



Analgescic and Anti-Inflammatory Activity of Ethanol Bark Extract of *Vitellaria paradoxa* in Wistar Albino Rats and Mice**Abdulrazaq A. Musa¹, Modupe I. Joseph¹, Fatima A. Goje¹, Hamidu A. Samira²**¹Department of Pharmacology & Toxicology, University of Maiduguri, Borno State, Nigeria.²School of Nursing and Health Sciences, University of Dundee.

Email: raz4ril@gmail.com

Abstract

Vitellaria paradoxa is used for the treatment of several ailments in different parts of Nigeria including pain and inflammation. *Vitellaria paradoxa*, has been reported to possess a variety of pharmacological properties. This study was undertaken to investigate the analgesic and anti-inflammatory activity of *Vitellaria paradoxa* ethanol bark extract through different animal models.

The acute toxicity (LD_{50}) of the extract was determined using Lorke's method. The analgesic activities were evaluated by hot-plate and acetic acid induced writhing tests. The anti-inflammatory effect was assessed by rat paw oedema test.

In the hot-plate test, *Vitellaria paradoxa* significantly extend pain threshold when compared to control group. The inhibitory rates of writhes ranged from 6.6% to 28.1% in *V. paradoxa*-treated mice. Treatment with *Vitellaria paradoxa* especially at 100mg/kg and 50mg/kg could significantly increase (ranging from 38 – 101 minutes) the latent time to heat sensation. In carrageenan-induced oedema test, the inhibitory rates were 7.0% and 4.9% ($p < 0.05$) at 180 minutes and 12 hours after administration of *Vitellaria paradoxa* (100mg/kg), respectively, while at an extract dose of 50mg/kg, the inhibitory percentages rates are 10.5% and 8.4% ($p < 0.05$) at 120 minutes and 12 hours after administration of *Vitellaria paradoxa* (100mg/kg), respectively.

Results from the analgesic and anti-inflammatory studies of *Vitellaria paradoxa* ethanolic bark extracts showed significant effects exhibited in a dose dependent manner.

Keywords: Pain, Inflammation, Induced writhing, oedema test, *V. paradoxa*

Introduction

Inflammation is a vital part of the immune system response to injury and infection. It is the body's way of signalling the immune system to heal and repair damaged tissues, as well as defend itself against foreign invaders, such as viruses and bacteria (Gyurkovska, 2011). Without inflammation as a physiological response, wounds would fester and infections become deadly.

Inflammation may be acute or chronic. Acute inflammation is often a short term response to an external stimulus with localized effects characterized by redness, swelling, heat and sometimes pain including loss of function (Izzo, 2012). Chronic inflammation however, has been linked to certain cardiovascular conditions such as heart disease, atherosclerosis or stroke and may also be

linked to autoimmune disorders, such as rheumatoid arthritis and lupus.

Vitellaria paradoxa is a traditional herbal medicine widely distributed in Northern part of Nigeria including Kano, Jigawa, Gombe and Katsina States. It is used for the treatment of several ailments in different parts of Nigeria including pain and inflammation. In vitro and in vivo studies have demonstrated its pharmacological effects, including antimicrobial and anti-oxidant activities. However, there are few reports about the analgesic and anti-inflammatory activities. Therefore, in this study, the analgesic effects of *Vitellaria paradoxa* were determined through the hot-plate test and acetic acid-induced writhing methods, and the anti-inflammatory activities were assessed through carrageenan induced paw oedema test.

Materials and Methods

▪ **Materials**

Measuring cylinder, filter paper, syringes (1ml, 2ml, 5ml), round bottom flask, beakers, test tubes, water bath, mortar and pestle, analytical weighing balance, thermometer, spatula, hot plate.

▪ **Reagents**

Ethanol 99.7%, distilled water (50 litres), normal saline, sulphuric acid 99%, acetic acid 99%, 10% hydrochloric acid, 10% ammonia solution, 10% sodium hydroxide solution, ferric chloride solution, Wagner's reagent, chloroform, Dragendorff's solution A and B, Mayer's reagent.

