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Research Article

ANTI-INFLAMMATORY, ANALGESIC AND ULCEROGENIC ACTIVITY OF *VIGNA MUNGO* LINN. LEAVES

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ABSTRACT

The anti-inflammatory, analgesic and ulcerogenic activity of extract of leaves of *Vigna Mungo* linn. (Leguminosae) were investigated as well as the mechanisms of action. The extract significantly ($P<0.05$) inhibited the formation of paw edema induced by carrageenan in rat and increased reaction latency to thermal pain in rat in a dose-dependent manner. The extract caused a significant ($P<0.05$) dose-dependent ulceration of rat gastric mucosa and concentration-dependent inhibition of hypotonicity-induced haemolysis of red blood cell. Also the extract significantly ($P<0.05$) inhibited the activates of the phospholipase A_2 and prostaglandine synthesis in a concentration related manner. These suggest that the leaves posses anti-inflammatory, analgesic and ulcerogenic activities mediated through sequential inhibition of the enzymes responsible for prostaglandine synthesis from arachidonic acid. Phytochemical analysis of the extract revealed the presence of glycoside, tannins, alkaloid, flavonoids and Saponins. Acute toxicity studies established an oral LD_{50} greater than 3000 mg/kg.

Keywords- *Vigna Mungo* Linn; Pharmacological evaluation; phytochemical investigation

1. INTRODUCTION

Medicinal plants with anti-inflammatory activity are considerably employed in the traditional treatment of several disorders of inflammation. The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair.[1] these different reactions in the inflammatory response cascade are therapeutic targets which anti-inflammatory agent including medicinal plants interfere with to suppress exacerbated inflammatory responses usually invoked in such disorders as rheumatoid arthritis, in infection or injury. Inhibition of the synthesis of pro-inflammatory prostaglandins is one of such therapeutic targets to which some of the potent anti-inflammatory agents of clinical relevance (NSAIDs) owe their activity[2]. several anti-inflammatory medicinal plants have also demonstrated the ability to inhibit the synthesis of prostaglandins[3].

Vigna Mungo Linn. (Leguminosae) is widely distributed in tropical west Africa and extensively cultivated all over India. It is commonly knows as Black gram, Urad and Udid. Hot aqueous extracts of the leaves are useds in the treatment of stomach, rhematic pain and

inflammatory disorderds. Indicating that the plants leaves may possess anti-inflammatory, analgesic and ulcerogenic properties among others. The young leaves are used as vegitable.[4]
The antiatherogenic nature of *Phaseolus Mungo* L. has been reported.[5] The enzymes IAA oxidase and peroxides are responsible for the production of IAA.[6] black gram fiber exhibits significant hypoglycemic action in experimental animals.[7]
The search for new anti-inflammatory agents from the vast array of medicinal plant sources in intensifying since they may hold promise for the discovery of therapeutic agents with beneficial effect not just in suppressing relevant aspects of the inflammatory cascade but also on diverse disease conditions where the inflammatory response is amplifying the disease process. This present study carried out to assess the validity of the folkloric uses of this plant in the management of pain and treatment of inflammatory disorders and establish the possible mechanisms of pharmacological action.

2. MATERIAL AND METHODS

2.1. Plant Materials: Fresh leaves of *Vigna Mungo* Linn. Collected in local area of Jalgaon. The plant was identified and authenticated from the Department of Botany, Rashtrasant Tukadoji Maharaj University, Nagpur. The Account no. of authentication certificate was 9146. The fresh leaves were cleaned, dried and pulverized. About 200 g of the powdered leaves was boiled and boiled in distilled water for 2 h. the filtrate was partitioned with chloroform: methanol (2:1) to remove traces of fatty constituents from the hot water extraction. The methanol fraction (15.29%) was concentrated and subjected to phytochemical analysis using the general method and evaluated for biochemical and pharmacological activity.
2.2. Animal and Tissues: Adult albino rats (150-200) of either sex. The animal were acclimatized for about 7 days under standard environmental condition and were maintained on a regular feed and water ad *libitum* Ox vesicles (OSV) and ox blood were obtained from healthy animals slaughtered in the local abattoir Nsukka. Strains of *Bacillus pulmilum* used for enzyme assay were obtained from the Dept. of Microbiology.
2.3. Chemical: The chemical used for this study included analytical grades of methanol, hydroquinone, ethyl acetate, sucrose, ethylene diamine tetracetate (EDTA), hydrochloric acid, sulphuric acid, sodium chloride, chloroform, tri-sodium citrate, alpha naphthol, glutathione, adenosine 5^1-diphosphate (ADP) and hemoglobin. Other reagents and solvent were also of analytical grade.
2.4. Acute toxicity and lethality (LD_{50}) test: Acute toxicity study was carried out according to OECD guidelines (Organization for economic co-operation and development).[8] The acute toxicity study of various extracts of *Vigna Mungo* Linn. Leaves shown signs of toxicity like tremor, convulsion and deep breathing at 2000 mg/kg b.w. $1/10^{th}$ of the same dose for all these extract were taken as therapeutic dose i.e.200 mg/kg b.w.
***2.5. Anti-inflammatory activity test*:** Anti-inflammatory activity was assessed in rat using a modification.[9] of the method of Winter *et. al.*[10] increase in paw volumes was used to assess inflammation. Four group of rats (n = 5) were deprived of food but not water for 18 h and then received i.p. injection of the extract (0.5 and 1 mg/kg) thirty minutes later, each animal received subplantar injection of carrageenan (0.1 ml of 2% suspension) in its right hind paw. Paw volume was measured by mercury displacement before and at 1.5 and 5.5 h after carrageenan injection. Control animals received either normal saline (5 ml/kg) or phenybutazone (150 mg/kg).
2.6. Analgesic activity test: Analgesic activity was tested in rats using the hot plate method. Twenty five rats of either sex were grouped in five (n = 5 per group). Each group received one dose of the extract (50, 100, 150 mg/kg), normal saline (5 ml/kg) or acetylsalicylic acid (100 mg/kg). at 15 and 60 min after extract administration, animal were lowered onto the

