Analgesic, anti-inflammatory and diuretic activities of *Macrotyloma uniflorum* (Lam.) Verdc.

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Abstract: *Macrotyloma uniflorum* (Lam.) Verdc. (Papilionaceae) is commonly known as Horse gram and *Kulthi*. The seeds are reported as anthelmintic, diaphoretic, diuretic and emmenagogue. It is also useful in asthma, bronchitis and urolithiasis. In the present study, analgesic, anti-inflammatory and diuretic effects of the methanol extract of *Macrotyloma uniflorum* seeds were evaluated in doses of 200 and 400mg/kg. Significant results were obtained in all activities.

Keywords: *Macrotyloma uniflorum*, methanol extract, analgesic, antiinflammatory, diuretic.

INTRODUCTION

Macrotyloma belongs to family Papilionaceae, is a genus of about 24 species, mainly distributed in Africa and Asia. It is widely cultivated in the tropics while some species are pantropical. *Macrotyloma uniflorum* (Lam.) Verdc. (Synonym: *Dolichos uniflorus* Lam.) is the only species found in Pakistan. In English it is called Horse gram and Madras gram, where as in Urdu it is known as *Kulthi*. The fruits of Horse gram are 3-5.5cm long and 6-8 mm broad. Seeds are oblong or orbicular-reniform in shape, 3-4.2 × 2.8-3.5mm, light or deep reddish brown (Ali, 1973). Traditionally the seeds are used as anthelmintic, diaphoretic, diuretic, emmenagogue and also useful in asthma, bronchitis, haemorrhoid, nephrolithiasis, splenomegaly and urolithiasis. The seeds of Horse gram when boiled and mixed with the ordinary gram, is regarded as a good food for horses, similarly green and tender parts of the plant are used as fodder. The chemical literature search of *Macrotyloma uniflorum* reveals the presence of caffeic acid, syringic acid, ferulic acid, sinapic acid, phenolic compounds, alkaloids, saponin, flavonoids, sterols and vanillic acid. The pharmacological studies reported *Macrotyloma uniflorum* as antihepatotoxic, anti pepticulcer, antilithiatic, anthelmintic, antidiabetic, larvicidal, antibacterial, antifungal and antioxidant (Sulochana *et al.,* 2009; Ahmed *et al.,* 2016).

MATERIALS AND METHODS

Plant material

The seeds of *Macrotyloma uniflorum* were procured from market. Identification and authentication was carried out by a Taxonomist, Department of Botany, University of Karachi with voucher specimen number (G.H. No.86483).

Preparation of extracts

Seeds (2kg) were cleaned, coarsely crushed then soaked in 5L methanol for a week, then filtered and the filtrate was concentrated under vacuum at 40ºC by rotary evaporator. This methanol extract was used for pharmacological activities. *Macrotyloma uniflorum* seeds' methanol extract= MUME

Animals

Swiss albino mice of 20-25g and Wistar albino rats of 150-200g were used in the study. They were kept under a normal day-night-cycle at 25±2°C in poly propylene cages with food and water. The animals were grouped (N=7) randomly as control, standard and test groups. All procedures were followed according to ethical standards and international guidelines on animal experimentation.

Chemicals and drugs

The chemicals and drugs used in the study were of analytical grade.

Pharmacological studies

Acute toxicity assay

Swiss albino mice were used in the toxicity study. Methanol extract of *Macrotyloma uniflorum* (MUME) in the doses of 100-2000mg/kg were orally administered and observed for the first 4h, then up to 24h for any change such as convulsions, grooming, hyperactivity, hypothermia, sedation and mortality. The doses of 200 and 400mg/kg, bw were selected for pharmacological studies (Lorke, 1983).

For analgesic, anti-inflammatory and diuretic activities of MUME the treatment of control, test and standard (mg/kg b.w.) by oral route for each activity to different animal groups N=7 is given below.

The analgesic activity was determined by three different methods *viz* acetic acid induced writhing test (Koster *et*

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Group I	Control	10ml/kg bw	Distilled water
Group II	Test	200mg/kg bw	MUME
Group III		400mg/kg bw	
Group IV	Standard	100mg/kg bw	Acetyl salicylic acid (analgesic activity)
		50mg /kg bw	Diclofenac sodium (anti-inflammatory activity)
		20mg/kg bw	Furosemide (diuretic activity)

al., 1959), tail immersion test (Schmauss and Yaksh, 1984) and hot plate test (Eddy and Leimbach, 1953), while anti-inflammatory activity (Winter *et al.*,1962) and diuretic activity (Zhao *et al.,* 2009) as reported in literature.

STATISTICAL ANALYSIS

Results were analyzed by one way ANOVA followed by Posthoc test (tukey HSD). Value of $P \leq 0.05$ was considered significant and $P \leq 0.01$ as highly significant.

