Analgesic and anti-inflammatory activities of fixed oil of *Macrotyloma uniflorum* (Lam.) Verdc. in mice and rats

Syeda Anum Fatima[1], Sadia Ghousia Baig[1]*, Muhammad Mohtasheem ul Hasan[2], Salman Ahmed[2] and Salma[1]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[2]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: *Macrotyloma uniflorum* commonly known as horse gram or kulthi bean is grown as a pulse for livestock and human consumption. The beans contain about 1.3% fat, 18% protein, 15% carbohydrate along with vitamins and minerals. In traditional medicine it is used as antihyperglycemic, antioxidant, antihypertensive and diuretic. Other important medicinal uses include treatment of renal stones, obesity, piles, oedema and fever. The present study evaluated analgesic (by acetic acid induced writhing, hot plate and tail flick tests in mice) and anti-inflammatory (carrageenan induced paw edema in rats) activities of *Macrotyloma uniflorum* fixed oil (MUFO). Four groups were included in study: Group-I: Normal Saline Control (2ml/kg), Group-II: MUFO (2ml/kg), Group-III: MUFO (4ml/kg), and Group-IV: Standard Acetyl salicylic acid (ASA 300mg/kg). All results were significant however delayed onset of action was observed in tail flick and paw edema tests. Acute oral toxicity of the oil was also checked in mice and was found safe upto 4ml/kg dose, as no signs of toxicity and mortality were observed. It is concluded that *Macrotyloma uniflorum* fixed oil may possess analgesic and anti-inflammatory activity which can be related with a peripheral mechanism of action.

Keywords: *Macrotyloma uniflorum*, fixed oil, acute oral toxicity, analgesic, anti-inflammatory.

INTRODUCTION

Plant derived drugs are used by 70-80% of world's inhabitants for various health problems (Ukwubile and Oise, 2016). It is estimated by W.H.O that 80% of the population of some African and Asian countries have herbal dependency for cure of their paramount medical issues. Medicinal plants have been used for centuries as a remarkable source of treating various types of diseases mainly because of the therapeutic value they have in their various active constituents. This gives rise to unrelenting focus to extract and the active constituents of plants that have a therapeutic value (Bharathi and Vijaya, 2015).

Macrotyloma uniflorum (Lam.) Verdc. (*Dolichos biflorus* L.) belongs to family Fabaceae and commonly known as *'Kulthi Daal'* in Hindi and 'Horse gram'in English (Das *et al*, 2014). The seed of *Macrotyloma uniflorum* is traditionally used as thermogenic, astringent, diaphoretic, anthelminthic, expectorant, tonic and diuretic. It is also effective in bronchitis, haemorrhoids, tumors, splenomegaly, asthma (Philip *et al*., 2009), dysuria, hepatomegaly, diarrhea, colic, leucorrhoea, kidney stones, hiccups and obesity. Flavonoids, phenolic acids and tannins have been reported in *Macrotyloma uniflorum* (Ahmed *et al*., 2016). According to a previous research, extract from *Macrotyloma uniflorum* seeds significantly inhibit *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* (Gupta *et al*., 2005). Many enzymes in the seeds of *Macrotyloma uniflorum* decrease oxidative injury when subjected to toxic levels of lead (Reddy *et al.*, 2005).

Pain is expressed in terms of unpleasant sensory and cognitive experiences happening in response to the primary or secondary tissue injury (Dubin and Patapoutian, 2010). There are various stages of pain which depend on the mode, frequency and site of the stimulus. Pain firstly can be described as stabbing, pricking or lancinating; secondly it can be quantified as more pervasive like throbbing, burning, itching and cramping (Price and Dubner, 1997). Inflammation is an immune response which is initiated in response to tissue damage (Dutta and Das, 2010). There is a worldwide use of NSAIDS (non steroidal anti-inflammatory drugs) for the treatment of fever, pain and inflammation. However, they incur many side effects such as gastric ulcers, renal damage, cardiac abnormalities and bronchospasm, therefore their use is limited or avoided. Thus, research on plants is important to use them as anti-inflammatory agents and pain relievers, as plant derived analgesics will be associated with less side effects (Safari *et al*., 2016). Pharmacological actions of *M. uniflorum* seed extracts have been studied extensively where as current study is focused on the analgesic and anti-inflammatory actions of fixed oil of *M. uniflorum* seeds.

