

## Acetaminophen-induced Liver Injury: Protective Effect of *Laportea aestuans* Aqueous Leaf Extract in Experimental Mice Model

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### Abstract

Acetaminophen commonly referred to as paracetamol is an analgesic and antipyretic drug commonly available as over the counter medications. Indiscriminate use of acetaminophen for management of pain and fever causes liver damage. The study evaluated the hepatoprotective effect of the *Laportea aestuans* aqueous leaf extract in acetaminophen-treated mice. Group 1: control, Group 2: APAP treated, Group 3, 4, 5, 6 and 7 received 25, 50, 100, 200 and 400 mg/kg bw of the extract for 7 days and then treated with 300 mg/kg bw APAP respectively, Group 8: silymarin group. Biochemical parameters were analyzed. Activities of liver function marker enzymes (gamma-glutamyl transferase, alkaline phosphatase, lactate and glutamate dehydrogenases, aspartate, and alanine aminotransferases) were significantly ( $p < 0.05$ ) lower in the serum of acetaminophen-treated mice pre-administered with the *Laportea aestuans* aqueous leaf extract when compared to the acetaminophen-treated control mice. Activities of antioxidant enzymes significantly ( $p < 0.05$ ) increased in groups pre-administered with the extract or silymarin. Histological micrographs also showed that the hepatic architectures of the pre-administered mice were maintained following treatment with APAP. Pre-administration of 100 mg/kg bw of *Laportea aestuans* aqueous leaf extract gave the best protective effect against APAP-induced hepatotoxicity. Findings from this study showed that *L. aestuans* leaf extract evidently protects mice against acetaminophen-induced liver injury, exhibiting antioxidant and hepatoprotective properties. This study supports the ethnobotanical use of *L. aestuans* for the prevention and treatment of liver diseases.

**Keywords:** Acetaminophen, antioxidant, hepatotoxicity, *Laportea aestuans*, liver injury

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### Introduction

Globally, chronic hepatic diseases including liver cirrhosis and drug-induced liver injury (DILI) are one of the leading causes of death (Ding & Yang, 2019). Approximately 2 million deaths per year are attributed to liver diseases, with over 1 million due to complications of cirrhosis (Asrani et al., 2019). The prevalence of liver disease is increasing in developing countries of sub-Saharan Africa. In Nigeria, liver disease is also one of the major causes of death (Obiajulu et al., 2019) and it is mainly due to overdose of analgesic drugs, in particular acetaminophen (also known as paracetamol, N-acetyl-para-aminophenol, APAP) and recently tramadol as

well as excess consumption of alcohol, infections such as HBV and autoimmune disorders (Licata et al., 2021). Liver injury because of acetaminophen, acute or cumulative overdose has prospect to progress to liver failure. DILI accounts for over 50 % of liver failure and about 39 % of DILI are due to acetaminophen (Satishchandran et al., 2018). Asymptomatic and slight biochemical abnormalities to severe hepatitis with jaundice are indices of DILI (Suk et al., 2012).

Myriads of drugs from antibiotics to diuretics are available for the management of liver diseases but many of these drugs produce some undesirable side effects and some are not

affordable to majority of the populace; these necessitated the need to explore alternative therapy for treatment in low-income countries. Plants such as milk thistle (*Silybum marianum*) are well known for the treatment of liver diseases. Extracts from *S. marianum* popularly referred to silymarin and its bioactive constituent, silybin have been scientifically proven for treatment of liver diseases with detailed review by Federico et al. (2017). Treatment of liver ailments and other infections using plant-derived medication and their by-products are remedy from natural sources (Bafor et al., 2018). In Nigeria, extract of *Laportea aestuans* is prescribed by traditional medicine practitioners for prevention and treatment of liver-related diseases. *Laportea aestuans* is an annual shrub that is native to sub-Saharan Africa and belongs to the family Urticaceae. It is usually grown on waste land and known in Nigerian local parlances such as "fiyafiya" by the Yorubas in the Southwest region and "bulsum fage" by the Hausas in the North of the country (Olufunke et al., 2008). Previous studies have demonstrated the antimicrobial, antioxidant, and gastro-protective potentials of extracts from *L. aestuans* (Oloyede and Oyelola, 2013; Christensen et al., 2015). Thus, this study was primarily designed to evaluate the protective properties of aqueous extract of *Laportea aestuans* leaf against acetaminophen-induced hepatotoxicity in mice.

