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Antidiabetic, Analgesic and Anti-Inflammatory Activities of Ethyl Acetate Fraction of *Pterocarpus erinaceus* on Albino Mice

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***Abstract* - Diabetes mellitus and the diseases that are associated with it is a major public health problem that affects approximately 5% of the world's population. The study aims at evaluating the antidiabetic, analgesic, and anti-inflammatory effects of ethyl acetate fraction of *Pterocarpus erinaceus* on Albino Mice. The acute toxicity study and phytochemical screening were carried out on the ethyl acetate fraction. Antidiabetic, Analgesic and Anti-inflammatory activity were also analyzed at different doses. The acute toxicity study showed LD_{50} of 5000 mg/kg while the phytochemical screening revealed the presence of flavonoids, tannins, steroids, and cardiac glycosides majorly. The two doses exhibited a significant reduction in blood glucose levels but were more pronounced in 250 mg/kg on the 4th day when compared with glibenclamide. The analgesic and anti-inflammatory properties of ethyl acetate fraction of *P. erinaceus* were investigated in Swiss albino mice with significant ($P < 0.05$) analgesic effects and ($P < 0.05$) anti-inflammatory effects.**

The doses of 200 mg/kg, and 400 mg/kg showed a better analgesic effect compared to the positive control (Pentazocine 10 mg/kg), with a significant difference in mean time reaction compared with the fractions. The 400 mg/kg showed a better anti-inflammatory effect compared to the positive control (Aspirin 100 mg/kg), with a significant difference in mean time reaction compared with the fractions. The present study validates the folk medicinal use of the plant in Jos, Plateau state for diabetes mellitus and pain.

***Index Terms* – *Diabetes mellitus*, Analgesic activity, Anti-inflammatory, *Pterocarpus erinaceus*, Streptozocin.**

I. INTRODUCTION

Diabetes mellitus is a major public health problem that affects approximately 5% of the world's population (Taylor, 1999) Hypersensitivity to painful stimuli (hyperalgesia) is one of the most common complications of diabetes mellitus (Obrosova, 2003). It is known that diabetic rats display hyperalgesic behaviour in response to noxious stimuli and this may serve as a model of painful diabetic neuropathy in humans

Recently, treatment of diabetes mellitus and its complications have focused on the use of plant products. The WHO had estimated that approximately 80% of the global population relies on traditional medicine for their primary healthcare needs, and most of this therapy involves the using plant extracts (Quintans et al., 2014).

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Pain is an unpleasant sensation and emotional experience associated with either acute or chronic tissue damage (Loeser, 2008). By acting in the central nervous system (CNS) or on the peripheral pain mechanism, analgesic compounds selectively relieve pain without substantial alteration of consciousness. Analgesics are applied when the noxious stimulus cannot be removed or as an adjuvant to a more etiological approach to pain (Sarker et al., 2016). *P. erinaceus* is a medicinal plant used to treat several diseases as the whole plant including the stem bark, leaves, roots, and resin. The stem bark has been used in traditional medicine to treat inflammation such as rheumatism, gastric ulcers, ulcers, and dermatitis (Nacoulma, 1996). The ethanolic extract of stem bark of *P. erinaceus* has shown pharmacological support for the folkloric use and safety of the plant's stem bark decoction in the treatment of bleeding disorders. The rise in the use of existing analgesic and anti-inflammatory drugs causing adverse reactions and other severe complications such as gastrointestinal bleeding, ulcers, dyspepsia, and anaemia has necessitated the search for new/alternative analgesics and anti-inflammatory drugs with less adverse effects and high potency to enable better health. Since the plant is used for the treatment of diabetes and pain by the folk medicinal practitioners of Nigeria, the objective of the present study was to evaluate the antihyperglycemic and antinociceptive activity in mice.

II. COLLECTION AND PREPARATION OF EXTRACTS

Plant Collection

The leaves of the plant *P. erinaceous* were collected from Jos North Local Government, Plateau State. It was identified and authenticated by Mr. Azila, of the College of Forestry, Jos. The leaves were allowed to dry, and the dried samples were then crushed using a pestle into a coarse powder.

Extraction and Fractionation

Extraction and fractionation were according to the method reported by (Maxwell et al., 2014) with some slight modifications in the choice of primary solvent (water) and partitioning/separating solvents (n-hexane). The powdered dry leaves (150g) were extracted with 250 ml 70% v/v of methanol within one week and the procedure was repeated three times using the same crude extract. The solvent was removed at 50 ^{o}C using a rotary evaporator under a vacuum.

