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ANALGESIC EFFECTS OF METHANOLIC SEED EXTRACT OF V*igna subterranea* (Fabaceae) IN ALBINO MICE

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ABSTRACT

Vigna subterranea is claimed traditionally to possess antioxidant and analgesic effects. This study is aimed at evaluating the possible analgesic claims of *Vigna subterranea*. An acute toxicity study of the extract was carried out using the Lorke's method. The central and peripheral analgesic effects were assessed using a hot plate and acetic acid-induced writhing tests respectively. The methanolic seed extract of *Vigna subterranea* (MEVS) at doses of 250 and 500 mg/kg produced significant central analgesic activity ($P<0.05$) when compared to the control. Similarly, the extract at doses 250 and 500 mg/kg significantly reduced the number of writhes. The percentage inhibition of writhing is in ascending order of 23.85, 35.98 and 43.09 % in a dose-dependent manner (125 mg/kg to 500 mg/kg). Findings from this research lend credence to the folkloric use of *Vigna subterranea* and may serve as a basis for development of pure analgesic compounds from it.

Keywords: Analgesic; *Vigna subterranea*; Seeds; Acetic Acid; Writhing; Hot Plate.

INTRODUCTION

Pain is felt when the brain responds to a potential harm (Lamont *et al*., 2000). Change in the physical condition of the organ whether there is tissue injury or not was said to be the cause of pain (Tashani and Johnson, 2010). This assumption agrees with the modern understanding of pain by the International Association for the Study of Pain (IASP): as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (IASP, 2020).

Epidemiological data showed that about 50% of people in developed countries is at the risk of chronic pain (Tashani and Johnson, 2010). Uncontrolled pain can affect the physical, mental, social, and financial conditions of an individual. (IASP, 2020). Postoperative acute pain can worsen into chronic pain if not properly treated (Wells *et al.*, 2012). Pain causes a significant increase in the rate of hospitalization and death (Torrance *et al.*, 2015). The aged, pregnant and nursing mothers, infants, people with substance abuse and the mentally ill are at a higher risk for inadequate pain management (Mędrzycka *et al.*, 2015).

Pain remains the most challenging and devastating health problem affecting 52.5 % of the adult population worldwide despite the availability of sufficient drugs (WHO, 2019). Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the standard drugs used in treating mild to moderate and severe pains respectively. However, these drugs have side effects such as gastrointestinal irritation,

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addiction, tolerance and dependency (Hanson *et al.*, 2014). Hence, searching for novel interventions which are efficacious with minimal side effects is an essential task (Adedapo *et al*., 2014).

Plant products are alternative approaches to chemical drugs (Rocha *et al*., 2005). Drug discovery originated from the management of diseases with herbs (Schulz *et al*, 2001). According to WHO, 80% of the people globally still depend on ethnopharmacology. Many of these natural products with analgesic activity had been used in recent years due to their wide range of pharmacological activities and fewer side effects (Schug *et al.*, 2013). *Vigna subterranea* (Bambara nut) is widely cultivated in northern Nigeria and used traditionally for managing various disease conditions and symptoms including pain. Gombe state is one of the largest producer regions of *Vigna subterranea* in Nigeria (Bamshaiye *et al.*, 2011). Scientific validations of the medicinal importance of the plant are scarce. This study aims at investigating the analgesic effect of the methanolic extract of *Vigna subterranea* seeds in albino mice.

MATERIALS AND METHODS

Sample Collection, Identification and Preparation of Plant Materials

Fresh seeds of *Vigna subterranea* were collected in January 2023 from Abuja-Bula village of Gombe State, Nigeria. It was identified and authenticated in the Department of Pharmacognosy and Drug Development, Gombe State University with voucher number PCG/GSU/00073. The seeds were dehulled and dried at room temperature (30 °C) for 22 days; milled into powder using mortar and pestle and stored at 4 °C. Then 1500g powder was weighed and partitioned in 1.5 litres of 99.5% methanol solution at room temperature for a period of 72hrs with regular shaking. Filtration was carried out through hydrophilic cotton followed by Whatman n°1 filter paper and concentrated by rotary evaporator at 70 °C. The concentrate was dried at 50 °C for 4 days and stored at 4 °C (Sasidharan *et al*., 2011).

