

## Acute and Subchronic Toxicities of Ethanol Root Extract of *Psidium guajava* (myrtaceae) in Experimental Animals.

Onyekwe N. G.<sup>1</sup>; Ilodigwe E. E.<sup>1</sup>; Ajaghaku D. L.<sup>1</sup>; Esimone C. O.<sup>2</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu, Anambra state, Nigeria

<sup>2</sup>Department of Pharmaceutical microbiology/ Biotechnology, Nnamdi Azikiwe University, Agulu, Anambra state, Nigeria.

**Abstract:-** The acute and subchronic toxicities of the ethanol root extract of *Psidium guajava*, a popular Nigeria traditional aphrodisiac was investigated. For the acute toxicity study, 1000-5000mg/kg of the ethanol root extract were orally administered to rats and obvious toxic symptoms and mortality 24 hours post-administration of the extract were determined. The median lethal dose (LD<sub>50</sub>) of the extract was determined. In subchronic study, 150-1200mg/kg of the extract were orally administered daily for 90 days. The food and water consumption, body weight changes, as well as haematological and biochemical parameters were determined periodically. The phytochemical constituents of the extract were also investigated. Phytochemical constituents revealed the presence of saponins, flavonoids, alkaloids and tannins. The estimated LD<sub>50</sub> of the extract was 1352mg/kg. During the period of the study, the animals showed signs of anorexia, weakness and sluggishness and significant (p<0.05) reduction in body weight; and mortality from the group receiving the highest dose of the extract was observed. The effects on haemoglobin concentration, PCV and RBC were non-significant (p>0.05) but there was significant dose-time dependent elevation of the WBC counts. The extract caused significant elevation in serum liver enzymes, AST, ALT and ALP. Hepatic photomicrographs showed dose-time dependent toxicities

**Industrial Relevance:** Impotence, weak libido and erectile dysfunction may require life-long management. In resource poor communities, aphrodisiac agents are not readily accessible. The use of medicinal plants especially *Psidium guajava* root is popular in Nigeria because it is readily available, cheap and relatively free from obvious adverse effects. The results of the resent study will enable the industry to produce from natural products an effective aphrodisiac that is affordable with little or no side effect.

**Key words:** *P. guajave*, toxicity, liver enzymes, haematological and histological indices.

### Introduction:

Medicinal plant products are very important in health delivery especially in resourced limited settings. About eighty percent of the world's population relies on traditional medicine for health care delivery [1,2]. This should be encouraged and supported especially in countries where access to the conventional treatment is inadequate in as much as efficacy and safety are assured [3]. There are uncertainties on the safety of medicinal plant preparations especially in situations of delayed or poor detectable symptoms of toxicities [4]. A number of studies have reported the toxic effects of herbal medicines [5].

*P. guajava* is widely distributed in Nigeria and other tropical regions of the world. The leaves are used for cough sedative, analgesic, anti-inflammatory, hepatoprotective and antioxidant activities [6]. studies on the pharmacological properties of the bark, fruit and leaves indicates antibacterial, hypoglycemic, antipyretic, spasmolytic and central nervous system depressant activities [7]. In rural communities of eastern Nigeria, the root decoction has been widely used as an aphrodisiac. Despite the popular use of this root, no information exists about its safety. Since sexual dysfunction may require chronic management, this study evaluated the acute and

subchronic toxicities of the ethanol root extract of *P. guajava* in rats.

### Materials and Methods:

**Plant Material:** The root of *P.guajava* was collected from the local Taxonomist in in Agulu town, Anambra State. The roots were authenticated by Mr. Ozioko Alfred, BDCP Research Centre, Nsukka.

### Preparation of Extract:

The roots were cleaned of sand particles, air-dried and pulverized. The powder (500g), was cold macerated with 5L of aqueous ethanol (70%) for one week. The resulting solution was filtered and the filtrate concentrated to dryness in vavuo using rotary evaporator at 40°C.

### Animals:

Swiss-male albino mice (20-27g) and rats (90-140g) were employed for this study. All the animals were obtained from the animal house of the department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were housed in standard laboratory conditions and fed with rodent feed (Guinea feed Nigeria Ltd). They were allowed free access to food and water *ad libitum*. The animals were acclimatized for two weeks and fasted over night with free access to water prior to experiments. All

animal experiments were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals (Pub. No. 85-23 Revised 1985) and approved by the Nnamdi Azikiwe University, Awka Ethical Committee for the use of Laboratory Animals.

**Phytochemical test:**

The phytochemical analysis of the root extract was carried out using standard methods [8,9].