RESULTS

The acute toxicity test was performed in mice. MUME did not show any symptom of toxicity and mortality when the extract up to 2000mg/kg b.w, p.o were given. Therefore the extract was considered safe. The doses of 200 and 400 mg/kg b.w. were used for pharmacological activities. In mouse writhing test, the extract significantly inhibited the writhes (table 1). The inhibition produced by MUME- 400 was 83.09%, whereas by MUME-200 was 76.76%. The standard drug Acetyl salicylic acid showed 68.30% inhibition. The pretreatment of animals in tail immersion test with extracts significantly delayed the reaction time to the nociceptive stimulus till 90 minutes (table 2). Similarly, the extract significantly increased the reaction time to the nociceptive response at 30 and 60 min, in hot plate test (table 3).

MUME showed anti inflammatory effect on the rat paw oedema (table 4). The significant anti-inflammatory effect started at 60 min by the extract in both doses and remained upto 240min. Diclofenac sodium (50mg/kg) decreased paw volume significantly.

MUME showed significant diuretic effect at the dose of 400mg/kg upto 24 hrs (table 5), while in 200mg/kg MUME showed significant effect only at 60min.

DISCUSSION

The acute toxicity assay revealed that MUME is nontoxic as no symptom of toxicity or mortality was found up to the dose of 2000mg/kg. The observed lack of toxicity confirms its use as fodder. Many pathophysiological conditions like arthritis, vascular diseases and cancer are associated with pain. In different traditional medicinal systems a large number of plants and their products are used to relieve and treat pain. Therefore any attempt to contribute an analgesic drug derived from easily available plants is always accorded without any reluctance. In the present study one chemical and two thermal pain models were used, so that central and peripheral action can be measured. The writhing test shows peripheral effect whereas tail immersion and hotplate tests reveal central activity. Injection of irritants such as phenylquinone and acetic acid in to the peritoneal cavity of mice induce nociception causing writhing. Acetic acid causes the release of histamine, kinins and prostaglandins etc. These mediators stimulate the nociceptive neurons which are sensitive to NSAID and opioids. As prostanoids are involved in this process therefore, the level of PGE_2, $PGF_2\alpha$ and lipoxygenase products were increased in peritoneal fluid (Sulaiman *et al.,* 2008). Therefore, analgesic action of the methanol extract of *Macrotyloma uniflorum* can be through inhibition of prostaglandin activity or synthesis.

The tail immersion and hotplate tests are commonly employed to assess central analgesic effect. Opioid agents exhibit their analgesic action both via spinal (μ_2, k_1, δ_2) and supraspinal (μ_1, k_3, δ_1, σ_2) receptors (Jinsmaa *et al.,* 2005). An increase in the reaction time in both tail immersion and hotplate methods by MUME indicate analgesic effect by central mechanism which involve the endogenous opioid peptides and also the biogenic amines as 5HT. Flavonoids have shown analgesic effect (Hosseinzadeh *et al.,* 2002) and the presence of flavonoids in the methanol extract of *Macrotyloma uniflorum* may also involve in the antinociceptive effect (Morris *et al.,* 2013).

The process of inflammation is very complex and generally associated with pain. In this condition there is an increase in vascular permeability, mononuclear cells migration, increase of granulocytes and granulomatous tissue. Anti-inflammatory agents act in different ways like they inhibit COX-2 or may decrease enzyme expression, decrease arachidonic acid release or some compounds may inhibit the release of previously formed mediators such as histamine, where as some block histamine receptor. Immnostimulation is another mode of action for anti-inflammatory compounds e.g. stimulation of phagocytosis or maturation of myeloid cells (Safayhi and Sailer, 1997). Prostaglandins are formed and released by mast cell types. The first step in the biosynthesis of PGs is catalyzed by cyclooxygenase (COX). Constitutive form of Cyclooxygenase-1 is involved in housekeeping cellular function. COX-2 (isoform of COX) increases as a result of different stimuli and also by inflammation process in different types of tissues. COX-3 (isoform of COX) is

Table 1: Analgesic effect of MUME in acetic acid induced writhing test

Treatment / Dose (mg/kg)	Number of writhings	Inhibition (%)
Control (vehicle)	28.40 ±3.77	-
MUME – 200	6.60±1.32**	76.76
MUME – 400	4.80±0.96**	83.09
Acetylsalicylic acid-300	9.00±1.37**	68.30

Table 2: Analgesic effect of MUME in tail immersion test

Treatment / Dose (mg/kg)	Latency period in sec.			
	0 min	30 min	60 min	90 min
Control (vehicle)	0.86±0.10	1.08±0.19	0.96±0.16	1.00±0.17
MUME-200	1.10±0.15	2.58±0.46*	2.56±0.12*	5.32±0.58**
MUME-400	1.12±0.21	5.32±0.34**	11.00±2.07**	9.80±1.15**
Acetylsalicylic acid-300	1.40±0.18	4.78±0.47**	11.60±3.17*	12.40±2.29**