Preliminary Phytochemical Investigation

Preliminary phytochemical investigation was carried out on the methanolic extract for the presence of various phytoconstituents (Sofowora, 1984).

Experimental Animals Grouping and Dosing

Fifty (50) albino mice, *Mus musculus*, weighing between 18 and 24 g of both sexes were used for the two experimental models. All the animals were obtained from the animal house of the Department of Pharmacology and Therapeutics, Gombe State University, Gombe, Nigeria. The mice were randomly assigned to five (5) groups of five (5) animals each per group for the hot plate model and kept fasting for 18 h before administration of treatment. Group I, the negative control was administered 10 ml/kg of normal saline, Group V , the positive control was treated with standard drug (Morphine 10mg/kg) while Groups II, III and IV, the test groups were given 125, 250 and 500 mg/kg of the extract respectively. Similarly, in the acetic acid-induced writhing test twenty-five (25) mice were randomly assigned to five (5) groups of five (5) animals each per group as described for the hot plate test. Aspirin (10mg/kg) was given to animals in group V.

Acute Toxicity Test

The acute oral toxicity test was conducted according to the Lorke's (1983) method modified by Shettima *et al.*, 2012. Healthy adult albino mice weighing between (25–35g) were divided into six (6) groups of two (2) animals each. The extract was dissolved in

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normal saline and administered via the oral route after the mice fasted overnight with free access to water. In the first phase, three groups of mice received 10, 100, and 1000 mg/kg of the methanolic extract of *Vigna subterranean* seeds (MEVS) while another three groups received 1600, 2900 and 5000 mg/kg respectively in the second phase. The behaviour of mice was observed continuously for 1h after the treatment and then intermittently for 4hrs, thereafter for 24hrs and then for 6 days following treatment for any sign of toxicity and mortality.

Hot Plate Test

Evaluation of the central analgesic activity of the extract in mice was carried out using Columbus hot plate analgesia meter (Columbus Instrument, 1440-E54, Ohio, USA) using the method of Eddy and Leimback (1953). Thirty mice were randomized into 5 groups (n=6); The animals were placed gently on the hot plate set at 55±1°C at 30, 60, 90, 120 and 150 minutes after administration of the treatments. The reaction time was recorded as the interval between the placement of the animal on hot plate and the time it licks its fore-paws or jumps off the plate. The maximum possible analgesia (MPA) was calculated as follows (Neto *et al*., 2011):

$$M\% = \frac{\text{Reaction time for treatment} - \text{Reaction time for saline}}{\text{Reaction time for saline}} \times 100$$

Acetic Acid-Induced Mouse Writhing Test

Acetic acid-induced writhing test was carried out to evaluate the possible peripheral analgesic effect of MEVS in mice. Thirty male albino mice were fasted for 12 h and assigned randomly to five groups (n=6); Group I: vehicle (10ml/kg, p.o.), Group 2-4: MEVS (120, 250 or 500 mg/kg, p.o., respectively), and Group 5: aspirin (10 mg/kg, p.o.). One hour post-treatment acetic acid 0.06%v/v (10 ml/kg, i.p.) was administered and five (5) minutes later the number of cumulative writhing behaviour including pulling of abdomen on the ground, for 20 min was recorded (Sanchez Mateo *et al.*, 2006).

Statistical Analysis

Data were analyzed using ANOVA, subjected to Fischer LSD post hoc test and expressed as mean ± standard error of the mean (SEM). The statistically significant difference between the mean values was determined at $p<0.05$.

RESULTS

Percentage Yield of Methanolic Seed Extract

Percentage yield was calculated as follows:

$$\frac{\text{Weight of extract}}{\text{Weight of seeds powder before extraction}} \times 100$$

$$= \frac{47g}{1500g} \times 100$$

$$= 3.13\%$$

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Preliminary Phytochemical Investigation

Carbohydrates, saponin, alkaloids, flavonoids, glycoside, anthraquinone and tannins were found present as the major phytoconstituents (Table 1).