**Acute toxicity study:**

Forty swiss male albino mice (20-27g) were divided into eight groups of five animals per group. After six hours fasting, groups 1-7 received oral administration of 100, 150, 500, 1000, 2000, 4000 and 5000 mg/kg doses of the extract respectively. Group 8, served as the control and received oral administration 10ml/kg of normal saline. The animals were observed for obvious toxic symptoms and mortality in each group was determined 24 hours post administration. The median lethal dose of the extract (LD<sub>50</sub>) was estimated using probit analysis [10].

**Sub-chronic toxicological studies:**

Seventy-five rats were randomly divided into five groups of fifteen rats per group. The extract was administered orally at doses 150, 300, 600, and 1200mg/kg to the test groups (IV) once daily for 90 days. The control group (group iv) received 10ml/kg of normal saline. Water and food intake patterns were determined every 24hrs. The rats were weighed every 7 days.

Five rats from each group were anaesthetized using chloroform on the 31<sup>st</sup>, 61<sup>st</sup> and 91<sup>st</sup> days of the study. Blood samples were collected through cardiac puncture and was used for the estimation of haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell count (WBC) [11].

For the estimation of the serum enzyme levels, the blood samples were allowed to coagulate for 30 minutes and the clear serum was separated by centrifuging at 2500 rpm for 10 minutes and was then used for analysis of biochemical hepatic markers- aspartate aminotransferase (ALT) [12], alanine aminotransferase (ALT) [12] and alanine phosphatase (ALP) [13]. Histopathological studies were done on liver isolates from different dose groups of the animals and at various times.

**Statistical Analysis:**

The results were analysed using SPSS version 15 and presented as mean ± SEM. Significance between control and extract treated group were determined using students t-test and two way ANOVA. A p value of less than 0.05 was considered significant.

**Results:**

**Phytochemical Analysis**

The extract gave positive reactions to alkaloids, tannins and saponins.

**Acute toxicity:**

The LD<sub>50</sub> of the extract was estimated to be 1,352mg/kg.

**Sub-chronic toxicity:**

At higher doses, the animals showed signs of anorexia, weakness and sluggishness. These observations however subsided after 24 hours. There was a progressive decrease

in body weight during the administration of the extract. Haematological studies revealed a dose and time dependent non-significant (p>0.05) elevation of haemoglobin concentration, packed cell volume and red blood cell count (**Tables 2, 3, 4, respectively**). The white blood cell count showed significant (p<0.05) elevation (Table 5). Biochemical studies showed a time dose-dependent significant (p<0.05) elevation of serum Aspartate aminotransferase (AST), Alanine aminotransaminase (ALT), and serum Alkaline phosphatase (ALP) (**Tables 6, 7 and 8 respectively**). Two deaths were recorded in the highest dose (1200mg/kg) group on the third month of administration. Hepatic photomicrographs revealed dose and time dependent liver toxicity (**Figure: 1-6**)

**Table 1: Effect of the extract on mean body weight reduction**

Mean body weight reduction (%)				
Treatment		31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Ext	150	12± 8.8	19± 7.2	27± 6.6
Ext	300	16± 5.3	23± 10.5	31± 9.2
Ext	600	22± 13.4	29± 7.1	40± 8.3
Ext	1200	33± 9.7	46± 9.8	61± 12.9
Dose of extract in mg/kg, n/gp = 5				

**Table 2: Effect of the extract on Haemoglobin level (Hb)**

Mean Haemoglobin (g/dl)					
Treatment		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		5.7 ± 0.5	5.7 ± 0.2	5.7 ± 0.5	5.5 ± 0.2
Ext	150	5.7 ± 0.2	5.7 ± 0.3	5.7 ± 0.3	5.5 ± 0.6
Ext	300	5.7 ± 0.2	5.3 ± 0.4	5.3 ± 0.5	5.4 ± 0.4
Ext	600	5.4 ± 0.3	5.2 ± 0.5	5.4 ± 0.5	5.4 ± 0.7
Ext	1200	5.6 ± 0.6	5.3 ± 0.4	5.3 ± 0.3	5.5 ± 0.2
Dose of extract in mg/kg, n/gp = 5					

