## A study of in vivo Biochemical estimation of ethanolic fruit extract of *Physalis angulate*

**ABSTRACT**

The fruits of *Physalis angulata* are recommended locally for treating infection, infertility, inflammation, postpartum infection, skin diseases. Some medicinal plants may contain toxins that affect the organs in the body, especially the liver and kidneys. In order to validate the use of *Physalis angulata* fruits in traditional medicine, it is important to evaluate its effect on liver and kidney functions in experimental animals.

Materials and Method: 18 adults female Wistar rats weighing 120 ± 20 g were grouped into 3 groups, with each group consisting of 6 rats. The control group was on a normal diet and distilled water while the other groups received 500 mg/bwt, and 1500 mg/ body wt of ethanolic extract of *Physalis* *angulata* for 28 days respectively. Animals were acclimatized for 2 weeks before commencement of the experiment. Blood samples were analyzed for alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Results: The concentration of AST in the control was significantly (p<0.05) lower than the values in the treatment groups. Concentrations of ALT and ALP concentrations were not significantly different among treatment groups. Serum electrolytes (potassium, sodium, and chloride ions) were not statistically significant. Significantly (p<0.05) higher concentrations of creatinine were observed in *Physalis angulate* 1500mg/kg group compared to the control. Conclusion: *Physalis angulate* fruit extracts is safe and non-toxic to the liver and kidney.

Keywords: alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, Serum electrolytes.

**INTRODUCTION**

“Natural plant-based compounds have been used by humans to treat a wide range of foods for thousands of years” (Parekh and Chanda, 2007). An important factor in the development of many human disorders is nutrition (Bayan et al., 2014). Many cultures believe that different diets are good for human health, and despite ethnic differences, there are some fundamental components that all healthy eating habits share (Benavides et al., 2007).

“Plant-based natural products contain physiologically active ingredients that have long been used in traditional medicine to treat a variety of illnesses” (Tion et al., 2018; Onyegeme-Okerenta et al., 2019). “According to World Health Organization, over 75% of the world's population relies on medicinal plants for their basic healthcare requirements, and roughly 25% of medications are plant-based” (WHO, 2001). Some medicinal plants may contain toxins that affect the organs in the body, especially the liver and kidneys (Reuben et al., 2022; Nasir et al., 2025).

“The fruits of Physalis angulate are recommended locally for treating infection, infertility, inflammation, postpartum infection, skin diseases” (Donkor *et al.,* 2012; Rengifo-salgado and Vargas-Arana, 2013). Ethyl acetate extract of it inhibits essential steps of metastasis including migration and invasion of human oral squamous carcinoma cells Hseu *et al.* (2011). [Hseu](https://pubmed.ncbi.nlm.nih.gov/?term=Hseu+YC&cauthor_id=21515352)*et al.* (2011) showed that *Physalis angulata* has an anti-metastatic and anti-angiogenic activity, which may contribute to the development of better chemo preventive agent for cancer and inflammation.

“Urea is an end product of protein metabolism and its product reflects diet protein intake and protein catabolic rate” (**Akanji** *et al.,* 2013). “It is a waste product that is left over from the breakdown of protein. Urea circulates in the blood until it is filtered out by the kidneys and excreted in the urine” (**Latha** *et al.,* 2016). “Kidney function was evaluated by means of serum urea, creatinine, and blood electrolyte concentrations” (Ukwubile & Oise, 2016). “Creatinine is an organic base formed during muscle protein metabolism as a degradation product of creatinine phosphate” (Mayes, 1988). Creatinine is filtered only but not reabsorbed. The major pathway of nitrogen secretion is urea synthesis in the liver which is released into the blood and cleaned by the kidney.

**Materials and Methods**

**Preparation of Plant extract**

Fruits of *Physalis angulate* were obtained from Orosi community in Obio/Akpor Local Government Area of Rivers State of Nigeria. The fruits were identified by a taxanomist in the department of Botany, University of Calabar. *Physalis angulata* fruit was air dried in a room, weighed and pulverized into fine powder using surface sterilized mortar and pestle. Known grams of the pulverized fruit were macerated by putting it into a container and introducing large quantity of 96 % ethanol (solvent) into the container. The container was covered and kept for 48 hours, slightly shaken from time to time. The extract was filtered with the aid of sterile cotton wool. The liquid extract was dried/concentrated under reduced pressure using rotary evaporator at 45oC to obtain crude extract.