MATERIALS AND METHODS

Plant material and identification

Dry seeds (2 kg) of *Macrotyloma uniflorum* (Lam.) Verdc. were purchased from a departmental store in Karachi. The

**Corresponding author:* e-mail: sadiaghousiabaig@yahoo.com

seeds were identified and authenticated by a taxonomist at Department of Botany, University of Karachi. The seeds were cleaned and crushed then soaked in 5L hexane for a period of one week. After filtration the hexane was evaporated by rotary evaporator to get fixed oil. A brownish-yellow color oil (25ml i.e. 1.25% of total seeds) was obtained.

Chemicals

Acetic acid (Merck, Germany), acetyl salicylic acid (Reckitt Benckiser), carrageenan (Fluka) and hexane analytical grade (Merck, Germany).

Animals

Healthy male and female swiss albino mice of 4-8 weeks, weighing between 20-25grams were obtained from animal house of Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. These animals were used for acute oral toxicity, writhing, tail flick and hot plate activity. Wistar albino rats of either sex weighing 180-200grams were used for carrageenan induced paw edema activity. Animals were housed in propylene plastic cages at a temperature of 22-25°C, 12 hour light and dark cycle and 50-60% humidity. The animals were fed with standard food and water *ad libitum* throughout the experiment. The experimental protocol was approved by institutional bioethics committee, Faculty of Pharmacy and Pharmaceutical Sciences on 17th April 2017 (IBCPH 17) and Board of Advanced Studies and Research (BASR), University of Karachi in November 2015.

Acute oral toxicity

As per OECD (Organization for economic cooperation & development) guideline 423 acute oral toxicity test was conducted in the mice using doses upto 4 ml/kg.

Analgesic activity

Acetic acid induced writhing test

Mice were divided in four groups (n= 7). The animals received different treatments as follows.
Group-I: Normal Saline (2ml/kg)
Group-II: Fixed Oil of *Macrotyloma uniflorum* (2ml/kg)
Group-III: Fixed Oil of *Macrotyloma uniflorum* (4ml/kg)
Group-IV:Standard(Acetyl salicylic acid=ASA 300mg/kg)

All treatments were given orally.

After 30 minutes of drug administration, 0.1ml of 1% acetic acid solution was given intraperitoneally (i.p.) to each group. The experiment was conducted by placing the mice in transparent cages where they were observed for at least 5 minutes or till the first writhe was seen after that each of the mice were further observed for 10 minutes counting the number of writhes per mice that occurred during this time. Percent inhibition was calculated by the following formula (Koster *et al*., 1959):

$$\% \text{ Inhibition} = \frac{\text{NWC - NWT}}{\text{NWC}} \times 100$$

Where,
NWC: Number of writhes in control group.
NWT: Number of writhes in test group.

Tail-flick test

Mice were randomly divided into 4 groups containing 7 animals per group. The animals received different treatments as follows.
Group-I: Normal Saline (2ml/kg)
Group-II: Fixed oil of *Macrotyloma uniflorum* (2 ml / kg)
Group-III: Fixed oil of *Macrotyloma uniflorum* (4 ml / kg)
Group-IV: Standard (ASA 300 mg / kg)

All treatments were given orally.

In this experiment a digital hot water bath was used which was kept constant at a temperature of 51±1°C. The tail flick method as described by Swell and Spencer (1976) was used to determine the analgesic effect of the test samples. One to two centimeter of the tail was dipped in hot water. The index of analgesia was determined from the time taken by the mice to flick their tails in reaction to the heat (latency period) and was determined at 0, 30, 60, 90, 120, 150 and 180 minutes.

Hot plate test

Method of Eddy and Leimbach (1953) was used to assess the analgesic activity through hot plate. Randomly the mice were divided into 4 groups consisting of 7 mice per group. The animals received different treatments as follows.
Group-I: Normal Saline (2ml/kg)
Group-II: Fixed Oil of *Macrotyloma uniflorum* (2ml/kg)
Group-III: Fixed Oil of *Macrotyloma uniflorum* (4ml/kg)
Group-IV: Standard (ASA 300mg/kg)

All treatments were given orally.