## Materials and Methods

### *Collection of plant materials*

*L. aestuans* leaves (LAL) were harvested on January 25, 2015, from the premises of University of Ilorin, Ilorin, Nigeria and authenticated at the herbarium unit of the Department of Plant Biology University of Ilorin, Nigeria (Voucher number UILH/001/1225).

### *Preparation of aqueous extract of Laportea aestuans leaf*

The plant leaves were harvested and washed in running tap water at the collection site to remove soil particles and further washed with distilled water in the laboratory. The leaves were air-dried and milled by means of a blender. The pulverized leaf sample (1 kg) was extracted exhaustively by maceration with distilled water (1:10 kg/L). The aqueous extract was freeze-dried, labeled appropriately as LAL extract and stored under refrigeration (-4 °C) until required for further analysis. Percentage yield of the aqueous extract of LAL

was also calculated prior to storage. Phytochemical analysis of the plant extracts was carried out using standard protocols (Adeniyi et al., 2009).

### *Experimental animals*

Thirty mice (male) weighing  $27.51 \pm 1.25$  g were obtained from Institute of Advanced Medical Research and Training (IAMRAT) unit of University College Hospital, Ibadan, Nigeria. The mice were acclimatized for a week in well ventilated cage at the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria, allowed to have access to standard pellet chow and water *ad libitum* and subjected to laboratory conditions of  $25 \pm 2$  °C; photoperiod of  $12 \pm 2$  hours light:  $12 \pm 2$  hours dark cycle throughout the period of the experiment.

### *Ethical clearance*

The research study follows strictly and conforms to the Principles of Laboratory Animal Care (NIH Book No. 85-23). University of Ilorin Ethical Review Committee approved the study with approval number UERC/ASN/2015/119

### *Animal grouping and extract administration*

Preliminary dose dependent study on aqueous leaf extract of *Laportea aestuans* by varying doses of 25, 50, 100, 200, 400 mg/kg bw of LAL extract on acetaminophen-induced mice was carried out to screen the LAL extract with best doses for hepatoprotective effect using alanine aminotransferase, gamma glutamyl transferase and histopathological indices as selected markers. For subsequent studies, thirty (30) male mice were randomly grouped into five and pre-administered orally with either 1 % dimethyl sulphoxide (DMSO) dissolved in normal saline, the LAL extract at 50 and 100 mg/kg bw or the reference drug, silymarin at 50 mg/kg bw once a day for 7 days. Acetaminophen (300 mg/kg bw) was administered after the 7th day of extract administration to all the mice except those in the group served as (non-induced) control.

### *Serum and tissue preparation*

The mice were sacrificed 6 hours after administration of acetaminophen following anaesthetization with diethyl ether. Blood were sampled and centrifuged to collect the serum. The serum supernatant was used within 24 h for analysis. The liver was immediately excised, blotted and small piece from the smaller quadrate lobe was stored in formalin solution for histological analysis. The livers were homogenized in ice-cold 0.25 M sucrose

solution (1:5 w/v) and centrifuged, the resulting supernatant was used for liver function and antioxidant enzyme assays.

#### Biochemical analysis

Activities of alanine (ALT) and aspartate aminotransferases (AST), glutamate (GDH), lactate (LDH) and glucose-6-phosphate dehydrogenases (G6PDH), gamma glutamyl (GGT) and glutathione transferases (GST), alkaline phosphatase (ALP), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were using assays kits from Randox Laboratories (Antrim, UK) and other reagents.

