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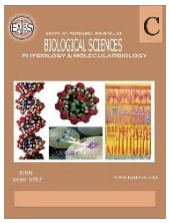

The Impact of Aqueous *Piliostigma thonningii* Fruit Extract on Certain Biochemical Parameters on Male Wister Albino Rats

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ABSTRACT

Introduction: *Piliostigma thonningii* is a medicinal plant known for its diverse biological activities. This study examined the impact of the aqueous extract of *P. thonningii* fruits on biochemical parameters in a rat model. **Methods:** Male Wister Albino rats were administered varying doses (200, 500, and 1000 mg/kg) of the *P. thonningii* fruit aqueous extract for 28 days. The levels of liver enzymes (AST, ALT, and ALP), total protein, lipids, glucose, and indicators of renal function (urea, creatinine) were evaluated using standard assay kits and colorimetric methods. **Results:** The fruit aqueous extract of *P. thonningii* fruit had a significant effect in reducing serum AST levels (p<0.01) at doses of 500 and 1000 mg/kg, and ALT levels (p<0.05) at a dose of 1000 mg/kg. The treated groups exhibited a significant (p<0.05) decrease in total protein and cholesterol. The administration of the highest dose (1000 mg/kg) resulted in a notable decrease (p<0.01) in serum glucose levels, indicating the presence of hypoglycemic characteristics. There were no significant alterations detected in the levels of ALP, urea, and creatinine. **Conclusion:** The aqueous extract of *P. thonningii* fruits exhibited favorable positive effects on biochemical parameters in rats, indicating its potential therapeutic applications.

INTRODUCTION

The African plum, Piliostigma thonningii (*P. thonningii*), is a woody plant that grows in sub-humid African forests and wooded fields. The natural habitats of this species are in Sudan, Kenya, Namibia, Senegal, Tanzania, Uganda, and Zambia (Orwa *et al.,* 2009). Comprehensive studies highlighted the phytochemistry and traditional medicinal uses of *P. thonningii*, paving the way for further investigation into the plant's potential benefits.

A preliminary phytochemical study on *P. thonningii* reveals high flavonoids, tannins, and alkaloids (Akindahunsi and Salawu, 2005). In addition to tannins, flavonoids in *P. thonningii* extracts may play a significant role in lipid mobilization and metabolism.

Cyril *et al.* (2021) and Dasofunjo *et al.* (2013) used male albino Wistar rats to test an ethanolic extract from *P. thonningii* leaves to see how it affected their blood and kidney function. The growth of grass cutters fed a mix of *Phyllanthus amarus* and *P. thonningii* leaf meal demonstrated the versatility of this plant extract (Alagbe *et al.,* 2020). It also assessed the animals' performance and serum biochemical markers (Ozolua *et al.,* 2009).

It is alleged that *P. thonningii's* crude extract has anti-lipidemic activities (Ighodaro and Omole, 2012), antibacterial (Akinpelu and Obuotor, 2000), and antihelmintic (Asuzu and Onu, 1994), besides possessing anti-inflammatory (Ibewuike *et al.*, 1997) effects. According to traditional Zambian medicine, *P. Thonningii* has antidiabetic properties. In contrast to glibenclamide, which caused blood glucose levels to drop from 24 mmol/L to 7.6 mmol/L, stem bark extract reduced the blood glucose of the experimental animals during the treatment period from 19.7 mmol/L to 8.8 mmol/L (Jere *et al.*, 2021).

Various countries have historically utilized different parts of *P. Thonningii* for diverse medicinal purposes. For instance, individuals conventionally employ twigs and leaves to remedy ailments such as malaria fever, skin infections, snake bites, and diarrhea (Kwaji *et al.,* 2010). *P. thonningii* stem barks are utilized for the treatment of intestinal, pulmonary, and inflammatory ailments (Kaigongi and Musila, 2015). Researchers have also used various parts of *P. thonningii* extracts for investigating its *in vivo* and *in vitro* medicinal purposes. They frequently use the leaves and stem bark extracts of *P. thonningii*, most likely through methanolic extraction methods (Ighodaro and Omole, 2012; Dasofunjo *et al.,* 2012; Dasofunjo *et al.,* 2013; Jere *et al.,* 2021; Moriasi *et al.*, 2020).

However, the beneficial use of *P. thonningii* fruit aqueous extract is rarely implemented. The study aims to investigate the effects of aqueous fruit extracts from *P. thonningii* on liver function measures in male albino rats.