The mice were placed on a hot plate which was kept constant at a temperature of 51±1°C. Sensation of pain was recorded when the mice either licked or flicked their limbs or tried jumping off the plate. The readings were recorded after 30, 60, 90, 120, 150 and 180 minutes. The reading taken at 0 minute was considered as baseline reading. A cut off time of 12 seconds was employed so that the animal do not suffer a permanent tissue damage.

Carrageenan induced paw edema test

The experiment was conducted by procedure of Winter *et al*, 1962. Rats were divided into 4 groups (n=7). The animals received different treatments as follows.
Group-I: Normal Saline (2ml/kg)
Group-II: Fixed Oil of *Macrotyloma uniflorum* (2ml/kg)
Group-III: Fixed Oil of *Macrotyloma uniflorum* (4ml/kg)
Group-IV: Standard (ASA 300mg/kg)

All treatments were given orally 40 minutes prior to carrageenan injection.

Intradermal injection of 0.1ml of carrageenan (1% in normal saline) was given into the plantar surface of the right hind paw of all rats. Edema volume (acute phase of inflammatory reaction) was then measured using plethysmometer prior to (at 0 hour) and 1, 2, 3, 4, 5 hours' post carrageenan injection.

STATISTICAL ANALYSIS

The experimental data is expressed as mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by tukey hsd. $P<0.05$ was considered statistically significant. SPSS 20 was used for the statistical analysis

RESULTS

Acute oral toxicity

Mortality rate was zero after 72 hours. No signs of seizures, sedation and unconsciousness were observed. Breathing and other behavioral patterns were normal (table 1).

Acetic acid induced writhing

As compared to control, acetic acid induced writhing is much reduced and very highly significant ($P\leq0.001$) results were obtained both in 2ml/kg and 4ml/kg *M. uniflorum* fixed oil (table 2).

Tail-flick

In tail flick, very highly significant ($P\leq0.001$) analgesic response for single dose or low dose (2ml/kg) was observed at 60 and 90 minutes. However, for high dose (4 ml/kg) very highly significant analgesic response was seen at 90, 120 and 150 minutes (table 3).

Hot plate

Maximum latency in the jumping response for low dose 2 ml/kg and high dose 4ml/kg was observed at 90, 120 and 180 minutes ($P \leq0.001$) (table 4).

Rat paw edema

Percent inhibition of paw edema volume was maximum at 3rd, 4th and 5th hour at 2ml/kg ($P\leq0.001$) whereas at 4 ml/kg 4th and 5th hour showed maximum inhibition ($P\leq0.001$) in inflammation (table 5).

Table 1: Acute oral toxicity of MUFO

Dose	Mortality	Seizures	Sedation	Respiratory change
2 ml/kg	0	0	0	0
4 ml/kg	0	0	0	0

Number of animals (n) =3; MUFO = *M. uniflorum* fixed oil

Table 2: Analgesic effect of MUFO in acetic acid induced writhing test

Groups	Number of writhes in 10 minutes	% inhibition
Control (2ml/kg)	47.3±4.4	---
MUFO (2ml/ kg)	25.3±0.8***	47
MUFO (4ml/kg)	22.3±1.6***	53
ASA (300mg/kg)	15.6±0.6***	67

Values are expressed as mean ± SEM; N=7; MUFO = *Macrotyloma uniflorum* fixed oil; ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at $P<0.001$ as compared to control; **= highly significant at $P<0.01$ as compared to control; *= significant at $P<0.05$ as compared to control.

DISCUSSION

In modern system of medicine treatment of pain mainly relies on NSAIDS which have gastric and cardiac adverse effects associated with them. Nowadays, people are referring to natural compounds as bioactive products to relieve mild to moderate pain. *Macrotyloma uniflorum* is a nutraceutical and has many therapeutic uses like diuretic, hepatoprotective, anti-inflammatory, analgesic, anti-obesity and antihyperlipidemic (Prasad and Singh, 2015).

