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Hepato-Safety Effect of the Poly-Herbal Aqueous Extract Using Animal Model

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Abstract

Poly-herbal formulation has been used all around the world due to its medicinal and therapeutic applications. These effects encompass mutual enhancement, mutual assistance, mutual restraint, and mutual antagonism.This research investigated the impact of a poly-herbal aqueous extract on the liver protective effect in Wistar rats. Plant samples were collected and processed into an extract. Twenty Wistar rats were divided into control group and graded doses (200, 400, and 800 mg/kg) of the poly-herbal aqueous extract. The rats were orally administered graded doses of the extract for 28 days. Various hepatic function tests (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin, Conjugated Bilirubin, Albumin, and Total Protein), were evaluated using standard protocol. The results indicated a significant increase in Aspartate Aminotransferase, total protein, and Globulin levels across the different doses when compared to the control group. A decrease in the Alkaline Phosphatase levels across the lowest doses. A significant decrease in Alanine Transferase, Total Bilirubin, Conjugated Bilirubin, and Albumin levels was observed across graded doses compared to the control group. In conclusion, this finding agreed with the report of the folklore findings on the poly-herbal aqueous extract with hepato-protective effect.

Keywords: Hepato-Safety, Poly-Herbal, Aqueous Extract, Animal Model

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INTRODUCTION

Zingiber officinale, commonly known as ginger, is a flowering plant that has a rich history of medicinal use spanning over 2000 years and is renowned for its diverse range of biological activities. Ginger is widely utilized as a spice, flavoring agent, and herbal remedy in various cuisines and beverages (Dhanik et al., 2017). *Zingiber officinale* has been traditionally utilized in various medicinal systems such as Ayurveda, Siddha, Chinese, Arabian, African, and Caribbean, employed to treat a wide range of ailments including nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion, and pain (Grzanna et al., 2005).

Annona muricata is an edible fruit that has been widely studied over the last decade for its therapeutic potential as well as its medicinal benefits (Moghadamtousi et al., 2014; Naik et al., 2020; George et al., 2014). It is recognized in different countries with different names such as in Nigeria, known called soursop or graviola (Boulogne et al., 2011) and in the West Indies as apple aqueous, kowoso, or soursop (Gómez-Estrada *et al.,* 2011). In traditional medicine, soursop fruit juice and infusions of its leaves or branches have been used to treat fever, act as a sedative, alleviate respiratory illnesses, combat malaria, address gastrointestinal problems, and manage liver, heart, and kidney issues (Magaa et al., 2010; Vandebroek et al., 2010; Waizel and Waizel, 2009; Boyom et al*.,* 2011; Atawodi, 2011).

Andrographis paniculata, commonly known as Kalmegh or "King of Bitters," is a medicinal plant that is abundant in the southern part of India. It is an annual plant and holds a prominent place in traditional systems of medicine like Unani and Ayurveda. It has been discovered to be traditionally useful for the treatment of colds, fever, laryngitis, and several infectious diseases ranging from malaria to dysentery and diarrhea in China, India, and other Southeast Asian countries. The plant is claimed to possess immunological, antibacterial, anti-inflammatory, antithrombotic, and hepato-protective properties (Nagalekshmi et al., 2011). The objectives of this study evaluate the impact of the poly-herbal aqueous extract on liver function parameters in Wistar rats and investigate the histopathological changes in liver tissues in rats.

MATERIALS AND METHODS

Plant Collection and Identification

The three chosen plants, *Annona muricata, Andrographis paniculata,* and *Zingiber officinale*, were sourced from Benin City, Edo State, Nigeria. Dr. Timothy from the Department of Plant Biology and Biotechnology at the University of Benin, Benin City, Edo State, was responsible for their identification. The voucher number (UBH-Z094; UBH-A411; UBH-A419) was issued.

Plant Preparation

The leaves of *Annona muricata* and *Andrographis paniculata* were washed and dried under shade for two weeks (14 days). The rhizomes of *Zingiber officinale* were also washed and sliced to facilitate quicker drying, after which they were dried under shade for the same duration of time (14 days). After proper drying, all plant samples were subjected to oven drying at 45℃ for 30 minutes.

Once the samples were completely dried, they were finely ground using an industrial blender. The powdered samples each weighing 300g were combined in a 1:1:1 ratio to create a poly-herbal mixture. This mixture was then subjected to a hot water maceration technique using distilled water, with intermittent stirring and shaking, for 72 hours.