**Table 3: Effect of the extract on Packed Cell Volume (PCV)**

Mean Packed Cell Volume (%)					
Treatment		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		44.9 ± 2.1	45.0 ± 2.9	44.8 ± 1.9	44.4 ± 2.9
Ext	150	44.0 ± 2.9	46.4 ± 2.5	45.6 ± 3.2	45.0 ± 2.2
Ext	300	44.8 ± 1.9	45.8 ± 2.9	44.2 ± 2.2	45.4 ± 1.1
Ext	600	44.4 ± 2.9	49.0 ± 1.4	46.6 ± 2.1	45.8 ± 2.6
Ext	1200	45.0 ± 2.2	43.4 ± 2.6	40.2 ± 3.1	40.0 ± 1.6
Dose of extract in mg/kg, n/gp = 5					

**Table 4: Effect of the extract on Red Blood Cell (RBC)**

Mean Red Blood Cell (x10 <sup>12</sup> /L)					
Treatment		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		5.6 ± 0.1	5.7 ± 0.2	5.7 ± 0.5	5.5 ± 0.2
E	150	5.4 ± 0.4	5.4 ± 0.3	5.4 ± 0.3	5.5 ± 0.6
E	300	5.5 ± 0.2	5.3 ± 0.4	5.3 ± 0.5	5.4 ± 0.4
E	600	5.6 ± 0.3	5.2 ± 0.5	5.4 ± 0.5	5.4 ± 0.7
E	1200	5.7 ± 0.5	5.3 ± 0.4	5.3 ± 0.3	5.2 ± 0.7
Dose of extract in mg/kg, n/gp = 5					

**Table 5: Effect of the extract on White Blood Cell**

Mean Red Blood Cell (x10 <sup>9</sup> /L)					
Treatment		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		5.8 ± 1.2	5.8 ± 1.0	6.0 ± 0.8	5.9 ± 0.8

Ex t	150	5.7 ± 0.9	6.5 ± 1.9	*7.0 ± 1.4	*6.7 ± 0.5
Ex t	300	5.6 ± 0.5	*6.8 ± 1.5	*6.8 ± 1.4	*6.9 ± 0.8
Ex t	600	5.8 ± 0.9	6.6 ± 1.7	*7.1 ± 1.3	*7.2 ± 1.2
Ex t	1200	6.0 ± 0.3	*7.3 ± 1.3	*7.0 ± 1.0	*7.7 ± 1.0
Dose of extract in mg/kg p.o, n/gp = 5. * P<0.05 compared with control					

Figure 1: Histological section of the liver that served as control showing a normal liver tissue with normal portal tract.

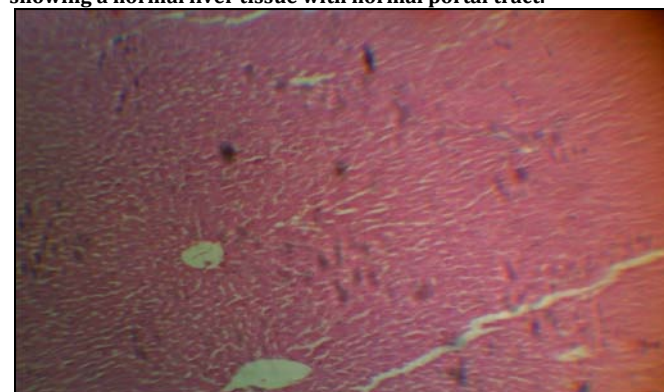


Figure 2: Histological section of the liver at dose 150mg/kg for the 1<sup>st</sup> month showing infiltrations of chronic inflammatory cells into the portal tract

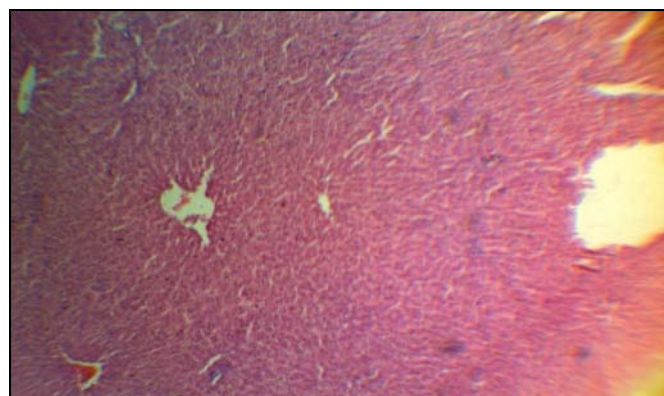


Figure 3: Histological section of the liver at dose 300mg/kg for the 2<sup>nd</sup> month showing no remarkable difference with 150mg/kg but with slight cellular distortion

