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Toxicological Status of the Hydroethanolic Extract of *Piliostigma thonningii* Leaves in Female Wistar Rats

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Abstract

This study aims to evaluate the toxicological impact of *Piliostigma thonningii* leaves on liver and renal function in rats and the plant extract was screened for heavy metals. Rats were divided into four groups, comprising five animals each, with the control group receiving distilled water orally and the other groups receiving 50, 100, and 200 mg/kg doses of the plant extract, orally, for seven days. On the eighth day, animals were sacrificed, and liver and kidney function parameters were assessed. Heavy metal screening revealed the absence of chromium and nickel, while trace amounts of lead and cadmium were detected. The liver body-weight ratio significantly increased ($p<0.05$) in rats treated with the plant extract at 100 and 200 mg/kg body weight. Liver functioning tests showed no significant ($p>0.05$) changes in total protein and albumin levels, suggesting no major adverse effects on protein metabolism or liver function. However, the significant ($p<0.05$) changes in Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) may be detrimental. Renal function tests revealed some variations, with significant ($p<0.05$) alterations in uric acid, creatinine, and urea levels which may affect renal function. The significant ($p<0.05$) decrease in phosphate ion and potassium ion levels further suggested potential side effects on renal functions.

Keywords: Toxicity, *Piliostigma thonningii*, Leaves extract, Hydroethanolic.

Introduction

The effectiveness, acceptability, affordability, safety, and low cost of herbs have led to their recognition as substitute medicines in treating a range of illnesses (Arya et al*.,* 2012). With the prevailing belief that natural products are inherently safe, there is a noticeable increase in public consumption of herbal formulations for treating numerous illnesses (Gunjan et al*.,* 2015). Another notion prevalent among individuals is that herbal treatments, derived from plants, are devoid of the negative or harmful side effects often associated with synthetic pharmaceuticals used in conventional medicine (Pushpa et al*.,* 2010), However, just as with properly developed

and researched conventional orthodox pharmaceuticals, it is important to look into the toxicity of genuine and confirmed herbal medicinal goods. The evaluation of toxicity in traditional herbal treatments is often overlooked, even though it should be a priority for legitimate and well-documented herbal medicinal products, just as it is for legitimate and extensively researched conventional drugs (Smart et al., 2011), Furthermore, herbal medications that are believed to be nontoxic may contain pollutants such pathogenic microbes, aflatoxins, and heavy metals based on the process of formulation or as a result of the acquisition of metals (such as cadmium) from the soil (Thanaboripat et al., 2007; Kneifel, 2002; Abou-Arab and Abou-Donia, 2000). For years, traditional medicines have witnessed increasing and determined efforts for their integration and adoption into the healthcare systems of both developing and developed countries (WHO, 2005). Interestingly, the demand for medicinal herbs is gradually increasing in both developed and developing countries (Abere et al., 2010). Despite an apparent lack of scientific evidence for their effectiveness, value and safety, African homemade herbal medications are widely utilized throughout the continent of Africa (Jadeja et al., 2011). For instance, most Nigerian, particularly in rural regions, rely on herbal therapy to manage a range of illnesses, including diabetes and cancer. The toxicity profiles of the majority of plants consumed locally in Nigeria have not been properly assessed (Agyare et al., 2009).

P. thonningii is a single-stem, deciduous leguminous tree in the Fabaceae family and Cercidoideae subfamily (Burrows *et al*., 2018). Its enormous, straightforward, two-section, fibrous leaves, which are perennial in habit give rise to the popular name "Camel's foot," as they are large, simple, and shaped like a camel foot. This plant is known locally in Nigeria by names like *abefe* (Yoruba), *kalgo* (Hausa), and *Okpoatu* (Igbo).

There are claims that the crude extract of *P. thonningii* contains antilipidemic, antibacterial, anthelmintic, anti-inflammatory and antiulcer properties (Ukwuani et al., 2012; Akinpelu and Obuotor, 2000). Currently, the hydroethanolic extract of this plant has not been investigated for its toxicological effects on female Wistar rats. Therefore, the purpose of the current investigation was to examine, in female Wistar rats, the toxicological effects of the hydroethanolic extract of *P. thonningii* leaves.

Materials and Methods

Collection and Identification of Plant Material

The fresh leaves of *P. thonningii* were gathered from the premises of Kebbi State University of Science and Technology, Aliero, Nigeria. The identification and authentication of these leaves were performed by Prof. Dharmendra Singh of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology. A voucher specimen (KSUSTA/PSB/H/109) was deposited.