▪ **Drugs and dosage**

Piroxicam (10mg/kg, Feldene[®] Pfizer Lot no: 1808018, exp. date July-20), Diclofenac injection (25mg/ml ampoule, Emporix[®], Lot no.181203, exp date: Dec-21), 1%

carrageenan solution (Sigma, St. louis, MO, 0.1mL/animal), Crude ethanol bark extract of *Vitellaria paradoxa* (25mg/kg, 50mg/kg and 100mg/kg). Physiological (normal) saline was used as negative control for each experiment.

▪ **Animals**

Male and female Wister albino rats (weighing 150-200g) and mice (weighing 25-35g) used for the study were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Maiduguri. They were housed in a well ventilated room and fed with standard diet (chicken feed; Amo Byny[®] feeds). All experiments were conducted in accordance with international regulations governing the use and care of laboratory animals.

▪ **Plant collection and preparation**

The fresh bark of *Vitellaria paradoxa* was collected from Dutse LGA of Jigawa State, Nigeria. It was identified and authenticated by Prof S. S. Sanusi (Professor of Botany, Department of Biological Sciences, University of Maiduguri). It was cleaned; shade dried and then grounded using mortar and pestle.

▪ **Preparation of extract (Maceration Method)**

The dried and ground bark (250g) of *Vitellaria paradoxa* was defatted with *n*-hexane and transferred into a large mouthed glass container which had a closure. 700ml of ethanol (70%) was poured into this container until the solvent completely saturated the plant material to form a layer above it. It was then left for 48 hours at room temperature (25^oc) with intermittent shaking at 2 h interval.

The mixture was stirred with a glass rod and then filtered using Whatman No 1 filter paper into a large round bottom flask. The residue obtained was air dried for 24 h and thereafter subjected to the same procedure three successive times. The filtrate was then poured unto a clean tray and allowed to dry (evaporation).

▪ **Preliminary Phytochemical Screening**

Preliminary screening of *Vitellaria paradoxa* on the ethanol bark extract was conducted using standard methods of analysis (Trease and Evans, 1989).

▪ **Acute Toxicity Study**

Mice (male and female) weighing between 25-35g was used to determine the acute toxicity of ethanolic bark extract of *Vitellaria paradoxa*. It was carried out using the modified Lorke's method (Lorke's, 1983).

▪ **Evaluation of Analgesic activity using hot plate method**

The hot-plate test was conducted according to methods previously reported (Younos *et al.*, 1990), (Zhang *et al.*, 2006). The temperature of electrical hot plate was set at $55 \pm 0.5^\circ\text{C}$. The mice were grouped into five groups of five mice per group. The first group was treated with 2ml each of physiological saline; the second group was administered diclofenac 75mg/kg. The third, fourth and fifth groups were administered the test extract at doses of 25, 50 and 100mg/kg respectively. The mice were put on an electrical hot-plate, and the time was recorded when the mice showed any nociceptive indicators. The pain indicators consisted of lifting, licking paw, and jumping. A pain threshold of the mice (the time when they first exhibit one of the pain indicators) within 5-30 s was used for the test.

▪ **Evaluation of Analgesic activity using Acetic Acid-Induced Writhing in Mice**

The acetic acid-induced writhing test was performed as described by Chao *et al.*, 2013. Mice were divided into five groups, with each group containing five mice. The first group was pre-treated with 2ml of physiological saline, the second group was administered piroxicam injection (20mg/kg), the third, fourth and fifth groups received 25, 50 and 100mg/kg respectively of the extract. The mice were intraperitoneally injected with 1ml each of acetic acid 30 minutes after the last administration.

The mice were then placed in an observation box, and the total number of writhes was recorded for 20 min after acetic acid administration. The percentage of analgesia was calculated by the following equation:

$$\% \text{ inhibition} = \frac{Nm - Nc}{Nm} \times 100$$

Where Nm is the number of writhes in control group and Nc is the number of writhes in the treated groups.