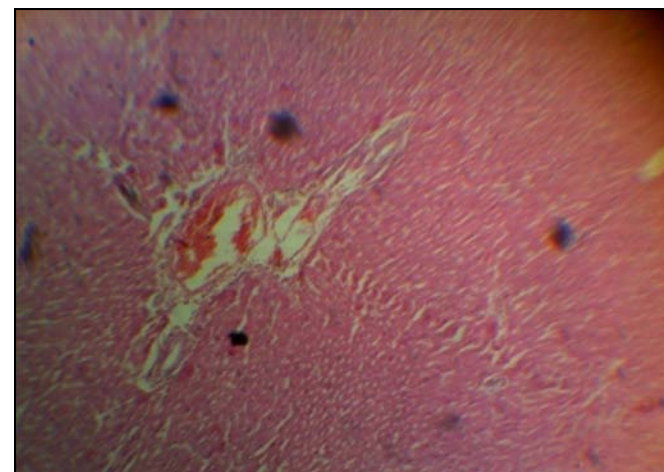


Figure 4: Histological section of the liver at dose 600mg/kg for the 2<sup>nd</sup> month showing a marked difference that worsened with time

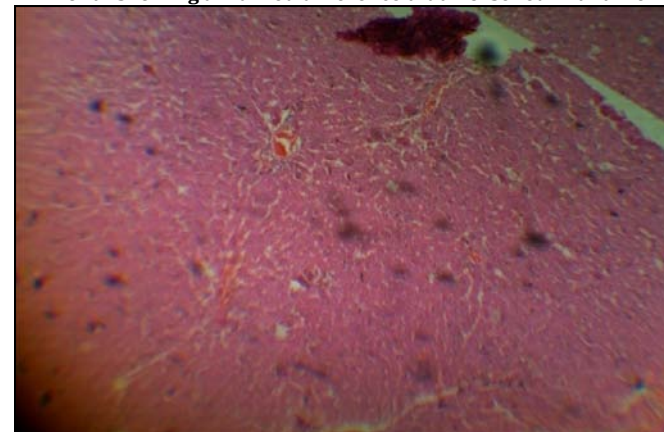


Table 6: Effect of the extract on Serum Aspartate Aminotransferase (AST)

Treatment		Mean Serum Aspartate Aminotransferase (U/L)			
		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		58.8 ± 5.0	64.0 ± 9.0	60.6 ± 6.5	68.5 ± 5.0
Ex t	150	62.8 ± 7.0	*100.8 ± 1.1	*134.0 ± 16.5	*193 ± 17.0
Ex t	300	72.4 ± 6.5	*153.4 ± 16	*192.4 ± 16.8	*235.0 ± 1.5
Ex t	600	58.0 ± 4.8	*184.0 ± 8	*226.4 ± 14	*311.3 ± 41
Ex t	1200	62.0 ± 3.0	*241.0 ± 13	*293.0 ± 40	*455 ± 66.0
Dose of extract in mg/kg p.o, n/gp = 5. * P<0.05 compared with control					

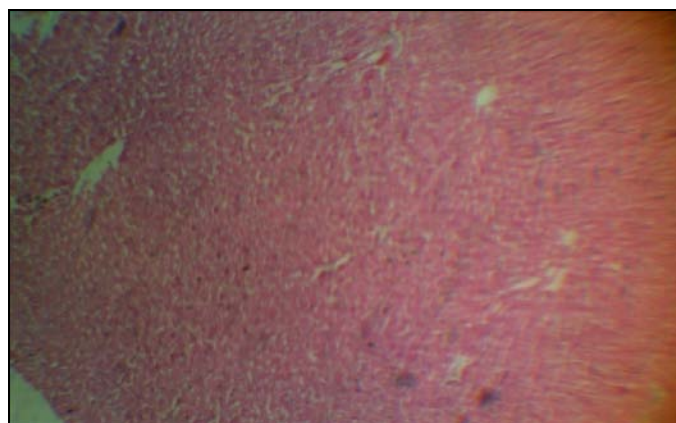
Table 7: Effect of the extract on SERUM Alanine Aminotransferase (ALT)

Treatment		Mean Serum Alanine Aminotransferase (U/L)			
		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		38.5 ± 2.0	33.5 ± 3.1	32.0 ± 7.3	36.2 ± 2.2
Ex t	150	32.0 ± 1.4	32.6 ± 1.7	*49.4 ± 9.4	*66.3 ± 4.0
Ex t	300	30.0 ± 2.1	*42.0 ± 5	*63.4 ± 7.4	*90.7 ± 11
Ex t	600	35.1 ± 1.7	*53.1 ± 2.9	*71.2 ± 7.3	*114.3 ± 2.9
Ex t	1200	34.8 ± 2.8	*74.8 ± 9.2	*91.2 ± 1.5	*150.0 ± 10
Dose of extract in mg/kg p.o, n/gp = 5. * P<0.05 compared with control					