Preparation of Plant Extract

The leaves of *P. thonningii* were washed under running tap water and then subjected to drying in an oven (Uniscope Laboratory Oven, SM9053, Surgifield Medicals, England) at a temperature of 40°C. Once dried, leaves were pulverized using an electric blender (FINLAB Nigeria Limited, Ilupeju Industrial Scheme, Lagos, Nigeria) and then kept in an airtight container until extraction. For extraction, 100 grams of the powdered sample was immersed in a solvent consisting of a 50:50 mixture of ethanol and water at room temperature. The mixture was shaken intermittently over a period of 48 hours and subsequently filtered using Whatman No.1 filter paper. The resulting filtrate was then subjected to lyophilization using a Zirbus Lyophiliser (Model VaCo 5-11, Zirbus Technology, Stephensonstraat, Germany), resulting in a yield of 29 grams (corresponding to a percentage yield of 29%). This lyophilized extract was reconstituted in distilled water to obtain the desired doses of 50, 100, and 200 mg/kg body weight, which were based on information obtained from an ethnobotanical survey and utilized in the present study.

$$\%\ \text{yield} = \frac{\text{weight of dried extract}}{\text{weight of powdered leaves soaked}} \times 100$$

Experimental Animals

Healthy, female Wistar rats (*Rattus norvegicus*) weighing 120.75 ± 2.33g were gotten from Markeen Nigeria Global Ventures, Ilorin, Kwara State, Nigeria. The animals were housed in clean wooden cages placed in well-ventilated housing conditions (temperature: 25°C – 27°C;

photoperiod: about a 12 h light and dark cycle; relative humidity: 45% – 50%). The animals were provided with unlimited access to clean rat pellets (Top Feeds Nigeria Limited, Ibadan, Nigeria) and tap water. Daily cleaning of the cages was also done.

Animal Grouping and Extract Administration

Twenty Wistar rats were divided into four groups, each consisting of five rats. The extract dosages were given orally to the rats using the following methods:

GROUP I: Administered 0.5 ml of distilled water only

GROUP II: Administered 0.5 ml of 50 mg/kg body weight of extract

GROUP III: Administered 0.5 ml of 100 mg/kg body weight of extract

GROUP IV: Administered 0.5 ml of 200 mg/kg body weight of extract

The animals received oral administration of the extract daily for 7 days using a cannula. Weight changes were also monitored throughout the experimental period. On the eighth day, the rats were sacrificed.

Markers of toxicity

Various parameters were assessed in the blood samples collected at the end of the acute toxicity experiment. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) levels were measured using the method established by Roy (1970), and bilirubin levels were determined as in Jendrassik and Grof (1938) method. Lactate dehydrogenase (LDH) levels were analyzed using the method described by Weisshaar *et al*. (1975), while albumin levels were determined as outlined by Rodkey (1965). Total protein concentration was measured using Weichselbaum (1946) method. Kidney parameters such as serum urea were determined using Young et al. (1997) berthelot colorimetric method. Serum creatinine levels were analyzed using Jaffe's method explained by Bartels and Bohmer (1971). The level of serum potassium and sodium ions was measured using flame photometry (Cheesbrough, 1998), while serum bicarbonate and chloride ions and phosphate were determined using the titration/volumetric method (Chapman, 1961). Serum uric acid concentration was determined using the method described by Henry *et al*. (1957).

Statistical Analysis

The data obtained from five replicated measurements were used to calculate the mean and then the standard error of the mean. Furthermore, a one-way Analysis of Variance (ANOVA) was performed on the data. Statistical significance was determined using GraphPad Prism version 6.01 software (GraphPad Software, Inc., San Diego, California, United States), with a significance level at $p < 0.05$.

Results

The results for the heavy metals screening of the extract revealed the absence of chromium and nickel while lead and cadmium are present in trace amounts (Table 1). The hydroethanolic extract of *P. thonningii* leaves significantly increased the liver body weight ratio in rats that are treated at 100 and 200 mg/kg. Though, treatment-related changes in the kidney body-weight ratio were not observed (Table 2).

The results of this study showed no significant difference ($p>0.05$) between the treatment groups' levels of albumin, and total protein and the control groups (Table 3). ALT, ALP, Total and Direct Bilirubin and LDH levels did, however, significantly drop ($p< 0.05$) in a dose-dependent manner. Conversely, at 200 mg/kg, the concentrations of aspartate aminotransferase increased significantly ($p< 0.05$) (Table 3).

The result of this study shows that the activity of urea, uric acid, creatinine, phosphate ion and potassium ion decreased significantly ($p< 0.05$) at 100 and 200 mg/kg body weight when compared to the control group (Table 4). Additionally, the study found no evidence of a difference in sodium, bicarbonate, calcium, or chlorine activities ($p>0.05$). The level of Phosphate significantly decreased ($p>0.05$) at 100 and 200 mg/kg.