MATERIALS AND METHODS

Plant Material:

The prospective plant, *P. thonningii*, was found in Western Sudan, where fruit samples were collected in multiple groups. The fruits were botanically identified and verified by Prof. Hatil Hashim EL-Kamali at the Herbarium of the Department of Botany, Faculty of Science and Technology, Omdurman Islamic University. The fruits underwent a process of washing, air-drying, and grinding using mortar and pestle to achieve a consistent and powdery texture (Handa *et al*., 2008). Concisely, a rough sample was immersed in distilled water for 3 days, with the extract being filtered and evaporated daily utilizing a rotating evaporator device while maintaining lowered pressure. The last portion was placed in an evaporating dish and thereafter exposed to the air to ensure full desiccation.

Preparation of Aqueous Extract *of P. thonningii:*

To obtain a sufficiently aqueous extract, 50 grams of *P. thonningii* plant powder was macerated in 250 mL of distilled water and left to sit at room temperature for 24 hours. The mixture underwent filtration using Whatman filter paper No. 1 and was

thereafter put into sterile freeze-drying flasks for lyophilization, which lasted for 48 hours. The dehydrated and lympholized extract was transferred into a sterile, moisture-free, pre-weighted, and labeled glass bottle.

As indicated by Truong *et al.* (2019) the yield percentage is calculated by dividing the weight of the extract by the weight of the macerated powder and then multiplying the result by 100. Subsequently, the extract was hermetically enclosed in a glass vial and kept at a temperature of 4 °C until it was utilized.

Animal Management and Administration of Extract:

A total of twenty (20) male Wister Albino rats, weighing between 100 and 150 g, were obtained from the National Centre for Research (NCR), Medicinal and Aromatic Research Institute, located in Khartoum, Sudan. The rats were kept in stainless steel wire mesh cages and allowed to acclimate for one week before the experiments. The animal's enclosure was equipped with a ventilation system and kept at a constant temperature of 22-24°C using a thermostat. The enclosure also had a natural light/dark cycle of 12 hours, and the animals had unrestricted access to water and food. Regularly cleaning and disposing of excrement and spilled feeds from the cages maintained a high level of hygiene.

The animals were selected in a random manner and then separated into four distinct groups (A, B, C, and D), with each group consisting of five rats. Group A (control) received daily administration of distilled water for 28 days. Groups B, C, and D received *P. thonningii* fruit extract orally at doses of 200 mg/kg bwt, 500 mg/kg bwt, and 1000 mg/kg bwt each day for 28 days, respectively. The investigation ensured that all animals had unrestricted access to standard laboratory feed and water.

Blood Sampling and Preparations:

Blood samples were taken from each group of rats individually at the beginning of the study by ocular puncturing the retro-orbital sinus of the eye into anticoagulant vials. Following a 28-day period, both the control and treated animals were fasted for one night, blood samples were taken once more, and the animals were promptly sacrificed by cervical dislocation to end the experiment.

To ascertain the biochemical parameters, five microliters of blood were obtained from the animals' eyes before and during treatment in sterile EDTA containers and microcentrifuge tubes. Plasma was acquired using centrifugation at 3000 rpm for 10 minutes at 4 °C and subsequently partitioned into aliquots to prevent freezing and thawing. Serum was acquired by permitting blood coagulation for 30 minutes before centrifugation at 3000 rpm for 10 minutes. The aliquots were preserved at -20° C until testing commenced.

Estimation of the Biochemical Parameters:

We utilized plasma for the biochemical evaluation of liver function tests (AST and ALT), renal function tests (urea and creatinine), total protein, cholesterol, and fasting blood glucose (FBG), whereas serum was employed for the quantitative measurement of ALP. We assessed the activities of ALT, AST, and ALP utilizing a commercially available kit, following the manufacturer's (Athenese-Dx, Tamil Nadu, India) guidelines. The absorbance at 340 nm was measured using a spectrophotometer (ULTROSPEC II, Pharmacia). The results were reported in units per liter (UI/L) format. The plasma concentration of total cholesterol (TC) was determined using the cholesterol oxidase method. The Biuret reagent was used to estimate the plasma total protein, and the calculation was performed according to the procedure outlined by Lubran (1978). The levels of plasma urea and creatinine were measured using assay kits obtained from Sigma Aldrich, USA. The FBS level was determined using the colorimetric glucose oxidase technique, as described by Kalungia *et al.* (2018).

Statistical Analysis:

The data were reported as the mean±SE (standard error of the mean) for the biochemical parameters. The study groups were compared using a one-way analysis of

variance (ANOVA). A significance level of less than 0.05 was used to determine statistical significance.