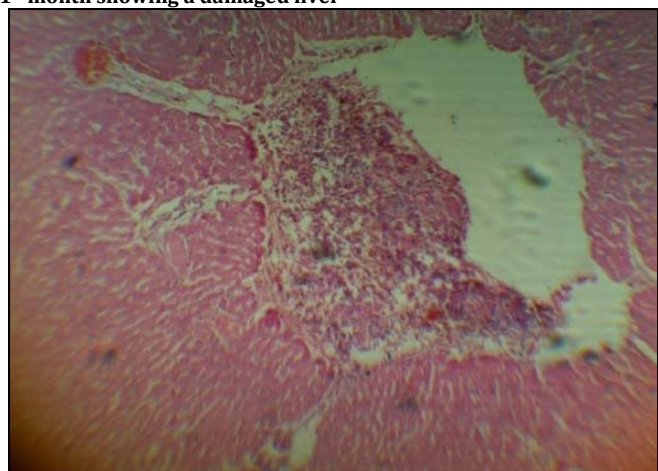
Table 8: Effect of the extract on Alkaline Phosphates (ALP)

Treatment		Mean Alkaline Phosphates (U/L)			
		Pre-treatment	31 <sup>st</sup> Day	61 <sup>st</sup> Day	91 <sup>st</sup> Day
Control (10ml/kg)		36.7 ± 0.8	33.7 ± 1.9	38.0 ± 0.8	33.4 ± 0.6
Ex t	150	36.6 ± 1.2	36.6 ± 1.1	38.3 ± 1.7	*41.0 ± 1.4
Ex t	300	37.9 ± 0.4	37.8 ± 0.6	*40.3 ± 1.2	*43.3 ± 0.7
Ex t	600	35.9 ± 0.7	*39.9 ± 1.0	*48.8 ± 2.0	*47.7 ± 1.6
Ex t	1200	36.4 ± 1.1	*42.4 ± 0.8	*47.5 ± 1.7	*51.9 ± 1.6
Dose of extract in mg/kg p.o, n/gp = 5. * P<0.05 compared with control					

**Histopathological Result:-**



**Figure 5: Histological section of the liver at dose 1200mg/kg for the 1<sup>st</sup> month showing a damaged liver**



**Figure 6: Histological section of the liver at dose 1200mg/kg for the 3<sup>rd</sup> month showing a huge cellular architecture of the liver with a large round inflammatory rupture and an enlarged portal tract.**

### Discussion:

The acute toxicity studies showed that the ethanol root extract has a toxic profile when administered orally, an indication of relative acute toxicity of the root extract [14]. The loss of weight exhibited by the animals during the sub-chronic toxicity studies may be attributed to the presence of anti-nutritional substances such as tannins and saponins in the extract. These substances have been reported to cause nutrient malabsorption [15]. The non-significant effect ( $P>0.05$ ) of the extract on Hemoglobin (Hb) concentration, red blood cell (RBC) count and Packed cell volume (PCV) indicated the unlikelihood of the extract to induce anaemia even after longterm use [11]. The extract showed a significant ( $P<0.05$ ) elevation in white blood cell (WBC) counts, probably due to normal responses to foreign bodies or as a result of inflammatory rupture on the liver cells as evidenced from the histological examination (Fig. 1-6). The increase in concentration of ALT, AST and ALP in the serum indicates a major permeability, congestion or cell rupture [16]. ALT is hepato specific and principally found in the cytoplasm of animals. The elevation of serum liver enzymes especially the aminotransferase may suggest liver congestion or injury. The histopathological examination of the liver (Fig 1-6) revealed significant and alarming changes on the liver, indicating severe dose-time dependent rupture of the liver architecture. The hepatotoxic effect can be attributed to high Alkaloid Content of the Plant. Pyrrolizidine alkaloids have been proven to produce liver injury in all the species of animals in which they have been tested [17,18]. The hepatotoxicity can as well be as a result of the ability of some plant species to absorb environmental toxins. A further study is needed to investigate the specific cause of the hepatotoxicity exhibited by the root extract of *P.guajava*. These results provide evidence for poor safety

profile of the ethanol root extract of *P. guajava* thus unresponsive of its validity as an aphrodisiac.

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**\*Corresponding Author:-**

Ajaghaku D. L.,  
Department of Pharmacology and Toxicology,  
Nnamdi Azikiwe University, Agulu, Anambra state, Nigeria



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