Table 1: Concentration of Heavy metals (mg/L) in hydroethanolic extract of *P. thonningii* leaves

Heavy metals	Concentration (mg/L)
Lead	0.04±0.02
Cadmium	0.02±0.01
Chromium	Not detected
Nickel	Not detected

Values are the mean of 3 replicates ± S.E.M

Table 2: Organ body-weight ratio (%) of rats administered hydroethanolic extract of *P. thonningii* leaves

Group/doses (mg/kg body weight)	Liver	Kidney
Control	5.87±0.65^{a}	0.66±0.08^{a}
50	5.72±0.83^{a}	0.59±0.08^{a}
100	7.84±0.37^{b}	0.67±0.03^{a}
200	7.56±0.73^{b}	0.68±0.12^{a}

Values are reported as mean ± S.E.M of five replicates. Values with the same superscript are not different significantly at (p>0.05)

Table 3: Effects of the hydroethanolic extract of *P. thonningii* on liver function parameters.

	Control	Plant Extract (mg/kg body weight)		
Parameters		50	100	200
Total Protein	29.92±1.40^{a}	30.00±0.11^{a}	29.72±0.14^{a}	30.24±0.87^{a}
Albumin	21.75±0.23^{a}	20.56±0.36^{a}	21.47±0.87^{a}	21.72±0.72^{a}
Direct Bilirubin	27.89±0.01^{a}	27.92±0.02^{a}	25.65±3.12^{a}	22.48±0.01^{b}
Total Bilirubin	48.38±0.28^{a}	46.70±0.33^{a}	37.81±0.09^{b}	43.58±0.08^{c}
Alanine Aminotransferase	37.46±0.44^{a}	35.43±0.59^{a}	31.29±0.50^{b}	28.60±0.43^{b}
Aspartate Aminotransferase	26.42±1.23^{a}	29.30±0.66^{a}	29.59±0.48^{a}	32.62±0.37^{b}
Alkaline Phosphatase	51.34±0.14^{a}	29.31±0.66^{b}	29.51±0.48^{b}	33.96±1.69^{c}
Lactate Dehydrogenase	34.07±0.26^{a}	35.00±0.45^{a}	25.18±0.36^{b}	19.93±0.95^{c}

Values are reported as mean ± S.E.M of five replicates. Values with the same superscript are not different significantly at (p>0.05)

Table 4: Effects of the hydroethanolic extract of *P. thonningii* on renal function parameters

Parameters	Control	Plant Extract (mg/kg body weight)		
		50	100	200
Urea	3.31±0.10[a]	3.01±0.09[a]	2.98±0.08[b]	2.93±0.04[b]
Uric acid	3.75±0.23[a]	3.56±0.36[a]	2.47±0.87[b]	1.72±0.72[b]
Creatinine	4.00±0.02[a]	3.30±0.11[b]	3.40±0.18[b]	3.70±0.05[b]
Phosphate ion	68.87±0.12[a]	67.60±0.15[a]	63.11±3.17[b]	61.52±2.95[b]
Sodium ion	66.62±0.36[a]	63.26±0.06[a]	64.06±0.37[a]	69.11±4.62[a]
Potassium ion	9.17±0.17[a]	9.54±0.21[a]	5.53±0.18[b]	5.89±0.08[b]
Calcium ion	64.29±0.61[a]	64.03±0.44[a]	65.13±0.79[a]	66.42±0.65[a]
Chloride ion	31.97±0.24[a]	30.31±1.48[a]	31.19±0.68[a]	32.57±0.63[a]
Bicarbonate	28.03±0.32[a]	27.57±0.22[a]	28.68±0.47[a]	28.93±0.49[a]

Values are reported as mean ± S.E.M of five replicates. Values with the same superscript are not different significantly at (p>0.05)

Discussion

There has been a notable upsurge in using herbal formulations by the general public, driven by the perception that they offer safer and more natural alternatives to conventional medications (Latha et al., 2010). However, concerns regarding the safety, efficacy, and possible toxicity of herbal medications persist. This study addressed these concerns by investigating the effects of hydroethanolic leaf extract of *P. thonningii* on liver and renal functions.

Heavy metals present in natural products are a thing of concern due to their potential toxicity. Lead (Pb) is a toxic heavy metal known for its harmful effects on human health. Even when exposure level is low, lead can accumulate in the system over time and cause adverse effects, particularly on the nervous system, cardiovascular system, and kidneys (Shukla et al., 2018). Cadmium (Cd) is another toxic heavy metal that can have damaging effects on the health of man. Chronic exposure to cadmium is associated with kidney damage, bone disorders, and potential carcinogenicity (Rahimzadeh et al., 2017). It is important to note that heavy metals, when chronically exposed to them, even at low levels, can be detrimental. The absence of chromium and also nickel in the extract could mean the metals were not detected or present below the detection limits of the analytical method used. Nickel and Chromium are known to have poisonous effects on man, with chromium being a potential carcinogen and nickel causing allergic reactions and respiratory issues (Nemery, 1990). The absence of detectable levels of these metals in this extract is reassuring in terms of impending toxicity.