**Experimental animals**

A total of 18 adults female Wistar rats were grouped into 3 groups, with each group consisting of 6 rats. Animals were housed in well ventilated wired cages at room temperature at the animal house, and had access to commercial standard rodent Pellets and cool clean water ad libitum. The experiments were conducted under standard experimental protocols. Animals were acclimatized for two (2) weeks before grouping.

**List 1-Animal Grouping**

|  |  |  |
| --- | --- | --- |
| Group | Number of Animals | Administration |
| Control | 6 | 0.5 mls of distilled water for 28 days |
| *Physalis angulata* (Low dose) | 6 | 500 mg/kg of *Physalis angulata* fruit extract for 28 days |
| *Physalis* *angulata* (High dose) | 6 | 1500 mg/kg of *Physalis angulata* fruit extract for 28 days |

**Preparation of serum, liver and kidney tissues**

Twenty-four hours after the last administration, the animals were anaesthetized under ketamine hydrochloride. Blood samples were collected by cardiac puncture, using sterile syringes with needles. Blood samples for serum preparation were collected into sterile plain tubes without an anticoagulant*.* Serum samples were separated from the clot by centrifugation at 3,000 g for 5 min using bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and both the serum. All analyses on serum samples were completed within 24 h of sample collection.

**Procedure for serum electrolytes**

**Determination of sodium concentration in serum using kits obtained from Agappe diagnostics based on Egbung *et al.* (2019)**

In this method sodium is precipitated as the triple salt, sodium magnesium Uranyl acetate, with the excess uranium then being reacted with ferrocyanide to produce a chromophore whose absorbance (color intensity) varies inversely as the concentration of sodium in the test specimen.

Uranyl ions + Mg ion + Na+ Uranyl Mg Na Precipitate

Free Uranyl ions + K4Fe (CN)6 Brown colored Complex

10µl of serum was drawn into plastic tubes, 1000µL of Sodium R1 (Precipitating reagent) was added, shaken vigorously and incubated at room temperature for 5 minutes, then centrifuged at 2000rpm for 2 minutes to obtain a clear supernatant. 20µL of the supernatant was transferred immediately after centrifugation and 1000µL of sodium R2 (Colour reagent), mixed well and allowed to stand at room temperature for 5 minutes. The absorbance of the standard and sample against reagent blank was measured.

**Determination of potassium concentration in serum using Agappe kits based on Orok *et al.* (2012)**

Sodium tetraphenylboron in a specially prepared mixture produces a colloidal suspension in the presence of potassium. The turbidity of which is proportional to potassium concentration in the range of 2 to 7 mEg\L.

Na+Tetraphenylborate + K+ K+ Tetraphenylborate + Na+

25µL of serum was added to 1000µL of potassium reagent was mixed and incubated for 5 minutes at room temperature. The absorbance of standard (Abs.S) and test sample (Abs.T) was recorded against distilled water was read at 578nm within 10 minutes.

**Determination of the concentration of chloride in serum using assay kits from Agappe (Egbung *et al.,* 2019)**

Chloride ions in the sample react with mercuric thiocyanate to release free thiocyanate ions, which forms a colored complex with ferric ions with color intensity proportional to the chloride concentration in the sample:

6 Cl- + 3Hg (SCN)2 3HgCl2 + 6 (SCN)-

 6(SCN + 2Fe3+ q112Fe (SCN) 3

1000 µl of reagent was added to 10 μl of the sample, mixed well, and incubated for 1 minute at room temperature. The absorbance of the sample and standard was measured against the reagent blank.