Table 1: The phytochemical constituents of methanolic extract of seeds of *Vigna subterranea* (MEVS)

Phytochemicals	Tests
Alkaloids	+
Anthraquinones	+
Tannins	+
Carbohydrate	+
Glycosides	+
Saponins	+
Flavonoids	+

Where + means present

Acute toxicity (LD_{50}) study

There were no mortality or toxic effects observed in all the groups up to 5000mg/kg of the extract (Table 2).

Table 2: Acute toxicity (LD_{50}) study of methanolic extract of *Vigna subterranea* seeds

Phase/Group	Dose (mg/kg)	*D/T
Phase I		
Group 1	10	0/2
Group 2	100	0/2
Group 3	1000	0/2
Phase II		
Group 1	1600	0/2
Group 2	2600	0/2
Group 3	5000	0/2

*D/T: Number of mice deaths/ total number of mice (n=2).

Effects of methanolic seed extract of *Vigna subterranea* and morphine on hot plate latency time in albino mice.

MEVS produced dose-dependent and significant ($P<0.05$) increase in pain threshold. The peak effect is at MEVS 500mg/kg (5.98± 0.12, 30 mins post-treatment). Similarly, the standard drug morphine (10mg/kg) increases the latency significantly from 2.90 ± 0.09 in control to 7.72± 0.12 at 30 minutes post-treatment (Table 3). The maximum possible analgesia of MEVS 500mg/kg and morphine (10mg/kg) at 30 mins post-treatment are 110.33% and 193.67% respectively (Table 4).

The effect of 99.5% methanolic seed extract of V*igna subterranea* and aspirin on acetic acid-induced writhing in albino mice.

Intraperitoneal administration of 0.06% acetic acid produced time course abdominal writhing 47.8 (±1.82) (Table 5). However, the pre-treatment of mice with MEVS inhibited writhing, with peak effect at 500 mg/kg (27.2±2.28; 43.09% inhibition) (Table 5). Similarly, aspirin reduced the mean number of writhes by 56.06% in comparison to vehicle-treated control.

Table 3: Effects of methanolic seed extract of *Vigna subterranea* and morphine on hot plate latency time in albino mice

Treatment/Dose	Reaction time at different time intervals (secs)				
	30 Mins	60 Mins	90 Mins	120 Mins	150 Mins
N/S (10ml/kg)	2.90(±0.09)	3.02(±0.20)	3.23(±0.42)	3.25 (±0.12)	2.00 (±0.28)
MEVS (125mg/kg)	4.20(±0.02)	4.18 (±0.05)	3.82 (±0.11)	3.74(±0.05)	3.20(±0.14)
MEVS (250mg/kg)	4.84(±0.06)*	4.72(±0.14)	4.27(±0.10)	4.04(±0.09)	4.25(±0.09)
MEVS (500mg/kg)	5.98(±0.12)*	5.62(±0.06)	4.90(±0.21)	4.54(±0.05)	4.31(±0.03)
MP (10mg/kg)	7.72(±0.12)*	6.80(±0.13)*	6.42(±0.08)*	6.23(±0.22)*	5.81(±0.19)*

Where ± SEM (Standard Error of Mean), n=5, N/S (Normal Saline), MP (Morphine), MEVS (Methanolic Seed Extract of *Vigna subterranea*), *P-Values ($P<0.05$).

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Table 4: Maximum Possible Analgesia (MPA) of methanolic seed extract of V*igna subterranea* and morphine in hot plate test in albino mice

Treatment/Dose	Maximum Possible Analgesia (MPA) %				
	30 Mins	60 Mins	90 Mins	120 Mins	150 Mins
N/S (10ml/kg)	_	_	_	_	_
MEVS (125mg/kg)	44.83	59.31	38.41	18.27	15.08
MEVS (250mg/kg)	75.00	66.90	56.29	32.20	24.31
MEVS (500mg/kg)	110.33	106.2	86.09	34.08	39.69
MP (10mg/kg)	193.67	166.21	125.17	98.76	91.67

Table 5: The effect of 99.5% methanolic seed extract of V*igna subterranea* and aspirin on acetic acid-induced writhing in albino mice

Treatment/Dose	Mean Number of Writhes in 20 Mins	% inhibition of Writhing
N/S (10ml/kg)	47.8 (±1.82)	-----
MEVS (125mg/kg)	36.4(±2.62)	23.83
MEVS (250mg/kg)	30.6(±1.99)*	35.98
MEVS (500mg/kg)	27.2(±2.28)*	43.09
ASP (10mg/kg)	21.0(±0.99)*	56.06

NOTE: n (5), ±SEM (Standard Error of Mean), N/S (Normal Saline), ASP (Aspirin), MEVS (Methanolic Seed Extract of *Vigna subterranea*),* P-Values (P<0.05).