The methanol extract obtained was dissolved in water and methanol in a ratio of 2:1 and exhaustively extracted by consecutive liquid/liquid partition with n-hexane (1.5 Litres),

and ethyl acetate (1.5 Litres) using a separatory funnel (500 ml). The ethyl acetate fraction was evaporated to dryness until a constant weight was obtained (Ganchi et al., 2003). The fractions obtained were tested to evaluate antidiabetic, analgesic, and anti-inflammatory effects.

III. EXPERIMENTAL ANIMALS

The mice of both sexes weighing 15 -25g were obtained from the animal house, University of Jos. They were kept in a well-constructed cage that allowed freedom of movement for one week for acclimatization before the commencement of the study. They were fed with standard feeds and allowed access to water. The study was undertaken after obtaining approval and ethical clearance from the institutional Animal Ethics Committee.

IV. METHODS

A. ACUTE TOXICITY STUDIES (Determination of LD_{50})

The Lorke method of lethal dose (LD_{50}) determination was used (Lorke, 1983).

B. EVALUATION OF HYPOGLYCEMIC ACTIVITY

Induction of Diabetes

Streptozotocin (STZ) was freshly prepared in distilled water, citric acid and sodium citrate maintain at a pH of 4.2. The animals fasted for 24 hours but were given water. Diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg BW).

After one week, blood was withdrawn for blood glucose estimation and monitored with a glucometer. The animals with blood glucose levels $\geq$ 150 mg/dl were considered diabetic and included in the experiment (Gupta et al., 2005).

C. HYPOGLYCAEMIC EFFECT OF THE FRACTION ON DIABETIC MICE.

The twenty mice were divided into a group of two, with six mice in each group. The following drugs were administered to each group orally as follows.

GROUP I: Diabetic group (Normal Saline 0.1ml/kg)
GROUP II: Glibenclamide (5 mg/kg)
GROUP III: 250 mg/kg of the ethyl acetate fraction
GROUP IV: 500 mg/kg of the ethyl acetate

D. SAMPLE COLLECTION AND ANALYSIS

Blood samples were collected from the tail vein of the mice and then applied to the glucose test strip in the glucometer and the concentration was displayed on the screen of the glucometer.

This was done after days 0, I, 4, and 7 after administration of the ethyl acetate fraction and standard drugs.

E. ANALGESIC SCREENING

25 mice were used for the analgesic screening. They were divided into 5 groups of 5 animals each. Group A was control and received normal saline. Groups B, C, and D were the test groups and received ethyl acetate fraction at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg intraperitoneally, while group E was the standard and received pentazocine (10 mg/kg).

Each animal was gently placed in a glass beaker on a hot plate maintained at a temperature of 55 ± 2 °C. The time taken for the animal to either lick its paw or attempt to jump out of the beaker was recorded as the reaction time. At no time were the mice allowed to stay on the hot plate for more than 60 secs to avoid tissue damage. The mean hot plate latency for each group was determined (Vaz,1997).

F. ANTI-INFLAMMATORY SCREENING

The anti-inflammatory activities of the ethyl acetate fraction of *P. erinaceus* were investigated using the induced paw oedema model with 25 mice. They were divided into 5 groups of 5 animals each. Before drug administration, the mice were labeled appropriately, and their paw sizes were taken using the electronic Vernier caliper and recorded. Groups Band, C, and D were treated with the fraction at 3 doses (100 mg/kg, 200 mg/kg, and 400 mg/kg respectively) by intraperitoneal injection. Group A was given Aspirin while Group E, the negative control was given normal saline.

After the treatment, all animals were kept for 1 hour and then injected with 0.1ml egg albumin in the right hind paw to induce inflammation. Oedema was assessed in terms of paw size, and this was measured using the electronic vernier caliper to measure the size of paw increase in each animal in each group immediately after egg albumin administration. Results were recorded at 0, 1, 2, 3, 4, and 5-hour intervals (Akah & Nwambie, 1994).

V. STATISTICAL ANALYSIS

Data are expressed as mean ±SEM, with 'n' indicating the number of animals used. Differences between the standard and the test groups were tested by two-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software, with the level of significance set at $p < 0.05$.

VI. RESULTS

Phytochemical screening (qualitative)

The ethyl acetate fraction contains tannins, and flavonoids (highly present), while steroids; and cardiac glycosides are moderately present. Alkaloids, saponins anthraquinones, and terpenes were absent.

Acute toxicity studies (Determination of LD_{50})

The acute toxicity study of the plant revealed that the ethyl acetate fraction of *P. erinaceus peroral* is safe at a dose of 2000 mg/kg.