The macerated poly-herbal samples were filtered and the filtrate was concentrated using crucibles in a water bath to concentrate into a semi-solid. Percentage yield was calculated with the formula ($\% \text{ Yield} = \frac{\text{Extract weight}}{\text{Powder sample weight}} \times 100$)

Experimental Animals

Twenty (20) adult Wistar rats, of male and female sexes, weighing between 200-250 g, were acquired from the Department of Pharmacology, Faculty of Pharmacy. The animals were accommodated in the Department of Biochemistry, with free access to food and water *ad libitum*. They were exposed to a 12-hour light-dark cycle. The animals were allowed to acclimatize for 14 days. Ethical considerations were strictly followed, adhering to the procedures approved by the ethical committee with the assigned ethical number LS21511

Experimental Protocol

The hepato-safety evaluation of the poly-herbal aqueous extract derived from was conducted on rats, focusing on hepatic parameters. A total of 20 adult male and female

Wistar rats were randomly divided into four (4) groups, containing five (5) rats (n = 5). Group 1 rats received distilled water (negative control), group 2 rats were orally administered 200 mg/kg of the poly-herbal extract, group 3 rats received 400 mg/kg of the poly-herbal extract, and group 4 rats were orally administered 800 mg/kg of the poly-herbal extract daily for 28 days.

Hepatic Function Test Procedure

Serum Alanine Aminotransferase (ALT) Determination

The serum alanine aminotransferase was determined using the method described by Reitman and Frankel (1957), and Magili and Bwatanglang (2018). The enzyme activity was measured by monitoring the concentration of pyruvate hydrazone formed through the reaction between α-oxoglutarate, L-alanine, and ALT at 540 nm and 37℃. A standard calibration table is provided in the manual of Randox Lab. Ltd, UK Reagent Kit was used to determine ALT concentration (U/L).

Serum Aspartate Aminotransferase (AST) Determination

The serum aspartate aminotransferase was determined using the end-point technique described by Reitman and Frankel (1957), and Magili and Bwatanglang (2018). The enzyme activity was measured by monitoring the concentration of oxaloacetate hydrazone formed through the reaction between α-oxoglutarate, L-aspartate, and AST at 540 nm and 37℃. AST concentration (U/L) was determined using the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

Serum Alkaline Phosphatase (ALP) Determination

The alkaline phosphatase activity in the serum was determined using the method described by Bassey et al. (1946) as modified by Magili and Bwatanglang (2018) with Randox kits. The enzyme activity was measured by monitoring the concentration of phosphate hydrazone formed through the reaction between para-nitrophenyl phosphate and ALP at 405 nm and 37℃.

Serum Total Bilirubin and Unconjugated Bilirubin Determination

Total bilirubin was determined using the acid diazo technique as illustrated by Doumas et al. (1973) with assay kits from Randox Laboratories Ltd. Unconjugated bilirubin levels were

calculated based on the differences between total bilirubin absorbance and direct bilirubin absorbance.

Serum Albumin Determination

Serum albumin was determined using the method of Doumas et al. (1971) with a Randox assay kit. The absorption spectra of bromocresol green (BCG) dye were altered in the presence of albumin, producing a blue-green color that is directly proportional to the albumin concentration.

Serum Total Protein Determination

Total protein levels were colorimetrically evaluated using a Randox assay kit based on the method described by Doumas *et al.* (1971).The absorbance at 540 nm was measured due to the formation of a colored complex, which is directly proportional to the concentration of protein.

Data Analysis

Results were analyzed with Graph pad Prism version 6. Data were presented as Mean ± S.E.M and statistical significance was calculated using one-way ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

RESULTS

The administration of the extract had different effects on the enzyme volume depending on the dosage. Animals given doses of 200 and 400 mg/kg experienced a reduction in enzyme volume, while those administered with 800 mg/kg showed an increase. These effects were compared to the control group of animals (Table 4.1).

Table 1: Effects of poly-herbal aqueous extract on Alkaline phosphatase (ALP) in Wistar rats

Groups	Doses (mg/kg)	Alkaline phosphatase (IU/L)
Control	0.5 ml	443.70 ± 9.39^{a}
PAE	200	159.70 ± 4.72^{a}
PAE	400	308.00 ± 8.58^{a}
PAE	800	629.70 ± 21.50^{b}

P -value > 0.05, all superscript 'a' are not statistically significant. **Key:** PAE (poly-herbal aqueous extract)

An increase in the volume of the enzyme in the different doses as compared with the control group. At 200 and 400 mg/kg, the increase was slightly different from the control, while the 800 mg/kg group showed a rather significant increase when compared with the control (Figure 1).