DISCUSSION

Available analgesics are effective but are with various side effects including gastrointestinal irritations, abuse and addictions etc. Therefore, the need to find more effective and safe drugs with fewer side effects in pain management (Schug *et al*., 2013). Plant materials are commonly used in the folkloric treatment of pain. Thus, this study aims at evaluating the analgesic potential of the methanolic extract of dehulled seeds of *Vigna subterranea* (Fabaceae).

The percentage yield of extract obtained was found to be 3.13%. There was no mortality or toxic effect in animals at up to 5000 mg/kg of the extract within 7 days in the acute toxicity study. It suggests that the mean lethal dose (LD50) of the methanolic extract *Vigna subterranea* was higher than 5000mg/kg body weight thus considered safe.

Peripheral and central nervous systems are the sites of action of analgesics for pain relief (Bannister and Dickenson, 2020). Paws of mice are sensitive to heat at temperatures which do not damage the skin; therefore the central analgesic mechanism of the extract was evaluated by placing mice on heated plate and by recording the reaction times (Neto *et al.*, 2011). In the hot plate model, 250 and 500 mg/kg of MEVS produced significant central analgesic activity (P<0.05) when compared with the negative control (normal saline). The maximum analgesic activities of all doses of the MEVS and the standard drug were observed at 30 minutes which indicated that the analgesic effect decreased with an increase in the duration of observation in a dose-dependent manner. This may imply that MEVS contain centrally acting analgesic compounds which have fast onset and short duration of action. The seed extract of *Vigna subterranea* is found to contain tannins and flavonoids which are reported to produce significant analgesic effect through opiodergic mechanism (Das *et al*., 1989). Thus, the tannins and flavonoids may be responsible for the central analgesic activity of the plant.

The peripheral analgesic activity of test compounds is commonly investigated with

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the acetic acid-induced writhing test due to its sensitivity and ability to detect analgesic effects of natural products and drugs at dose levels which are inactive for other models (Sanchez Mateo *et al.,* 2006). The methanolic extract of *Vigna subterranea* seeds at the doses of 250 and 500mg/kg significantly reduced the number of writhes. The methanolic extract might inhibit the release of pain mediators that depend on acetic acid including arachidonic acid, prostaglandins, etc.

In a related analgesic study of *Vigna unguiculata* of the same genus as our plant, Tazin *et al*., 2014 reported that methanolic extract of *Vigna unguiculata* at doses 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg significantly reduced the number of writhes in mice induced by acetic acid. *Vigna mungo* leaves extract was also investigated by Rageeb *et al*., 2012 for analgesic activity in Wistar rats. It was reported to possess a significant analgesic activity at a dose of 50mg/kg. Findings from this research conform with a recent study on a luteolin glycoside isolated from of *Vigna subterranea* seeds. This compound is found to possess ability to inhibit lipoxygenase (Dutsadee and Natee, 2022). Earlier studies had reported the possibility of lipoxygenase as a pain mediator (Pascanus *et al*., 2018). Peripheral pain may be alleviated by inhibition of cyclooxegenase and lipooxygenase (Marshall *et al*, 1991). The peripheral analgesic effect of the methanolic extract of *Vigna subterranea* could be related to such enzymes.

CONCLUSION

The findings from this study indicated that the methanolic extract of the seeds of *Vigna subterranea* (Fabaceae) may possess central and peripheral analgesic potential in a dose-dependent manner. This lends credence to the folkloric use of the Bambara seeds in pain management. More investigations are required on *Vigna subterranea* to detect compounds responsible for its analgesic activity and explain their mechanisms.

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