TABLE 1
EFFECT OF ETHYL ACETATE FRACTION OF *P. erinaceus* ON BLOOD GLUCOSE CONCENTRATION IN STREPTOZOTOCIN INDUCED DIABETIC MICE

Treatment	Dose mg/kg	Blood Glucose Concentration (mg/dl)			
		Baseline	Day 1	Day 4	Day 7
Control(Tween 80)	0.1	161.40±7.35	203.40±7.37	204.40±11.07	182.80±22.40
Glibenclamide	5	166.25±11.16	72.00±3.67	115.75±13.18	132.50±12.57
EA	250	166.80±7.61	271.20±36.47	117.00±3.93*	116.80±9.03*
EA	500	192.00±25.10	278.60±24.47	105.00±10.98*	115.00±10.32

Values are Mean ± SEM, n=5, *indicates a significant difference ($p<0.05$) in mean blood glucose concentration in the study group compared to the control group. EA- Ethyl acetate.

TABLE 2
ANALGESIC ACTIVITY OF THE ETHYL ACETATE FRACTION OF *P. erinaceus* ON THERMALLY INDUCED PAIN IN ALBINO MICE.

Group	Treatment	Dose mg/kg	Reaction Time (minutes)				
			0	30	60	90	120
1	Normal saline	0.1	4.58±0.59	4.18±0.88	4.88±1.42	4.28±1.18	3.94±0.93
2	Pentazocine	10	4.16±0.68	8.0±2.68*	9.14±2.81*	8.72±1.96*	8.476±1.59*
3	Fraction	100	3.92±0.48	8.30±0.88*	9.34±1.52*	9.26±1.89*	8.44±2.04+*
4	Fraction	200	4.50±0.75	7.74±1.69*	9.26±3.08*	10.92±4.31*	10.20±2.56+*
5	Fraction	400	3.72±0.75	9.17±1.71*	10.66±1.70*	12.27±3.32*	12.44±3.09+*

*= Significant difference in mean time reaction compared within the group ($P < 0.05$)
+= Significant difference in mean time reaction compared to pentazocine ($P < 0.05$)

TABLE 3
THE EFFECT OF THE ETHYL ACETATE FRACTION OF *P. erinaceus* ON FRESH EGG ALBUMIN INDUCED INFLAMMATION IN ALBINO IN MICE PAW SIZE.

Groups	Treatment	Dose mg/kg	Basal	Change in paw oedema (Mean ± SEM) mm					
				0hr	1hr	2hr	3hr	4hr	5hr
1	Normal saline	0.1	1.86 ± .06	0.93 ±.13	0.66 ±.09	0.38 ±.19	0.45±.06	0.42 ±.05	0.22±.09
2	Acetylsalicylic acid	100	1.78±.18	1.06 ±.14	0.73 ±.14	0.39 ±.06	0.27±.10	0.17 ±.09	0.05±.06
3	Fraction	100	1.95 ± .09	0.18±.14^{+}	0.84±.23	0.78±.20	0.55±.18	0.26±.22	0.06±.03
4	Fraction	200	1.96±.09	0.08±.05^{+}	0.87±.17	0.79±.18*	0.47±.20	0.25±.15	0.07±.02
5	Fraction	400	1.67±.77	0.31±.13^{+}	0.90±.16	0.60±.13*	0.43±.14	0.12±.01	0.01±.01

$^{+}$= Significant difference in mean time reaction compared to pentazocine ($P < 0.05$)
*= Significant difference in mean time reaction compared within the group ($P < 0.05$)

VII. DISCUSSION

Diabetes is a chronic metabolic disorder affecting a major population worldwide. A sustained reduction in hyperglycaemia will decrease the risk of developing microvascular complications (Kim et al., 2007). The conventional therapies for diabetes have many shortcomings like unwanted side effects and a high rate of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy without the side effects like that of conventional drugs. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future. (Cervero, 2012). Most pain resolves once the noxious stimulus is removed and the body has healed, but it may persist despite removal of the stimulus and apparent healing of the body. Sometimes pain arises in the absence of any detectable stimulus, damage or disease (Raj, 2007). Inflammation is a natural response that promotes the survival of a host in the presence of a variety of internal and external insults (Hunter et al., 2009). However, abnormal regulation of inflammatory processes can result in the destruction of cells or may disturb cellular metabolism and thus contribute to the development of chronic diseases. Macrophages play a central role in the innate immune response and chronic inflammation processes by secreting pro-inflammatory cytokines such as interleukin (IL) - 6 and tumor necrosis factor-α (TNF-α) (Hunter et al.,2009, Laroux, 2004). Macrophages activate inflammation-related genes that are regulated via nuclear factor-κB (NF-κB) signaling (Baeuerle & David, 1996). The phytochemical screening revealed the presence of tannins, steroids, flavonoids, and cardiac glycosides for the ethyl acetate fraction. These phytochemical constituents have been reported to possess analgesic and anti-inflammatory properties in various experimental animal models (Yuan et al., 2006). Thus, the observed analgesic and anti-inflammatory effects of *P. erinaceus* are attributable to the constituents (Table 2 and Table 3). Azwanida (2015) reported that ethyl acetate fraction has both medium polarities and some polar compounds such as flavonoids, tannins, and some terpenoids. The presence of pharmacologically active constituents of the various fractions agrees with those of previous investigators (Ekam et al., 2013; Zakaria et al., 2016). The value obtained for the median lethal dose for the ethyl acetate fraction of *P. erinaceus* is safe at a dose of 2000 mg/kg. Thus, it can be said that the fraction is safe to a high degree and not toxic and this agrees with the European chemical industry ecology and toxicology guidelines which state that LD_{50} of 2000 mg/kg is likely to be non-toxic (IRAC, 2004). Ethyl acetate fraction of the plant showed a decrease in blood glucose concentration in the animals across the groups (Table 1). The blood glucose lowering activity of the plant might be due to its ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibition of the intestinal absorption of glucose which may be due to its phytochemical constituents such as flavonoids, tannins, steroids, and cardiac glycosides. These constituents are frequently implicated as having anti-diabetic effects (Malviya et al., 2010). Flavonoids have also been found to cause pancreatic beta cell re-granulation and enhance insulin release (Modak et al., 2007, Sharma et al., 2010). Over the decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some plant as a whole or their identified ingredients with antioxidant properties have substantial protective effects on diabetes (Sabu & Kuttan, 1982), cardiovascular and renal disorders (Anderson et al., 2000) and several other human