▪ **Evaluation of Ant-inflammatory activity using Carrageenan-Induced Rat Paw Oedema**

The carrageenan-induced paw oedema test was conducted according to the method reported by Khodabakhsh, *et al.*, 2015. Wister albino rats were randomly grouped into five with five rats per group. The left hind paw sizes of each group were measured with the aid of Vernier caliper (baseline readings). Each group was pre-treated with 0.1ml carrageenan solution.

An hour later, rats in group one received physiological saline, group two received piroxicam injection (10mg/kg). Groups three, four and five received graded doses of the

extract (25, 50 and 100mg/kg respectively). Half an hour later, the sizes of the inflammation produced was measured and the procedure was repeated at 60, 120, 180 min and 12 hours later. The oedema degree was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{V_1 - V_2}{V_2} \times 100$$

$$= \frac{\text{edema degree}(\text{control}) - \text{edema degree}(\text{treated})}{\text{edema degree}(\text{control})} \times 100$$

Where V_1 is the average volume of paw swelling at different times per group. V_2 is the average volume of paw before injecting carrageenan solution per group.

▪ *Statistical Analysis*

All statistical analysis was determined using SPSS 19.0 statistical software and one-way analysis of variance (ANOVA) in Duncan's test. < 0.05 was regarded as statistically significant and < 0.01 was considered very significant. The data were expressed as mean value \pm standard deviation ($M \pm SD$).

Results

▪ *Extractive Value*

Percentage yield = (quantity recovered \div total quantity) $\times 100$

$$= (12 \div 250) \times 100 = \underline{4.8\%}$$

The percentage yield from 250g powered bark of *Vitellaria paradoxa* was found to be 4.8% (Table 1)

Table 1: Extractive Value

Weight of powered plant	Weight of extract	Percentage yield
250g	12g	4.80%

▪ *Acute toxicity study*

Table 2 shows the result of the oral acute toxicity study of the ethanol bark extract of *Vitellaria paradoxa* (phase I and II) within 24 hours of extract administration.

Table 2: Result of Lethal Toxicity (LD₅₀)

Phases	Mice	Dose (mg/kg)	Observation
Phase I	Mouse I	10	No death
	Mouse II	100	No death
	Mouse III	1000	Death
Phase II	Mouse I	200	No death
	Mouse II	400	Death
	Mouse III	800	Death

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_{100} = Highest dose with no death

D_0 = Lowest dose with death

The LD_{50} was found to be 283mg/kg.

▪ **Preliminary photochemical screening**

Results from the preliminary phytochemical screening of the ethanol bark extract of *Vitellaria paradoxa* revealed the presence of some secondary metabolites as shown in table 3:

Table 3: Result of Preliminary Phytochemical Screening

Test	Results
Carbohydrates	
(Molisch's Test)	+
Saponins	
Frothing test	+
Fehling's Solution Test	
Tannins	+
Flavonoids	
(ferric chloride test)	+
Alkaloids	
Mayer's test	+
Wagner's test	
Dragendoff's test	
Cardiac glycosides	-
(Keller Killani's Test)	
Anthraquinones	+
(Borntrager's Test)	
Steroids and Terpenes	+

NOTE + = Present, and - = Absent.

▪ **Analgesic Activity (hot plate test)**

Table 4: Effects of *Vitellaria paradoxa* on percentage increase in latency Time

Treatment	Dose	% increase in latency time (minutes)			
		0	30	60	120
Control	5ml/kg	0	8	9	10
Diclofenac	2 mg/kg	0	72	106	156
EVP	25mg/kg	0	12	14	14
EVP	50mg/kg	0	38	60	70
EVP	100mg/kg	0	54	80	101

Note: All values are expressed in percentage, EVP=Ethanol Bark Extract of *Vitellaria paradoxa*.