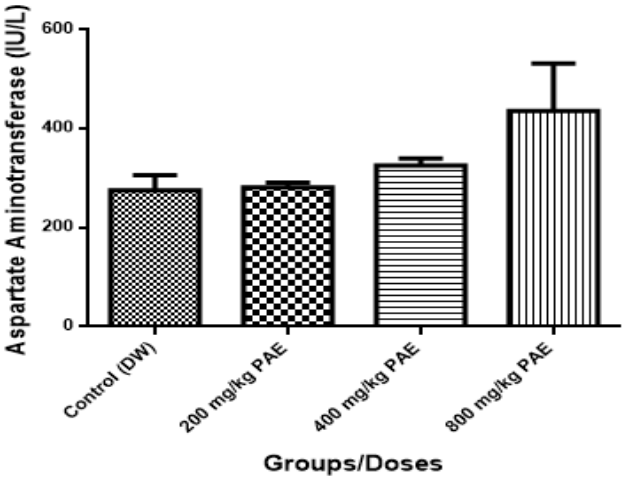


Figure.1: Effects of poly-herbal aqueous extract on aspartate aminotransferase (AST) in Wistar rats.

Key: PAE (poly-herbal aqueous extract)

A decrease in the volume of the enzyme across the different doses when compared with the control group (Table 2)

Table 2: Effects of poly-herbal aqueous extract on alanine transaminase (ALT) in Wistar rats

Groups	**Doses (mg/kg)**	**Alanine transaminase (IU/L) Mean ± SEM**
Control	0.5 ml	136.00 ± 10.82[a]
PAE	200	117.30 ± 6.87[a]
PAE	400	98.33 ± 7.25[b]
PAE	800	113.70 ± 22.00[a]

P -value > 0.05, all superscripts 'a' are not statistically significant. **Key:** PAE (poly-herbal aqueous extract)

A significant decrease in the total bilirubin level across all doses when compared with the control (Figure 2).

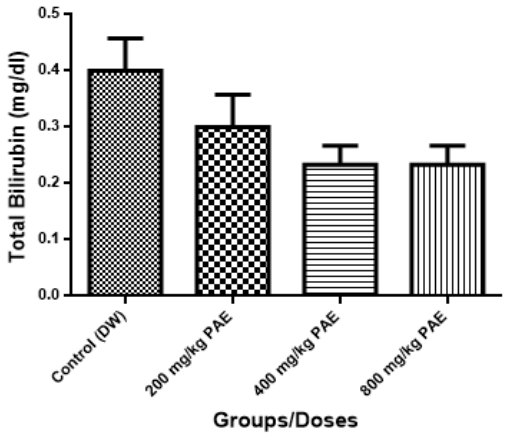


Figure 2: Effects of poly-herbal aqueous extract on total bilirubin (TB) in Wistar rats.

Key: PAE (poly-herbal aqueous extract)

A slight increase in the conjugated bilirubin level across all doses when compared with the control (Table 3).

Table 3: Effects of poly-herbal aqueous extract on conjugated bilirubin (CB) in Wistar rats

Groups	Doses (mg/kg)	Conjugated bilirubin (mg/dl)
Control	0.5 ml	$0.20.00 \pm 0.0^{a}$
PAE	200	0.13 ± 0.03^{a}
PAE	400	0.10 ± 0.00^{b}
PAE	800	0.10 ± 0.00^{b}

P -value > 0.05, all superscript 'a' are not statistically significant. **Key:** PAE (poly-herbal aqueous extract)

A slight increase in the total protein level across all doses when compared with the control (Figure 3).

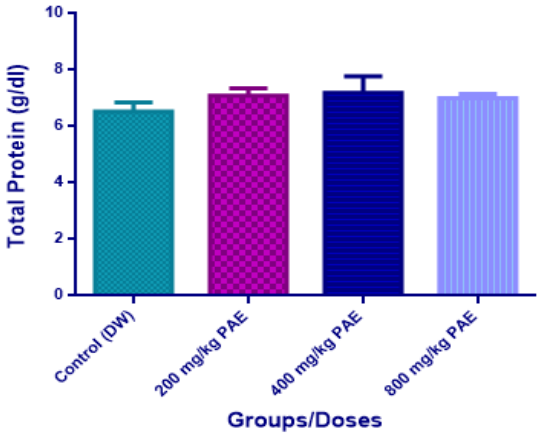


Figure 3: Effects of poly-herbal aqueous extract on total protein in Wistar rats.

Key: PAE (poly-herbal aqueous extract)

A slight decrease in the Albumin level across the various doses when compared with the control. Although the decrease was not statistically significant ($p > 0.05$) (Table 4)

Table 4: Effects of poly-herbal aqueous extract on albumin (ALB) in Wistar rats

Groups	Doses (mg/kg)	Albumin (g/dl)
Control	0.5 ml	3.53 ± 0.09^a
PAE	200	3.10 ± 0.04^a
PAE	400	3.13 ± 0.02^a
PAE	800	3.03 ± 0.04^a

P -value > 0.05, all superscript a are not statistically significant. **Key:** PAE (poly-herbal aqueous extract)

At various doses resulted in a rise in the volume of globulin within the animal as compared to the control (Figure 4).