ailments (Lampee, 2003). However, the antidiabetic effect of plant extracts is believed to largely depend on their antioxidant properties (Meliai et al., 2011). Also, radical scavengers such as phenolics like flavonoids are reported to be effective in preventing diabetes in animal models. It is believed that the constituents such as flavonoids, tannins, glycosides, and steroids which are present in the ethyl acetate fraction of *P. erinaceus* could be responsible for the therapeutic efficacy (Williamson, 2001).

The ethyl acetate fraction of *P. erinaceus* showed dose-dependent analgesic, and anti-inflammatory properties, and significantly delayed the reaction time of thermally induced pain using the hot plate test (Table 2). The hot plate test is selective for centrally acting analgesics and indicates narcotic involvement with opioid receptors (Turner, 1965). The fraction showed a dose-dependent reduction in pain compared to the normal saline at the normal, lower and higher doses but in comparison to the reference standard pentazocine, there was a significant difference in mean reaction time which indicates that the fraction has analgesic properties but not as effective as the standard reference drug (Table 2). Analgesic and anti-inflammatory actions in this plant extract could be attributed to the presence of flavonoids, tannins, and steroids. Flavonoids are also reported to target prostaglandins which are involved in the late phase of acute inflammations and pain perception. In addition, tannins are known to act upon certain receptors in antinociceptives and anti-inflammatory activities therefore, the presence of these biological principles in the plant may be responsible for the analgesic activity of the fraction. Steroids act intracellularly to reduce inflammations, which is why drugs like prednisolone which is a glucocorticoid are indicated for oedematous diseases such as rheumatoid arthritis and other pathological inflammatory conditions, so the presence of steroids in the ethyl acetate fraction of the plant could also be responsible for the anti-inflammatory activities of the plant. The ethyl acetate leaf extract of *P. erinaceus* significantly reduced oedema of the mouse hind paw induced by fresh egg albumins (Table 3).

This dose-dependent action was comparable to that of acetylsalicylic acid, a cyclo-oxygenase inhibitor in this study at 5 h and other studies (Singh et al., 1996). Flavonoids which are some of the constituents of the ethyl acetate fraction of *P. erinaceus* have reported anti-inflammatory properties in literature (Trease & Evans, 1989). Oedema is attributed to the release of histamine, 5-HT, kinins, and prostaglandins (Vane & Bolting, 1987), and the anti-inflammatory action of this extract may be due to the inhibition of the release of the above-mentioned autocoids. The anti-inflammatory effects of the salicylates are due primarily to the blockade of prostaglandin synthesis at the thermoregulatory centre in the hypothalamus and peripheral target sites (Richard et al., 1997).

The extract might be acting via a similar mechanism as aspirin though this hypothesis is not confirmatory as a thorough biochemical analysis of the extract is required to fully understand its mechanism of action.

The present study also validates the folk medicinal use of the plant in Jos, Plateau State for diabetes and pain.

VIII. CONCLUSION

The ethyl acetate fraction of *P. erinaceus* possesses comparable antidiabetic effects in STZ-induced diabetic mice to the standard drug, glibenclamide with anti-inflammatory and analgesic effects probably mediated via inhibition of various autocoids formation and release. Further studies are needed to elucidate the exact mechanism by which *P. erinaceus* ethyl acetate fraction reduces blood glucose levels and inhibits inflammation and pains.

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