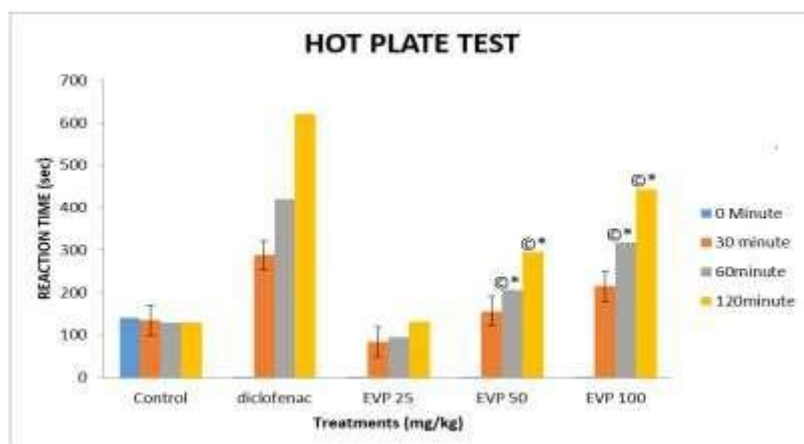


Fig 1: Effects of ethanol bark extract of *Vitellaria paradoxa* on reaction time of mice.

Note: Values represent mean ±SEM (n = 4)

* = significantly different (p<0.05) when compared with control.

© = significantly different (p<0.05) when compared with diclofenac.

▪ **Analgesic Activity (acetic acid test)**

Table 5: Effects of *Vitellaria paradoxa* on percentage decrease in writhing/ abdominal constriction

Treatment	Dose	% writhing/abdominal constriction	
		0	10
Control	5ml/kg	37.4	62.6
Piroxicam	2 mg/kg	14.0	9.3
EVP	25mg/kg	23.4	28.1
EVP	50mg/kg	16.8	23.4
EVP	100mg/kg	6.5	14.0

Note: All Values are expressed in percentage, EVP=Ethanol Bark Extract of *Vitellaria paradoxa*

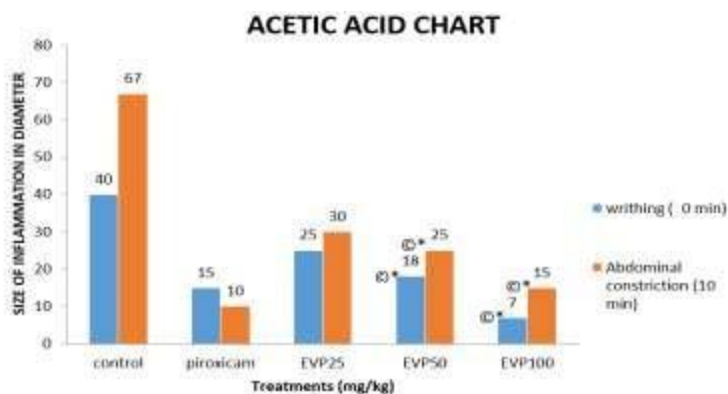


Fig 2: Effects of ethanol bark extract of *Vitellaria paradoxa* on reaction time of mice using Acetic acid test.

Note: Values represent mean ±SEM (n = 4)

* = significantly different (p<0.05) when compared with the control.

©= significantly different (p<0.05) when compared with piroxicam.

▪ **Anti-inflammatory Activity**

The effect of ethanol bark extract of *Vitellaria paradoxa* in carrageenan-induced inflammation showed a dose dependent activity in inflammation reduction.

Table 6: Effects of *Vitellaria paradoxa* on percentage decrease in size of inflammation of rat paw.

Treatment	Dose	% decrease in inflammation					
		0 mn	30 mn	60 mn	120 mn	180 mn	12hr
Control	5ml/kg	13.6	13.9	16	16.7	18.8	20.9
Piroxicam	2 mg/kg	13.6	4.2	4.2	4.2	4.2	4.2
EVP	25mg/kg	13.6	13.5	13.5	13.3	12.6	10.5
EVP	50mg/kg	13.6	12.6	11.9	10.5	9.8	8.4
EVP	100mg/kg	13.6	10.6	9.1	8.4	7	4.9

Note: All values are expressed in percentage, EVP= Ethanol Bark Extract of *V. paradoxa*