surface of a hot plate (50±2 ^{0}C) enclosed with cylindrical glass and the time for the animal to raise or lick the fore limb was noted as the reaction time (RT). Cut off time in the absence of a response was 90 sec to prevent the animal from being burnt.[11]

2.7. Gastric ulcerogenic activity test: The ulcerogenic activity of the extracts was investigated using the method of Cashin *et. al.*[12] rat of either sex were fasted for 18 h with access to water. At the end of the fasting the animal received the extracts (250 or 500 mg/kg; n = 5) orally control animal received indomethacin (30 mg/kg) or normal saline (5 ml/kg). Eight hours after drug administration, animals were scarified and stomachs opened along the greater curvature. The stomach mucosa was examined for ulcer lesion using a hand lens (x20 magnification). The length of lesions on the gland at portion were estimated and summed up to calculate the ulcer index using the method.

2.8. Phospholipase A_2 activity test: The preparation of Phospholipase A_2 from *B. pulmilus* and assay of the extract on its activity were performed using the method of Vane.[13] aliquots (0.5 ml) of re-suspended erythrocytes were mixed with normal saline containing 2 Mm calcium chloride and the enzyme preparation and incubated either in the absence or presence of the extract (0.37, 0.74, 1.10 mg/kg) at 37^{0}C for 1 h. the incubated reaction mixture was centrifuged at 3,000 g for 10 min and the absorption of the supernatant read against the blank at 418 nm. Prednisolone, a known inhibitor of the enzyme was used as control.

2.9. Prostaglandine synthesis activity test: Prostaglandine synthesis was isolated from ox-seminal vesicle using the method of Nugteren et. al.[14] the enzyme activity was assayed using a modification method of Yoshimoto et. al.[15] and Flower et. al.[16] the enzyme activity was monitored at 278 nm due to the formation of PGB from PGE_2 by concentrated alkali treatment. The reaction mixture consisted of 1.5 ml cofactor solution and 0.5 ml arachidonic acid as substrate. After incubating at 37^{0}C for 2 min, the reaction was stopped by adding 1.5 ml of 0.2 M citric acid, extracted twice with 5 ml ethyl acetate and centrifuged at 2,500 g for 10 min. each time 4 ml aliquots of the top organic layer were pipette into a clean test tube.

***2.10. Membrane stabilization activity tes*t:** The membrane stabilization effect of the extract was evaluated using hypotonicity induced haemolysis of red blood cells. Briefly, citrated ox-blood sample were centrifuged at 3,000 g for 10 min. the pellets containing the red blood cells were re-suspended in volumes of saline equal to those of the plasma. An aliquot (0.5 ml) of the red cell suspension was added to 4 ml water and incubated at 37^{0}C for 1 h.

3. STATISTICAL ANALYSIS

Data was analyzed using ANOVA, further subjected to Fischer LSD post hoc test and expressed as Mean ± SEM. Differences between means were regarded significant at $P<0.05$.

4. RESULTS AND DISCUSSION

4.1. Phytochemical tests: Phytochemical tests on the extract have positive reaction for glycosides, tannins, alkaloids, flavonoids and Saponins.