RESULTS

In terms of liver enzymes, the 500 mg/kg and 1000 mg/kg groups significantly reduced the levels of AST (aspartate aminotransferase) compared to the control group (215.4±7.4 vs. 177.4±11.5, p= 0.006; 215.4±7.4 vs. 170.6±3.9, *p*= 0.001, respectively). This suggests a potential hepatoprotective effect at these particular doses. However, the 1000 mg/kg group showed a significant (19.4±0.8 vs. 16.8±1.2, *p*= 0.043) decrease in ALT (alanine aminotransferase) levels, suggesting improved liver function at this particular dose. The 200 mg/kg and 500 mg/kg groups showed ALT levels comparable to the control group (19.4±0.8 vs. 18.8±0.9; 19.4±0.8 vs. 19.0±0.01; *p*> 0.05). In terms of ALP (alkaline phosphatase), levels were increased in high doses but didn't reach significant differences between any of the treated and the control group (Table 1). These findings indicate that the dose of 1000 mg/kg of the *P. thonningii* extract may have the most favorable effects on liver function, as evidenced by the reductions in both AST and ALT levels.

As shown in Table 1, the treated groups (200, 500, and 1000 mg/kg) significantly reduced their total protein (8.4±0.3 vs. 6.4±0.2, *p*= 0.0001; 8.4±0.3 vs. 6.7±0.4, *p*= 0.01; 8.4±0.3 vs. 5.6±0.3, *p*= 0.000, respectively) and cholesterol levels (97.2±6.1 vs. 69.6±5.5, *p*= 0.004; 97.2±6.1 vs. 69.6±5.5, *p*= 0.001;97.2±6.1 vs. 76.4±1.6, *p*= 0.011, respectively) compared to the control group. Furthermore, the 1000 mg/kg group significantly reduced (88.6±1.9 vs. 61.8±2.0, *p*= 0.000) their glucose levels compared to the control group. These results suggest that the *P. thonningii* extract may have beneficial effects on lipid and carbohydrate metabolism, particularly at higher doses. In terms of kidney function, the levels of urea and creatinine, which serve as indicators of kidney function, showed no significant differences between any of the treatment groups and the control group. This suggests that the *P. thonningii* fruit extract did not negatively impact on kidney function at the tested doses.

Table 1: The effect of *P. thonningii fruits aqueous extract* on biochemical parameters after treatment of rats for 28 days**.**

Biochemical parameters	Group A (Control)	Group B (200 mg/kg)	Group C (500 mg/kg)	Group D (1000 mg/kg)
AST (IU/L)	215.4±7.4	189.4±13.3	177.4±11.5**	170.6±3.9**
ALT (IU/L)	19.4±0.8	18.8±0.9	19.0±0.01	16.8±1.2*
ALP (IU/L)	113 ±1.7	105 ± 5.9	118.6±9.5	114.0±2.9
Protein (g/dL)	8.4 ±0.3	6.4 ±0.2**	6.7 ±0.4**	5.6 ±0.3**
Cholesterol (mg/dL)	97.2 ±6.1	69.6 ±5.5**	97.2 ±2.9**	76.4 ±1.6*
Glucose (mg/dL)	88.6 ±1.9	79.4 ±3.9	85.4 ±2.6	61.8 ±2.0**
Urea (mmol/L)	72 ± 3.2	65 ± 3.2	76.8 ± 0.9	72.6 ±2.5
Creatinine (mg/dL)	1.4 ±0.9	1.2 ±0.2	1.2 ±0.2	1.2 ±0.4

Results are expressed in mean ± SE (n = 5); AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphate; body weight. * = $p<0.05$; ** = $p<0.01$

DISCUSSION

The present investigation demonstrated a notable decrease in AST and ALT levels, especially when high doses of P. *thonningii* fruit aqueous extract were administered. This suggests an enhancement in liver function, which aligns with the hepatoprotective benefits reported in a previous study conducted by Adebayo *et al.*, (2016). Simultaneously, the ethanolic extract of *P. thonningii* leaf caused a noteworthy decrease in blood AST and ALT levels when given at various doses to rats (Dasofunjo *et al.,* 2012). Taofeek (2011) conducted a study that showed the ability of P. *thonningii* leaf extract to improve liver damage caused by

carbon tetrachloride in rats. This effect is likely due to the extract's antioxidant and anti-inflammatory properties.

Despite the confirmation of the previously indicated findings about the decrease in liver marker enzymes, conflicting reports suggest that prolonged consumption of the ethanol stem bark extract of *P. thonningii* increases liver AST and ALT levels (Awhin *et al.,* 2013).

The researcher found that the extract of *P. nitida,* a species closely similar to the subject of study, effectively decreased the increased levels of liver enzymes, including AST and ALT.

A separate study conducted by Olorunnisola *et al*., (2012) examined the effects of *P. thonningii* stem bark extract on liver injury induced by paracetamol in rats, specifically focusing on its hepatoprotective and antioxidant properties. The data indicated that the extract effectively reduced the elevated levels of AST, ALT, and ALP induced by the paracetamol administration, confirming our results.