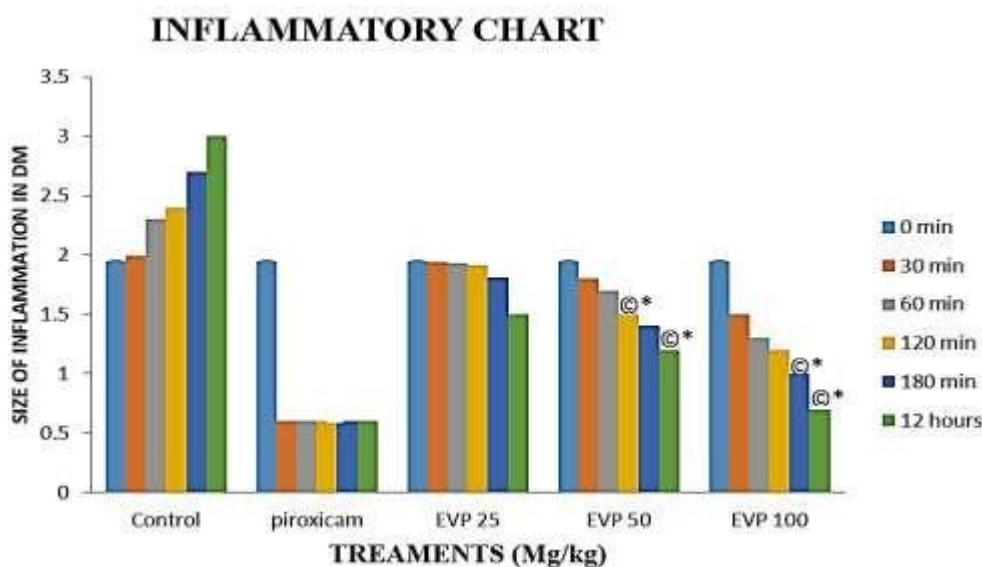


Fig 3: Effects of ethanol bark extract of *Vitellaria paradoxa* on rats using carrageenan test.

Note: Values represent mean ±SEM (n = 4)

* = significantly different (p<0.05) when compared with the control.

© = significantly different (p<0.05) when compared with piroxicam.

Discussion

Preliminary scientific evaluation of natural products for pharmacological effects is often accompanied by their toxicity investigation. Till now very limited toxicity studies have been carried out on plant derived products. However, not only their adverse effects but also its safe doses can be determined by acute oral toxicity test (Aziz, 2015). In this study, LD₅₀ of the plant extracts was found to be 283mg/kg, as mortality was observed at a dose of 400mg/kg and no death at 200mg/kg of the ethanol bark extract of *Vitellaria paradoxa*. Therefore, the test comparative doses (25, 50 and 100 mg/kg) were used for in vivo studies.

The hot plate-induced jumping and paw-licking test is a trustworthy method for investigating nociception as well as possible mechanism of analgesia. A distinct mechanism associated with this behaviour of pain in the test is attributed to neurogenic pain, which is induced by direct stimulation. The pain may arise from the spinal neuro-hyperactivity involving various mediators, which are started by the C-fibres. Peripherally and centrally acting drugs act on these phases to elicit analgesia (Azadmehr *et al.*, 2014).

Effects of ethanol bark extract of *Vitellaria paradoxa* in hot plate test showed a dose-dependent activity in pain reduction. The reaction times for the negative control were found to be 3.3, 2.25, 3.1 and 4.1 sec at 0, 30, 60 and 120 minutes respectively. When diclofenac (2mg/kg) was administered to the mice the reaction times increased from 3.3 to 10 sec, 2.25 to 288 sec, 3.40 to 420 sec and 5.10 to 620 sec at 0, 30, 60 and 120 minutes, respectively. The reaction times produced when the test extract were administered at doses of 25, 50 and 100mg/kg body weight

were 5, 6 and 8 sec at 0 minutes respectively. At 30 minutes, the reaction times increased to 84, 156, 216 seconds following administration of 25, 50, and 100mg/kg body weight of the ethanol bark extract of *Vitellaria paradoxa*, respectively. The highest reaction time following administration of the extract was recorded at 120 minutes when 100mg/kg of ethanol bark extract of *Vitellaria paradoxa* was administered which produced a reaction time of 444 sec (fig 1).