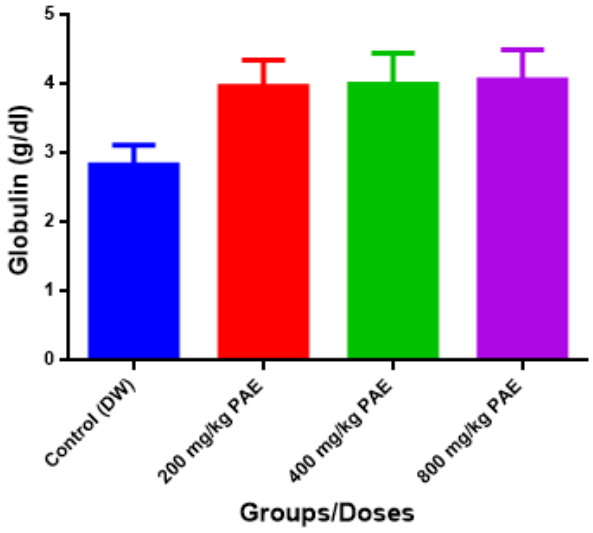


Figure 4: Effects of poly-herbal aqueous extract on globulin (GLO) in Wistar rats. **Key:** PAE (poly-herbal aqueous extract)

DISCUSSION

The liver is equipped with varieties of enzymes, including; AST, ALP, and ALT. These enzymes play a crucial role in maintaining the liver's functional state (Morosetti et al., 2017). Hepatotoxic drugs induce harm to the liver cell membrane, the functional enzymes are released into the bloodstream, leading to elevated levels in the serum, further highlighting the extent of the damage (Kumar et al., 2014; Arablou et al., 2014).

The result of the present study showed that the poly-herbal aqueous extract caused a significant decrease in Alkaline phosphatase (ALP) specifically at 200 and 400 mg/kg of the extract, with a slight at 800 mg/kg, compared with the control. This is similar to the results obtained from Alanine Transaminase (ALT) with a significant decrease across the graded doses of the extract when compared with the control. (Tables 4.1 and 4.2) (Aslam et al., 2016). This finding agrees with the report of Coria-Téllez et al. (2018). Their result showed that the pretreatment of rats with herbal formulation at a dose of 100 and 200 mg/kg b.w for 5 days resulted in a significant ($P<0.01$) decrease in the level of ALT, AST, and ALP (Akimoto et al., 2015).

The observed reduction in the liver enzymes indicated a potential stabilization of the hepatic cellular function. This could be implicated in the modulation of oxidative stress, inflammation, and cellular apoptosis, which are common mechanisms associated with liver injury (Helfer et al., 2014). Total

bilirubin (TB), Total protein, and albumin assays can be used as a consistent and responsive marker of liver statutory function. The results obtained from the Total bilirubin and conjugated bilirubin showed a significant decrease in their levels across graded doses of the extract compared with the control, similar to the work of Khandouzi *et al.* (2015) whose results showed that the pretreatment of rats with herbal formulation at a dose of 100 and 200 mg/kg b.w for 5 days resulted in significant ($p < 0.01$) decrease in the level of TB (Nazim et al., 2015; Ngo et al., 2014). The results revealed that hepato-protective herbal formulation was potent and found to be equipotent with the standard. The obtained results of the total protein and albumin levels had a slight increase, respectively (Figure 4.3 and Table 4.4) when compared with the untreated group. A decrease in albumin levels could be an indication of liver dysfunction or damage this agreed with the work of Radji et al. (2015) whose results showed that the pretreatment of rats with herbal formulation at a dose of 100 and 200 mg/kg b.w for 5 days resulted in significant ($P<0.01$) decrease in the level of albumin (Rajasekaran et al., 2015).

CONCLUSION

This study offers proof of the potential of a combination of *Andrographis paniculata, Zingiber officinale,* and *Annona muricata* as a hepato-protective agent. The observed effects on the liver enzymes and histopathological changes support the folklore reports as a potent hepatoprotective effect. Further research, including clinical studies, is necessary to validate these findings and explore their application to human health.

Recommendation

Further findings as regards hepatoprotective effect in novel drug development. The plant should be considered a traditional drug for health remedy. The extract elicited therapeutic effect boosting liver cells

Acknowledgment

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Competing Interests

Authors have declared that no competing interests exist.

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