**Determination of urea concentration in serum using Agappe assay kit (Urease-Berthelot method) based on Osigwe *et al.* (2017)**

In the presence of the enzyme urease, urea is hydrolyzed to ammonia and carbon IV oxide. The ammonia whose concentration is in proportion to the initial concentration of urea in sample is quantitated photometrically by Berthelot’s reaction.

Urea + H2O urease  2NH3 + CO2

NH3 + hypochloride + phenol indophenol (blue compound).

10 µl serum was added to 1 ml of the reagent and the change in absorbance was recorded at 340 nm for 3 min. For standard, 10 µl standard solution of urea (40 %) was added to 1 ml reagent.

 **Determination of creatinine concentration using Agappe assay kit based on Orok *et al.* (2012)**

Creatinine reacts with picric acid to produce a colored compounds creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration. 1 ml of reagent was added to 100 μl of sample, mixed well and absorbance was read at 505mm after 60 seconds (T1) second reading (T2) taken after 60 seconds from the first one.

 **Assay procedure for liver enzyme parameters**

 **Estimation of alanine amino transaminase (ALT) activity using Agappe kit based on Oyesola *et al.* (2016)**

Α-oxoglutarate + L- alanine ALT L-glutamate + pyruvate

Pyruvate whose concentration depends on the amount of L-alanine transaminated and hence the activity of ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nM. 100 µl of serum was added to 1 ml working reagent. After mixing tubes were incubated for 1 minute at 37 0C. The change in absorbance per minute during 3 minutes was recorded against blank at 340 nm.

 **Estimation of aspartate amino transaminase (AST) activity using Agappe kit based on Oyesola *et al.* (2016)**

α-oxoglutarate + L- aspartate AST L- glutamate + oxaloacetate

Oxaloacetate, with concentration in proportion to aspartate consumed by enzyme and hence its activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

100 µl serum was added to 1 ml of working reagent. The tubes were incubated for 1minute at 37 0C after mixing. The change in absorbance per 20 second during 1 `minute was recorded against blank at 340 nm. Distilled water was used as blank.

 **Estimation of aspartate alkaline phosphatase (ALP) activity using Agappe kit based on Alhassan and Baraaj (2018)**

P – Nitrophenylphosphatase ALP p-Nitrophenol + Phosphate

Alkaline phosphatase in serum catalyses the hydrolysis of *P. Nitrophenyl* phosphate to *P. Nitrophenol* and phosphate. The rate of formation of *P. Nitrophenol* is measured as an increase in the absorbance which is proportional to the ALP activity in the sample.

20 µl of serum was added to 1ml of working reagent. The tubes were incubated for1 minute at 370C. The change in absorbance per minute during 3 minutes was recorded against blank at 405 nm.

**Results**

 **Effect of *Physalis angulata* on liver enzymes**

The concentration of AST (IU/L) in the control was 121.60±4.79. It was significantly (p<0.05) lower than the values in the treatment groups. Concentrations of ALT and ALP concentrations were not significantly different among the different experimental groups (Table 1).

 **Effect of *Physalis angulate* fruit extract on serum electrolyte**

Serum electrolytes (potassium, sodium, and chloride ions) were not significantly different among the control and the group administered with *Physalis angulate* extracts. Serum urea concentrations in control (34.57±4.34) and *Physalis angulate* 500 mg/kg, 26.95±1.50 were not significantly different. Significantly (p<0.05) higher concentrations of creatinine were observed in *Physalis angulate* 1500mg/kg group compared to the control (Table 2).

TABLE 1 Liver enzymes concentrations of control and *Physalis angulate* fruit extract treated animals

|  |  |  |  |
| --- | --- | --- | --- |
|  | AST(IU/L)Normal (50-150 IU/L) | ALT(IU/L)Normal (40 IU/L) | ALP(IU/L)Normal (30-130 IU/L) |
| Control | 121.60±4.79 | 69.49±6.11 | 38.96±1.99 |
| Physalis (Low dose) | 113.26±12.98\* | 67.00±8.83 | 40.85±1.47 |
| Physalis (High dose) | 114.66±11.16\* | 68.09±5.30 | 42.12±3.86 |

Values are expressed as mean ±SEM, n = 5.