This is further illustrated in table 4, which shows a dose dependent percentage increase in latency time in mice. The percentage increase in latency when normal saline was administered is 8%, 9% and 10% at 30, 60 and 120 minutes, respectively. Diclofenac (2mg/kg) produced 72%, 106% and 156% increase in percentage latency time at 30, 60 and 120 minutes, respectively. However, ethanol extract of bark *Vitellaria paradoxa* (25mg/kg) produced 12%, 13.7% and 13.9% percentage increase in latency time at 30, 60 and 120 minutes, respectively. At a dose of 50mg/kg, the ethanol bark extract of *Vitellaria paradoxa* produced 38%, 60% and 70% increase in latency time at 30, 60 and 120 minutes, respectively. Similarly, 100mg/kg of the extract dose produced a percentage increase in latency time of 54%, 80% and 101% at 30, 60 and 120 minutes, respectively.

Globally, acetic acid induced writhing test is primarily used in the assessment of antinociceptive activity of natural composites (Mazid *et al.*, 2010). The release of different endogenous noxious mediators such as bradykinin, serotonin, histamine, and substance P is induced by acetic acid. Contraction of abdominal muscle goes together with expansion of the forelimbs and

body elongation that characterize the pain that is brought about by acetic acid (Mazid *et al.*, 2010; Rauf *et al.*, 2014). Local peritoneal receptors as well as prostaglandin pathways are thought to be responsible for abdominal contraction (Rauf *et al.*, 2014). To suppress pain conventionally, nonsteroidal anti-inflammatory drugs are prescribed. These drugs are used for treating various diseases such as arthritis, headache, pain and orthopaedic conditions. Analgesic drugs such as diclofenac relieve pain peripherally/centrally by inhibiting cyclooxygenase enzyme (COX-1 and COX-2). Inhibition of cyclooxygenase enzyme reduces the production of pain mediators such as prostaglandins, substance P, histamine, serotonin, and Bradykinin. Pain sensation is eventually reduced in the nociceptors (Inotai *et al.*, 2010).

In this study, the effect of ethanol bark extract of *Vitellaria paradoxa* in acetic acid test showed a dose dependent activity in pain reduction. When normal saline was administered to the mice, the number of abdominal constrictions and writhing were 67 and 38 (37.4% and 62.6%) at 0 and 10 minutes, respectively. However, following administration of piroxicam (2mg/kg) to the mice, there was a potential decrease in number of abdominal constrictions and writhing to 10 and 15 (14.0% and 9.3%) at 0 and 10 minutes respectively. Abdominal constrictions produced when the extract was administered at a dose of 25mg/kg were 35 and 26 (23.4% and 28.1%) at 0 and 10 minutes, respectively. At a dose of 50mg/kg, the number of abdominal constrictions and writhing were 28 and 18 (16.8% and 23.4%) at 0 and 10 minutes respectively. Similarly, the highest decrease in number of abdominal constriction and writhing in mice was 15 and 7 (6.5% and 14.0%) following the

administration of extract was observed at 0 and 10 minutes when 100mg/kg body weight of the ethanol bark extract of *Vitellaria paradoxa* was administered (fig 2, table 6).

These findings strongly suggest that the ethanol bark extract of *Vitellaria paradoxa* possess peripheral or central analgesic property. Perhaps acting in a similar manner as conventionally used therapeutic drugs that reduce the pain perception in nociceptors by inhibiting production of prostaglandins. These results concur with other research studies on the evaluation analgesic activity of herbal plants extract using laboratory animals.