For the evaluation of peripherally acting analgesic drugs the acetic acid induced writhing test is a useful method. Inflammatory mediators such as bradykinin, substance P, prostaglandins as well as pro-inflammatory cytokines like IL-1, IL-6, IL-8 and TNF-α are triggered by the intraperitoneal injection of acetic acid thereby stimulating the peripheral nociception activity (Silva *et al*., 2015). In this study, it is seen that both doses of MUFO produced significant analgesic response by reducing the number of writhes but maximum analgesic response was observed in standard group. *Macrotyloma uniflorum* fixed oil contains α linolenic acid, linoleic acid and oleic acid (Ahmed *et al.*, 2016). Alpha linolenic acid which is an omega 3 fatty acid has been linked with analgesic activity (Kaithwas *et al*., 2011).

In this study the central analgesic effects of MUFO were assessed using tail flick and hot plate models for nociception. The tail flick method mediates spinal reflexes in response to some nociceptive stimuli while higher brain functions are involved in hot plate method, it has a supra-spinal response. In both of these tests MUFO produced marked, however delayed analgesic effect which indicates that may be the mechanism of analgesic action is not centrally mediated or dose dependent.

The anti-inflammatory response from carrageenan induced paw edema occurs in two phases. The first phase being the non-phagocytic edema (1-1.5 hour) and the

Table 3: Analgesic effect of MUFO in tail flick test

Groups	Latency of flicking response in seconds						
	Basal	30 min	60 min	90 min	120 min	150 min	180 min
Control (2ml/kg)	0.78±0.08	0.98±0.09	0.83±0.05	0.90±0.08	0.88±0.04	0.81±0.03	0.83±0.05
MUFO (2ml /kg)	0.82±0.08	2.80±0.39	5.98±1.02***	3.54±0.40***	2.10±0.36	2.14±0.43	2.66±0.54*
MUFO (4ml/ kg)	0.88±0.18	2.80±0.46	3.60±0.34*	4.51±0.38***	4.60±0.59***	4.45±0.60***	3.79±0.59*
ASA(300mg/kg)	0.66±0.08	4.38±0.66***	4.53±0.53***	3.96±0.53***	3.40±0.20***	2.82±0.08*	2.40±0.13

Table 4: Analgesic effect of MUFO in hot plate test

Groups	Latency of jumping response in seconds						
	Basal	30 min	60 min	90 min	120 min	150 min	180 min
Control (2 ml/kg)	7.3±0.3	7.6±0.3	7.3±0.3	6.6±0.6	7.3±0.3	6.6±0.3	6.6±0.3
MUFO (2ml/kg)	9.3±0.3	13.0±0.7*	13.9±1.0	14.3±0.6***	16.1±0.2***	14.2±0.5*	11.6±0.4***
MUFO (4ml/kg)	7.3±0.8	13.0±2.0*	20.3±3.1*	17.6±0.8***	17.0±1.1***	16.6±2.4*	13.6±0.3***
ASA(300mg /kg)	7.6±0.3	8.6±0.6	12.0±0.6	13.3±0.8*	14.3±1.2*	11.0±0.6	9.3±0.3*

Table 5: Anti-inflammatory effect of MUFO in carrageenan induced paw edema test.

Groups	Paw edema (ml)					
	0 h	1 h	2 h	3 h	4 h	5 h
Control (2ml/kg)	1.5±0.0	2.9±0.1	3.8±0.1	4.5±0.0	5.3±0.1	5.8±0.1
MUFO (2 ml/ kg)	1.7±0.0	1.9±0.1	2.1±0.1*	1.8±0.2***	1.8±0.2***	1.9±0.2***
MUFO (4 ml/ kg)	1.7±0.0	2.2±0.4	2.3±0.3*	2.2±0.4*	2.1±0.4***	1.9±0.3***
ASA(300mg/kg)	1.7±0.0	1.8±0.0*	1.8±0.1***	1.7±0.0***	1.7±0.0***	1.7±0.0***

Values are expressed as mean ± SEM; N=7; MUFO = *Macrotyloma uniflorum* fixed oil; ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at P <0.001 as compared to control; **= highly significant at P <0.01 as compared to control; * = significant at P <0.05 as compared to control

second phase (2-5 hour) in which there is increased edema formation due to significant increase in inflammatory markers like TNF-α, Prostaglandins, Interleukin and Nitric oxide (Mansouri *et al.*, 2015). MUFO may have a role in down regulating these inflammatory mediators as in this study both doses of MUFO caused inhibition in paw edema volume during 3 – 5th hour. In seeds of *Macrotyloma uniflorum*, Proteinase inhibitors such as Bowman-Birk type inhibitors (BBIs) are present (Richardson, 1991). The specific BBI present in horse gram is HGI-III which is stable at cooking temperature (Sreerama *et al.*, 1997). BBIs inhibit trypsin and chymotrypsin and cause antipyretic and anti-inflammatory effects (Duranti, 2006).