The liver and also the kidney are vital organs responsible for metabolic processes, detoxification, and elimination of waste products from the body. A change in the organ body-weight ratio is a useful indicator of potential toxicity or alterations in organ size and function (Knight et al., 2006). Comparing the control group to the experimental groups, there are notable variations regarding the organ body-weight ratios. An increase in liver body-weight ratios of Rats treated with 100 mg/kg body weight of extract and those treated with 200 mg/kg body weight of extract, in comparison to the control suggests a possible hypertrophic or hyperplastic feedback by the liver to the treatment. Such an increase in liver weight may indicate liver cell proliferation or accumulation of fat, potentially reflecting liver damage or altered metabolic functions (Knight et al., 2006; Tsukada et al., 2006). The absence of substantial alteration in the body-weight ratios in other groups suggests that the treatments did not significantly impact their kidney size or weight, indicating a relative lack of toxicity toward the kidney at the tested doses. However, it is imperative to note that organ body-weight ratios alone cannot provide a complete assessment of organ toxicity. Additional investigations, such as biochemical markers, would be necessary to comprehensively evaluate the impending toxic effects on both the liver and kidney.

Total protein and albumin are indicators of overall protein status and liver function (Johnson et al., 2013; Oettl et al., 2008). In this study, the absence of substantial changes in total protein or

albumin levels across the different doses tested suggests that there may be no impact on protein metabolism or hepatic protein synthesis due to the extract. Direct bilirubin and total bilirubin levels are markers used for liver functioning and degradation of red blood cells (Pratt et al., 2000). The substantial reduction in direct bilirubin and total bilirubin may indicate a potential beneficial function of plant extract on bilirubin metabolism or liver function, as lower levels of bilirubin can be indicative of improved liver health.

ALT and AST are enzymes primarily found in liver cells, and their elevation in the bloodstream can be an indication of liver damage or injury (Pratt et al., 2000). The elevation in AST levels and reduction in ALT levels especially in the group receiving the highest dose (200 mg/kg) suggests a possible hepatocellular injury or stress in response to the higher plant extract dose.

ALP is an enzyme present in various tissues, including the liver, bones, and intestines. Changes in ALP levels can indicate liver dysfunction or bone-related issues (Kuo et al., 2017). The extract at different doses caused significant variations in ALP levels compared with the control group. These changes may reflect alterations in liver or bone metabolism prompted by the extract. LDH however, is an enzyme needed during energy production, its elevation in the bloodstream can be associated with tissue damage or cell death (Brady et al., 2010). This reduction in LDH at the highest dose of (200 mg/kg) may suggest a potential protective effect on cellular integrity and tissue damage.

The findings signify that plant extract at the tested doses did not exert major harmful effects on protein metabolism or liver function, as evidenced by the relatively stable levels of total protein and albumin as well as the reduction in the levels of LDH, Direct Bilirubin, and Total Bilirubin. However, changes observed in AST, ALT and ALP suggest potential dose-dependent side effects on liver health and cellular integrity.

Urea, uric acid, and creatinine are waste products that are excreted by kidneys and their intensity in blood can indicate renal function (Wani and Pasha, 2021). The significant decreases in urea levels and also uric acid at doses of 100 mg/kg and 200 mg/kg suggest a potential influence of the plant extract on the reabsorption of metabolic products in renal tubules. Moreover, creatinine levels were significantly decreased across all doses of the plant extract, indicating a potential disruption in kidney filtration or clearance (Brown et al., 2015).

Phosphate ion, potassium ion, sodium ion, chloride ion, calcium ion, and bicarbonate are electrolytes involved in various physiological processes, including fluid balance and acid-base regulation (Hoorn et al., 2013). The significant decrease in Phosphate and potassium levels in the groups receiving 100 mg/kg and 200 mg/kg suggests a potential alteration in their metabolism, renal reabsorption, or excretion by the kidneys. Bicarbonate, Sodium, Chloride, and Calcium ion levels remained relatively stable across all doses of extract, some of these electrolytes are involved in maintaining acid-base balance indicating no significant impact on their metabolism or homeostasis. The plant extract at the tested doses might have exerted some harmful effects in the reabsorption of metabolic products in renal tubules, as evidenced by the significant decreases in urea, uric acid, creatinine, Phosphate ion and potassium ion levels.

Conclusion

The results put forward that the plant extract at the tested doses poses some threats to liver functionalities. It may also affect renal function, as evidenced by the changes observed in urea, creatinine, and uric acid levels. Additionally, the variations in phosphate ion and potassium ion levels suggest potential side effects on electrolyte balance and renal handling of these ions which might be a potential dose-dependent side effect on cellular integrity and renal functionality.

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