Inflammation has four cellular processes, which are changes in blood flow by changing in smooth muscle cell function that are accountable for vasodilatation, alteration of the vascular permeability, migration phagocytic leukocytes to the site of inflammation, and phagocytosis (Barbosa *et al.*, 2006). Carrageenan-induced paw oedema test is done as acute inflammatory test. In addition, carrageenan can release inflammatory mediators such as bradykinin, histamine, and serotonin. These mediators are responsible for oedema as they enhance vascular permeability and improve vasodilation (Barbosa *et al.*, 2006) Fluid accumulation occurs at the treatment site, which is shown by the carrageenan-induced paw oedema test and inhibition of this fluid accumulation is considered as anti-inflammatory effect. Topical steroids or nonsteroidal anti-inflammatory agents inhibit phospholipase A₂ that can be evaluated by the carrageenan-induced paw oedema test (Aziz, 2015).

The effect of ethanol bark extract of *Vitellaria paradoxa* in carrageenan-induced

inflammation showed a dose dependent activity in inflammation reduction. Before and after administration of normal saline the paw size of inflammation were 1.95 cm, 1.99 cm, 2.30 cm, 2.40 cm, 2.70 cm and 3.0 cm at 0, 30, 60, 120, 180 minutes and 12 hours respectively (fig 3). However when piroxicam (2mg/kg) was administered to the rats, the size of inflammation were 1.95 cm, 0.60 cm, 0.60 cm, 0.50 cm, 0.50 cm and 0.30 cm at 0, 30, 60, 120, 180 minutes and 12 hours respectively. The size of inflammation produced when the test extract was administered at a dose of 25mg/kg were 1.95 cm, 1.94 cm, 1.93 cm, 1.91 cm, 1.60 cm, and 1.50 cm, at 0, 30, 60, 120, 180 minutes and 12 hours respectively. Also at a dose of 50mg/kg of test extract, the size of inflammation produced were recorded as 1.95 cm, 1.80 cm, 1.70 cm, 1.50 cm, 1.40 cm and 1.20 cm at 0, 30, 60, 120, 180 minutes and 12 hours respectively. Similarly at a dose of 100mg/kg of test extract, the size of inflammation produced were also recorded as 1.95 cm, 1.50 cm, 1.30 cm, 1.20 cm, 1.0 cm and 0.70 cm at 0, 30, 60, 120, 180 minutes and 12 hours respectively (fig 3).

Table 6 further illustrates the dose dependent percentage decrease in size of paw inflammation in rats. The percentage increase in size of inflammation when normal saline was administered were 13.60%, 13.88%, 16.04%, 16.74%, 18.83% and 20.92% at 0, 30, 120, 180 minutes and 12 hours respectively. Piroxicam (2mg/kg) produced 13.60%, 4.18%, 4.18 %, 4.18%, 4.18% and 4.18% decrease in percentage at 0, 30, 60, 120, 180 minutes and 12 hours respectively. However, when the test extract was administered at a dose of 25mg/kg, it produced a percentage inflammatory decrease of 13.60%, 13.46%, 13.32%, 12.62%, and 10.46% at 0, 30, 60, 120, 180

minutes and 12 hours respectively. At a dose of 50mg/kg, the extract produced 13.60%, 12.55%, 11.85%, 10.46%, 9.76% and 8.37% percentage decrease in inflammation at 0, 30, 60, 120, 180 minutes and 12 hours respectively. Similarly, at a test extract dose of 100mg/kg the percentage decrease recorded was 13.60%, 10.46%, 9.07%, 8.37%, 6.97% and 4.88% at 0, 30, 60, 120, 180 minutes and 12 hours respectively. Here the significant reduction of paw oedema may be due to the blockage of phospholipase A₂, reduction of vascular permeability, and vasodilation. However, extensive study is required to assure the exact mechanism, through which the extract suppressed oedema.

Vitellaria paradoxa bark extract was found to possess important phytochemicals such as carbohydrates, saponins, tannins and terpenoids. It possesses significant analgesic and anti-inflammatory effects. *Vitellaria paradoxa* provides a good potential for use in management of pain and inflammation.

