sorrentino, E. (1997).Isoquinoline alkaloids from *Argemone mexicana* reduce morphine withdrawal in guinea pig isolated ileum. *Planta Medica*., 63(4):326-328.

[18]. Vogel, G. H. and Vogel, W. H. (1997).Drug Discovery and Evaluation; Pharmacological assays. Springer-Verlag, Berlin. Pp 267-268

[19]. Singh,Y. N. (1986). Traditional Medicine in Fiji : Some Herbal Folk Cures Used By Fiji. *Journal of Ethnopharmacology*15 (1): 57 – 88

[20]. Eming, S.A., Krieg, T. and Davidson, J.M.(2007) Davidson, Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol*. 127(3): 514-525.

[21]. Ashcroft, G.S. (1999a). Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol*,. 1(5):.260-266.

[22]. Ashcroft, G.S.(1999b). Bidirectional regulation of macrophage function by TGF-beta. *Microbes Infect*, 1 (15): 1275-1282.

[23]. Werner, F. (2000) Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. *J BiolChem*,.275(47): 36653-36658

[24]. Sato, Y., Ohshima, T. and Kondo, T. (1999), Regulatory role of endogenous interleukin-10 in cutaneous inflammatory response of murine wound healing. *Biochem Biophys Res Commun*, 265(1): 194-199.

[25]. Serhan, C.N. (2008). Controlling the resolution of acute inflammation: a new genus of dual anti-inflammatory and proresolving mediators. *J. Periodontol*, 79(8 Suppl): 1520-1526.

[26]. Greenhalgh, D.G. (1998). The role of apoptosis in wound healing. *Int. J. Biochem. Cell Biol*, 30(9): 1019-1030.
Jiang, D. (2005). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat. Med.*, 11(11): 1173-1179.

[27]. Teder, P. (2002), Resolution of lung inflammation by CD44. *Science* 296(5565): 155-158. .

[28]. Jiang, D. (2005). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat. Med.*, 11(11): 1173-1179.

[29]. McQuibban, G.A. (2000) Inflammation dampened by gelatinaseA cleavage of monocyte chemoattractant protein-3. *Science*, 289 (5482): 1202-1206.

Table 1: Effect of acacia gum soluble portion of Ethanolic extracts of the roots of *Argemone mexicana* L on acetic acid induced writhing

	Dose (mg/kg body weight)	No. of Abdominal constrictions	% Inhibition	P value
Normal saline		15.4± 2.8		
Extract (*i.p*)	50	[a]9 ± 1.6	42%	P<0.05
Extract (*i.p*)	100	[a] 5.2 ± 1.6	52.3	P<0.05
Analgin (*i.p*)	50	[b] 2 ± 0.3	87.0	P<0.001

Table 2: Mean paw volume produced by the roots extract and the control at various time post albumin – induced edema in rats.

		Paw volume (ml) at various time (minutes)						
Treatment	Dose mg/ kg body wt.	0	20	40	60	80	100	120
Normal saline (negative control)		0.09 ± 0.013	0.44 ± 0.047	0.53 ± 0.078	0.59 ± 0.053	0.52 ± 0.0.64	0.36 ± 0.041	0.34 ± 0.044
Extract	50	[b] 0.13 ± 0.018	[b] 0.38 ± 0.072	[a] 0.46 ± 0.072	[a] 0.37 ± 0.048	[a]0.38 ± 0.066	[a] 0.43 ± 0.087	[b]0.42 ± 0.074
Extract	100	[b]0.17 ± 0.028	[b]0.41 ± 0.061	[a]0.43 ± 0.042	[a]0.47 ± 0.050	0.42 ± 0.068	[a] 0.39 ± 0.049	[b]0.37 ± 0.048
Aspirin (positive control)	150	[a]0.17 ± 0.042	[a]0.33 ± 0.044	[b]0.30 ± 0.049	[b] 0.28 ± 0.043	[b] 0.30 ± 0.058	[a] 0.30 ± 0.078	[b]0.26 ± 0.076

Data presented as Mean ± S.E.M, using student t – test, n = 5, a, b are significantly different from control at P< 0.05 and P< 0.001 a = p < 0.05, b = p < 0.001

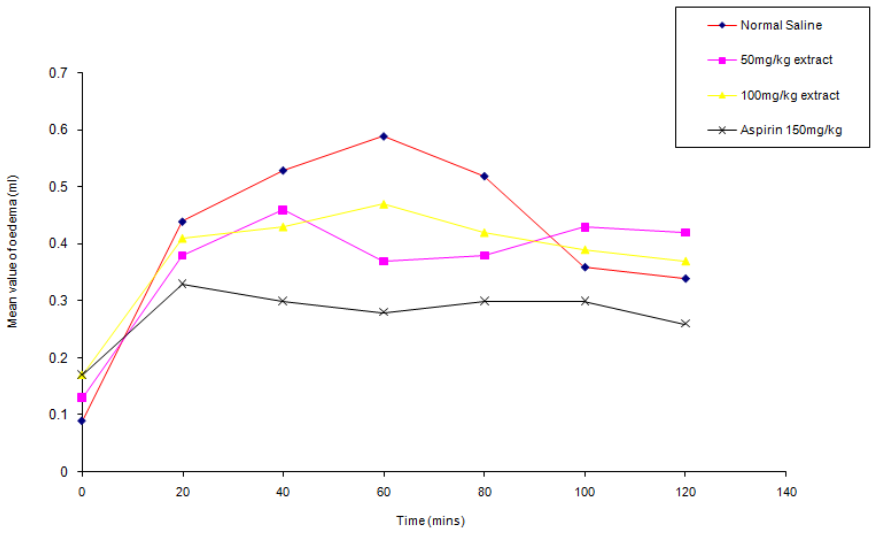

Fig. 1. Effects of ethanol extracts of *Argemone mexicana* Linn Roots on egg-albumin induced oedema in rats