It is concluded that MUFO has analgesic and anti-inflammatory activities in 2ml and 4 ml/kg doses. Further study is required to investigate the active principles responsible for these activities and their exact mechanism of action.

REFERENCES

Ahmed S, Hasan MM and Mahmood ZA (2016). Macrotyloma uniflorum (LAM.) VERDC. (Papilionaceae): A review of medicinal uses, phytochemistry and pharmacology. *WJPPS.,* **5**(2):51-62.

Bharati V and Vijaya AA (2015). A review on *Macrotyloma uniflorum*. *Am. J. Pharm. Tech Res.,* **5**(3): 126-130.

Das S, Vasudeva N and Sharma S (2014). Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS Spectroscopy. *Org. Med. Chem. Lett.,* **4**(13): 1-4.

Dubin AE and Patapoutian A (2010). Nociceptors: The sensors of pain pathway. *J. Clin. Invest,* **120**(11): 3760-3772.

Duranti M (2006). Grain legume proteins and nutraceutical properties. Fitoterapia. **77**: 67-82.

Dutta S and Das S (2010). A study of the anti-inflammatory effect of the leaves of *Psidium guajava*

Linn on experimental animal models. *Pharmacognosy Res.,* **2**(5): 313-317.

Eddy NB and Leimbach D (1953). Synthetic analgesics. II. Dithienylbutenyl- and di thienl butyl amines. *J. Pharmacol. Exp. Ther.,* **107**: 385-393.

Philip A, Athul PV, Charan A and Afeefa TP (2009). Anthelmintic Activity of Seeds of *Macrotyloma uniflorum*. Hygeia, **1**(1): 26-27

Prasad SK and Singh MK (2015). Horse gram- an underutilized nutraceutical pulse crop: *A review. J. Food Sci. Technol.,* **52**(5): 2489-2499.

Price DD and Dubner R (1997). Mechanism of first and second pain in the peripheral and central nervous systems. *J. Invest. Dermatol.,* **69**(1): 167-171.

Reddy AM, Kumar SG, Jyothsna kumari G, Thimmanaik S and Sudhakar C (2005). Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.)(Verdc.) and Bengalgram (*Cicer arietinum* L.). *Chemosphere*, **60**: 97-104.

Richardson M (1991). The complete amino acid sequence of the major Kunitz trypsin inhibitor from the seeds of *Prosopis juliflora*. *Phytochemistry,* **30**: 2829-2833.

Safari VZ, Kamau JK, Nthiga PM, Ngugi MP, Orinda G and Njagi EM (2016). Antipyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Aloe volkensii* in Albino Mice. *Med. Aromat. Plants*, 5(3): 1-7.

Sewell RD and Spencer PS (1976). Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail immersion test in mice and rats. *Neuropharmacol.,* **15**(11): 683-688.

Silva JC, Araújo CDS, Lima-Saraiva SRGD, Oliveira-Junior RGD, Diniz TC, Wanderley CWDS, Palheta-Júnior RC, Mendes RL, Guimarães AG, Quintans-Júnior LJ and Almeida JR (2015). Antinociceptive and anti-inflammatory activities of the ethanolic extract of Annona vepretorum Mart. (Annonaceae) in rodents. *BMC Complement. Altern. Med.,* **15**(197): 1-10.

Sreerama YN, Das JR, Rao DR and Gowda LR (1997). Double-headed trypsin/chymotrypsin inhibitors from horse gram (*Dolichos biflorus*): Purification, molecular and kinetic properties. *J. Food Biochem.,* **21**: 461-477.

Ukwubile CA and Oise IE (2016). Analgesic and Anti-inflammatory Activity of *Physalis angulata* Linn. (Solanaceae) Leaf Methanolic Extract in Swiss Albino Mice. *Int. Biol. Biomed. J.,* **2**(4): 167-170.

Winter CA, Risley EA and Nuss GW (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.,* **111**: 544-547.