References

- Ayankunle BA, Kolawole OT, Adesokan AA and Akibinu MO (2012). Antibacterial activity and sub chronic toxicity studies of *Vitellaria paradoxa* stem bark. *Journal of Pharmacology and Toxicology* 7: 298-304.
- Azadmehr A, Sofiabadi M, and Hajiaghaee R (2014). Analgesic effect and immunomodulation response on pro-inflammatory cytokines production by *Scrophularia megalantha* extract. *Tropical Journal of Pharmaceutical Research* 12(6): 935–939.
- Aziz MA (2015). Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and

- antipyretic activities of *Microcos paniculata* barks and fruits. *Journal of Integrative Medicine* **3**(3): 173–184.
- Barbosa-Filho JM, Piuvezam MR, and Moura MD (2006). Anti-inflammatory activity of alkaloids: a twenty-century review. *Revista Brasileira de Farmacognosia* **16**(1): 109–139.
- Calixto JB, Campos MM, Otuki MF and Santos AR (2004). Anti-inflammatory compounds of plant origin part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta medica* **70**(2): 93-103.
- Chao J, Liao JW, Peng WH, Lee MS, Pao LH, and Cheng HY (2013). Antioxidant, analgesic, anti-inflammatory, and hepatoprotective effects of the ethanol extract of *Mahonia oiwakensis* stem. *International Journal of Molecular Sciences* **14**: 2928–2945.
- Gyurkovska V, Alipieva K, Maciuk A, Dimitrova P, Ivanovska N, Christiane H, Thomas B and Georgiev M (2011). Anti-inflammatory activity of Devils claw in vitro systems and their active constituents. *Food Chemistry* **125**: 171-178.
- Hosseinzade H, Ramezani M, Salmani GA (2000) Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria boiss* extracts in mice and rats. *Journal of Ethnopharmacology* **73**(3): 379-385.
- Inotai A, Hanko B, Meszaro A (2010). Trends in the non-steroidal anti-inflammatory drug market in six central-eastern European countries based on retail information. *Pharmacoepidemiology and Drug Safety* **19**: 183-190.
- Izzo AA (2012). Limited value of traditional Chinese medicine in improving quality of life in cancer patients. *Alternative and contemporary therapies* **17**(4): 228-229.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.* **54**: 275-287.
- Khodabakhsh P, Shafaroodi H, and Asgarpanah J (2015). Analgesic and anti-inflammatory activities of *Citrus aurantium* L. blossoms essential oil (neroli): involvement of the nitric oxide/cyclic-guanosine monophosphate pathway. *Journal of Natural Medicines* **69**: 324–331.
- Kumar S, Bajwa BS, Kuldeep S, and Kalia AN (2013). Anti-inflammatory activity of herbal plants: a review. *International Journal of Advances in Pharmacy, Biology and Chemistry* **2**: 272-281.
- Mazid MA, Datta BK, Nahar L *et al.*, (2010). Analgesic and diuretic properties of α -santalone from *Polygonum flaccidum*. *Phytotherapy Research* **24**(7): 1084–1087.
- Newman DJ and Cragg GM (2014). Marine-sourced anti-cancer and cancer pain control agents in clinical and late preclinical development. *Marine Drugs* **12**: 255-278.
- Rauf A, Uddin G, Siddiqui BS, Muhammad N and H. Khan (2014). Antipyretic and antinociceptive activity of *Diospyros lotus* L. in animals. *Asian Pacific Journal of Tropical Biomedicine* **4**(1): 382–386.
- Risso WE, Scarminio IS and Moreira FG (2010). Antinociceptive and acute toxicity evaluation of vernonia condensate baker leaves extracted

- with different solvents and their mixtures. *Indian journal of experimental Biology* **48**: 811-816.
- Trease GE, Evans WC (1989). *Pharmacognosy, Bailliere Tindall, London*. **11**: 45-50
- Younos C, Rolland A, Fleurentin J, Lanhers MC, Misslin R and Mortier F (1990). Analgesic and behavioural effects of *Morinda citrifolia*. *Planta Medica* **56**: 430–434.
- Zhang X, Hung TM, Phuong PT, Ngoc TM, Min BS and Song KS (2006). Anti-inflammatory activity of flavonoids from *Populus davidiana*. *Arch Pharm Res* **29**(12): 1102-1108.