Table 3: Analgesic effect of MUME in hot plate test

Treatment / Dose (mg/kg)	Latency period in sec.	
	30 min	60 min
Control (vehicle)	0.84±0.20	0.94±0.15
MUME-200	3.17±0.40*	3.28±0.48*
MUME-400	3.80±0.86*	7.85±2.8*
Acetylsalicylic acid-300	4.20±1.49*	2.98±0.53*

Table 4: Anti-inflammatory effect of MUME in rat paw edema test

Treatment/Dose (mg/kg)	Changes of paw oedema volume in ml				
	0 min	60 min	120 min	180 min	240 min
Control (vehicle)	1.76±0.09	2.70±0.07	3.70±0.05	4.10±0.05	4.50±0.15
MUME-200	1.78±0.05	2.96± 0.09**	3.05±0.07**	3.54±0.12**	3.86±0.09**
MUME-400	1.6±0.05	1.99± 0.05**	2.43±0.08**	3.00±0.05**	3.46±0.14**
Diclofenac sodium- 50	1.89±0.02	2.18±0.08*	2.32±0.10**	2.42±0.10**	2.45±0.10**

Table 5: Diuretic effect of MUME in rats

Treatment/Dose (mg/kg)	Volume of urine in ml				
	30 min.	60 min.	90 min.	120 min	24 hr
Control(vehicle)	0.00	0.03±0.03	0.200.0	0.41±0.00	1.16±0.33
MUME-200	0.00	0.16±0.03*	0.266±0.17	0.43±0.12	2.80±0.23
MUME- 400	0.06±0.03	0.53±0.03**	1.00±0.00**	1.03±0.03**	3.76±0.03**
Furosemide- 20	0.56±0.03**	0.9±0.05**	0.90±0.05**	1.00±0.00**	4.16±0.16**

Values are expressed as mean ± SEM., N = 7; *p<0.05 (significant) and **p<0.01(highly significant) compared with control

present in the cortex, brain and cardiac tissues (Chandrasekharan *et al.,* 2002; Teather *et al.,* 2002).

Rat paw oedema is a very common test for acute inflammation to investigate new anti-inflammatory agents. The method consists of two phases. The first phase (1-2 hr) is mediated by serotonin, histamine and also by an increased synthesis of prostaglandins in the damaged tissue area, whereas the second phase is sustained by prostaglandin release (Antonio and Brito, 1998).

The results of anti-inflammatory activity indicate that MUME inhibited carrageenan induced rat paw odema significantly at both doses in the first hour of experiment and sustained it upto 4th hour. The antioxidant activity of *Macrotyloma uniflorum* has been reported (Ahmed *et al.,* 2016) and it is also well known that at the site of inflammation high concentration of free radicals and oxidants are present and involve in the process of inflammation. Therefore the antioxidant compounds of MUME may also involve in preventing the process of inflammation. Similarly the flavonoids in the extract also

involve in anti-inflammatory effect as the flavonoids can block the activities of lipoxygenase and cyclooxygenase and therefore diminish the formation of inflammatory metabolites (Nijveldt *et al.,* 2001). Beside flavonoids the alkaloids, saponins and sterols present in the extract (Sulochana *et al.,* 2009; Atchibri *et al.,* 2010) may also involve in anti-inflammatory effect of the extract.

Diuretics are the drugs used in ascites, cirrhosis, congestive heart failure, hypertension, pulmonary diseases, nephritic syndrome, renal failure and toxemia of pregnancy. These diuretics alter renal function by promoting the formation of urine and therefore eliminate excess liquid and toxic substances from the body. Diuretics currently in use such as furosemide and thiazides cause various side effects like electrolyte imbalance, metabolic alterations, hyperuricemia, acidosis, gastric irritation, hyperglycemia, impotence, fatigue and weakness (Hullatti *et al.,* 2011; Nayak *et al.,* 2013).

The results of the methanol extract of *Macrotyloma uniflorum* (MUME) in diuretic activity reveal that the extract has significant diuretic activity in 400mg/kg and is comparable to standard (furosemide) group. The diuretic action produced by MUME may be related with inhibition of tubular reabsorption of water by mediators or by neurohumoral mechanism. All these possibilities of diuretic action is due to secondary metabolites in the extract (Galati *et al.,* 2002; Nayak *et al.,* 2013)

CONCLUSION

The methanol extract of the seeds of *Macrotyloma uniflorm* (MUME) did not show any symptom of change in behaviour or mortality upto 2000mg/kg oral dose that indicates therapeutic safety of the pharmacologically active doses. In analgesic activity extract exhibited both central and peripheral antinociceptive activity. Similarly in anti-inflammatory activity the extract has the potential to inhibit lipoxygenase and or cyclooxygenase, involved in arachidonic metabolism. The extract produced significant diuresis when rats were treated acutely which support the use of *Macrotyloma uniflorm* as diuretic agent in ethnomedicine. Taking these findings into account, it seems quite possible that *Macrotyloma uniflorum* (Lam.) Verdc. contains chemical compounds with analgesic, anti-inflammatory and diuretic activities. Further studies are required for the isolation and characterization of the active compounds and to determine their mode of action.

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