4.2. Acute toxicity: The acute toxicity studies revealed an oral LD_{50} greater than 2000 mg/kg.

4.3. Effect on carrageenan induced paw edema formation: The extract significantly ($P<0.05$) inhibited the formation of paw edema in rats. The magnitude of inhibition increased with time with the effect of the extract comparing well with that of phenybutazone (Table No.1)

4.4. Effect on reaction latency to thermal induced pain in rat: The extract caused a dose-dependent increase in reaction latency to thermal pain. At 15 min, the extract (50 mg/kg) significantly ($P<0.05$) evoked a longer reaction latency than aspirin and also significantly ($P<0.05$) prolonged the reaction latency at 60 min (Table No.2)

4.5. Effect on gastric ulcerogenic activity: The extract caused a significantly (P<0.05) dose-dependent ulceration of the rat gastric mucosa. The ulcerogenic effect of the higher dose was comparable to indomethacin (Table No.3)

4.6. Effect on phospholipase A_2 activity: The extract significantly (P<0.05) inhibited phospholipase A_2 activity in a concentration related manner provoking inhibition comparable to that pf Prednisolone (Table No.4)

4.7. Effect on prostaglandine synthesis activity: The extract evoked a significant (P<0.05) concentration related inhibition of prostaglandine synthesis activity (Table No.5)

4.8. Effect on hypotonic-induced haemolysis: The extract significantly (P<0.05) inhibited hypotonicity-induced haemolysis of red blood cells in a concentration-dependent manner (Table No.6)

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Table No. 1: Effect of extract on carrageenan induced paw edema in rats.

Extract	Dose (mg/kg)	Edema (ml)		Inhibition of edema (%)	
		1.5 h	5.5h	1.5 h	5.5 h
Control	_	0.48±0.01	0.41±0.01	_	_
Methanol extract	500	0.02±0.01	0.07±0.01	58.3	82.9
	1000	0.42±0.01	0.07±0.01	12.5	82.9
Phenybutazone	150	0.03±0.01	0.02±0.01	37.5	51.2

*P<0.05 compared to control (ANOVA; LSD post hoc) n = 5.

Table No. 2: Effect of extract on latency of pain reaction in rats.

Extract	Dose (mg/kg)	Reaction latency (T) (s)	
		15 min	60 min
Control	_	6.3±0.6	6.1±0.25
Methanol extract	50	5.1±0.8#	6.1±0.25
	100	7.0±1.40	13.8±1.83*
	150	8.0±1.00	15.3±0.58*
Acetylsalicylic acid	100	8.30±0.90	12.0±0.60*

#*P<0.05 compared to acetylsalicylic acid and control respectively (ANOVA; LSD post hoc).

Table No. 3: Gastric ulcerogenic activity of extract.

Extract	Dose (mg/kg)	Ulcer index (mm)
Control	_	0±0.00
Methanol extract	250	8±1.15*
	500	14±1.73*
Indomethacin	30	15±1.15*

Values of ulcer index shown are Mean ± SEM., *P<0.05 compared to control (ANOVA; LSD post hoc) n = 5.

Table No. 4: Effect of extract on phospholipase A_2 activity.

Extract	Dose (mg/kg)	Absorbance	Inhibition of enzyme activity (%)
Control	_	1.33±0.06	00.0
Methanol extract	0.37	0.64±0.01*	51.88
	0.74	0.14±0.00*	89.47
	1.10	0.02±0.01*	98.49
Prednisolone	1.00	0.11±0.01*	99.13

Values of absorbance shown are Mean ± SEM of triplicate determination.
*P<0.05 compared to control (ANOVA; LSD post hoc).

Table No. 5: Effect of extract on prostaglandine synthesis activity.

Extract	Concentration (mg/kg)	Absorbance	Enzyme activity	Inhibition of enzyme activity (%)
Control	_	1.570±0.040	7.15	0.00
Methanol extract	0.1	0.110±0.005*	5.54	22.52
	0.5	0.055±0.003*	3.07	57.06
	1.0	0.004±0.001*	1.08	84.89
	5.0	0.020±0.006*	0.54	92.45
Indomethacin	4.0	0.030±0.003*	2.00	72.02

Values of absorbance shown are Mean ± SEM of triplicate determination.
*P<0.05 compared to control (ANOVA; LSD post hoc). Percent inhibition enzyme activity was calculated relative to control.

Table No. 6: Effect on extract on hypotonic-induced haemolysis.

Extract	Concentration (mg/kg)	Absorbance	Inhibition of haemolysis (%)
Control	_	0.46±0.02	0.00
Methanol extract	0.1	0.21±0.01*	54.35
	0.2	0.20±0.01*	56.52
	0.4	0.15±0.00*	67.39
Indomethacin	0.2	0.15±0.01*	67.39
	0.4	0.13±0.01*	71.74
	0.6	0.16±0.01*	65.22
Distilled Water	_	0.65±0.28	_

Values of absorbance shown are Mean ± SEM of triplicate determination.
*P<0.001 compared to control (Normal saline) and distilled water (ANOVA; LSD post hoc).
Percent inhibition of haemolysis was calculated relative to control.