\* = significantly different from control at p<0.05

TABLE 2 Serum electrolytes, urea, and creatinine concentrations of control and Physalis fruit extract treated groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | K+(mmol/L) | Na+(mmolL) | Cl-(mEq/L) | Urea(mg/dL) | Creatinine(mg/dL) |
| Control | 8.77±0.45 | 129.42±6.26 | 98.32±4.34 | 34.57± 4.34 | 0.23±0.03 |
| Physalis (Low dose) | 8.03±0.49 | 127.96±2.35 | 98.42±3.91 | 26.95±1.50 | 0.24±0.02 |
| Physalis (High dose) | 8.42±0.50 | 128.49±3.68 | 105.76±7.38 | 22.31±1.77\* | 0.29 ±0.01\* |

Values are expressed as mean ±SEM, n = 5.

\* = significantly different from control at p<0.05

b = significantly different from Physalis (high dose) at p<0.05

**DISCUSSION**

“The liver is responsible for most of the metabolic activities that occur in the body, and the use of unknown concentrations of plant extract is increasing” (Okpara et al., 2022).
“Aspartate Transaminase (AST) was significantly (p<0.05) lower in *Physalis* angulate-treated groups compared with control. This implies that the plant extract may not cause liver damage. This was in contrast with” (Okpara et al., 2022). They reported that a significant increase in serum enzymes such as ALT, ALP, and AST are the measures of liver injury.

 Alanine transaminase (ALT) and Alkaline phosphate (ALP) concentrations were not significantly different among the different experimental groups. This may suggest that the extract is safe. This agrees with **Olaitan** *et al.* (2013). “They reported that extracts administrations capable of causing a non-significant increase in Liver enzymes are said to be safe and non-toxic to the liver.

Changes in the ALT and ALP activities were not observed between the treated groups compared with the control. The significant decrease in the serum AST level observed in the study indicates

that the mitochondria of the liver cell may have been affected by the extract since AST is found in the mitochondria and cytoplasm and ALT in the cytoplasm.

Assessment of serum urea, creatinine, and electrolytes (Na+, K+, HCO3-, Cl-) are important and sensitive biochemical markers involved in the diagnosis of renal impairments” (Reuben et al., 2022). “In the present study, serum electrolytes such as potassium, sodium, and chloride ions were not significantly different between the control and *Physalis* *angulata* extracts groups. This suggests that the plant extract may not alter kidney function. A slight but not significant increase was observed in the levels of urea, sodium, and potassium by previous researchers explaining that such plant extract may not alter kidney function” (Eziejiofor *et al.,* 2013).

When the plasma urea concentration is increased, plasma creatinine will normally increases due to the reduction of GFR. This increase in plasma creatinine signifies pathology of the kidney (Reuben et al., 2022). “Increase in blood creatinine was observed in the present study is a good indicator of uncompromised kidney function. Creatinine concentration in the rats treated with *Physalis angulata* fruit extracts was significantly higher when compared with the control. This increase in creatinine level in the blood may suggest the functional excretory mechanisms of the kidney nephron in ensuring the removal of toxic materials from the body” (**Ezeugwunne** *et al*. 2017). “Serum creatinine is elevated when there is a significant reduction in the glomerular filtration rate or when urine elimination is obstructed” (Gounden and Bhatt, 2023).

“If the kidneys are not functioning properly, there will be excess urea levels in the bloodstream. In the present study, the urea concentration in the rats treated with *Physalis angulate* fruit extracts significantly decreased compared to the control. This suggests that the continuous administration of a high dose of the plant extract may not induce renal impairment” (**Yusuf** *et al.,* 2020).

**Conclusion**

 *Physalis angulate* fruit extracts is safe and non-toxic to the liver, no adverse alterations were seen in liver enzyme parameters such as serum AST, ALT, and ALP, in Wistar rats. This fruit extract may not alter kidney function, no significant increase was observed in the levels of sodium, chloride ions and potassium. The serum level of urea decreased significantly.

**Ethical Approval**

Animal Ethic committee approval has been collected and preserved by the author(s)

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