The data we collected indicated a rise in ALT levels, but there were no notable differences between the study groups. This finding contradicts earlier research that revealed significant increases in ALT levels in a manner that depended on the dosage (Dasofunjo *et al.,* 2012; Dasofunjo *et al.,* 2013). The contentious outcome of the ALP is likely attributable to the utilization of distinct experimental extracts.

Notably, the leaves of *P. thonningii* have been discovered to possess hepatoprotective and antioxidant capabilities, successfully protecting the liver from damage caused by hepatic and oxidative factors. This is accomplished via their capacity to scavenge free radicals inside an organism, stimulate antioxidant enzymes, and inhibit excessive activation of antioxidant enzymes and lipid peroxidation (Ajiboye, 2011). The antioxidant and anti-inflammatory activities of *P. thonningii* and related species are responsible for the hepatoprotective effects they exhibit. The plant extracts contain bioactive phytochemicals, including flavonoids, tannins, and saponins. Researchers believe these compounds play a significant role in mitigating oxidative stress and inflammation, which significantly contribute to liver damage (Adebayo *et al.,* 2016; Olorunnisola *et al.,* 2012). Cautious should be applied when giving long-term extracts of *P. thonningii* stem barks, as it can harm the liver of adult Wistar rats (Adjene *et al.,* 2013).

Given the relatively safe nature of *P. thonningii* extract and its potential for multi-targeted effects on liver health, it could be explored as a complementary or integrative therapy alongside conventional treatments for various liver-related disorders. The extract could be investigated for its ability to enhance the efficacy of standard therapies or to provide supportive care for patients with liver disease.

The experimental groups in our dataset had markedly reduced levels of total protein, which contradicts the findings of Dasofunjo *et al.* (2013). In their study, they observed a considerable rise in blood protein levels in rats fed with an ethanol extract of *P. thonningii* leaf. Additional investigations are necessary to validate the results due to the conflicting data.

The significant decrease in plasma cholesterol levels observed in our data aligns with the findings reported by Ighodaro and Omole in 2012. Previous studies have reported that *P. thonningii* seeds and leaves contain a substantial amount of crude fibers, reaching up to 35.0% w/w (Ighodaro *et al.,* 2012; Mustapha et al., 2012). Consuming these fibers may have positive physiological effects by reducing cholesterol levels, which are closely linked to diseases like coronary heart disease and hypertension (Muhammad *et al.,* 2015).

According to our results, the experimental group that received a dosage of 1000 mg/kg experienced a substantial decrease in their glucose levels compared to the control group. A prior investigation demonstrated that the utilization of stem bark extract from *P. thonningii* had a notable hypoglycemic impact in diabetic rats (Jere *et*

al., 2021). The findings indicate that the extract derived from *P. thonningii* may have advantageous impacts on the control and treatment of diabetes.

The negligible disparities in urea and creatinine levels observed in our experimental study suggest that the administration of *P. thonningii* fruits' aqueous extract, even at large doses, had limited effects on kidney functioning. Nevertheless, it is important to mention that the administration of *P. thonningii* leaf extract in rats caused significant changes in uric acid, creatinine, and urea levels, potentially affecting renal function (Nurudeen *et al.*, 2023). In addition, Dasofunjo *et al.* (2013) found that the extract reduced renal function indices, suggesting the possibility of nephropathy and kidney dysfunction.

The risk connected with *P. thonningii* fruit extract is quite minimal, and it can provide various therapeutic effects. The findings of our study offer additional confirmation of the growing body of evidence regarding the therapeutic properties of *P. thonningii*. This plant has the potential to protect the liver, lower lipid levels, and reduce glucose levels, especially when administered in high doses. It could be explored as a supplementary or integrated therapeutic approach alongside conventional treatments for various disease conditions. The positive benefits of this extract may be attributed to the existence of bioactive components, including flavonoids and other phytochemicals. Nevertheless, additional research is necessary to clarify the exact processes by which this plant extract works and to assess its potential for use in clinical settings.

In conclusion, the findings of this study regarding the significant reduction of biochemical markers, specifically liver enzymes (AST and ALT), total proteins, and cholesterol, corroborate previous research on the hepatoprotective and other therapeutic attributes of P. *thonningii* and its related species. This adds to the growing body of evidence advocating for the ongoing research of P. *thonningii* as a prospective natural resource for health-promoting applications. Further research may explore the underlying mechanisms responsible for the hepatoprotective and metabolic effects observed. It may also investigate the optimal dose regimen to enhance beneficial outcomes while reducing potential unwanted consequences.

Declarations:

Ethical Approval: The study was approved by the Ethical Board of the Faculty of Science and Technology, Omdurman Islamic University.

Conflict of interest: The authors declare no conflicts of interest.

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