#### Statistical analysis

The results were expressed as means  $\pm$  SEM of six replicates. Data were computed on Graph

Pad Prism 6 software and statistically analysed by means of one-way analysis of variance (ANOVA) monitored by Tukey' multiple comparisons test. Differences across group means were considered to be least significant at  $p < 0.05$

#### Results

##### Yield and phytochemicals in LAL extract

The aqueous extraction of *L. aestuans* leaf afforded 116 mg/g of freeze dried LAL extract. Table 1 shows phytochemicals found in LAL extract. The extract had high concentration of flavonoids (2.45 mg/g) and other classes of phytochemicals quantified in appreciable quantity were tannins > alkaloids > phenolics > saponins > glycoside.

**Table 1:** Composition of Secondary Metabolites in Aqueous Extract of *Laportea aestuans* Leaf

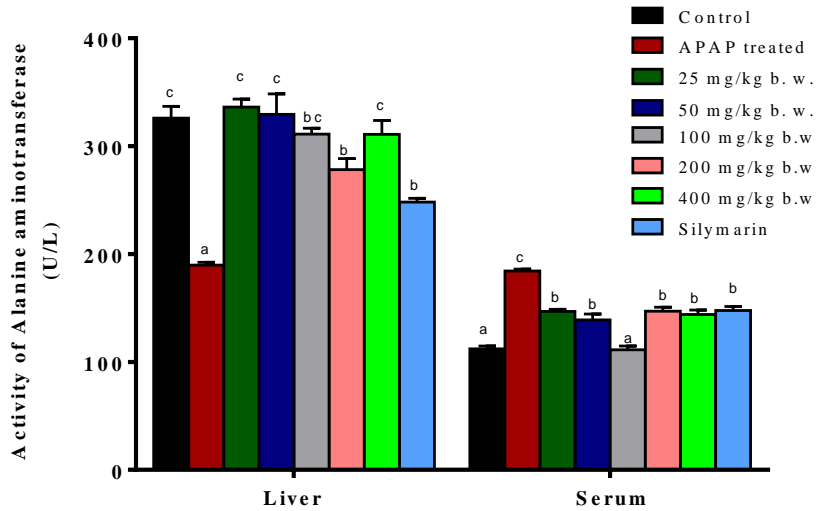
Secondary Metabolites	Composition
Alkaloids (mg/g)	0.720 $\pm$ 0.01
Saponins (mg/kg)	127 $\pm$ 0.00
Flavonoids (mg/g)	2.450 $\pm$ 0.05
Phenolics (mg/100g)	49.860 $\pm$ 0.00
Tannins (mg/g)	0.994 $\pm$ 0.50
Glycoside (mg/g)	0.003 $\pm$ 0.00
Steroids	ND
Anthocyanins	ND

ND: Not detected

#### Effects of LAL extract on liver function marker enzymes

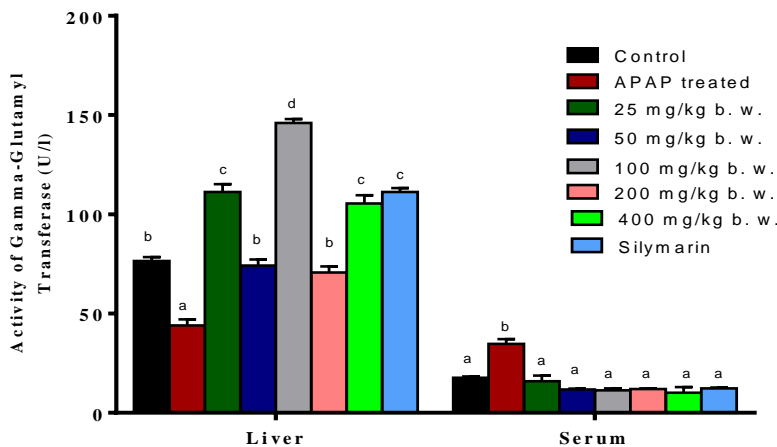
Activities of ALT and GGT in the liver of mice treated with acetaminophen were significantly ( $p < 0.0001$ ) decreased, whereas activities of the enzymes in the serum were significantly ( $p < 0.0001$ ) increased when compared to the non-induced control group as depicted in Figure 1 and 2. Pre-administration of 25 - 400 mg/kg bw of LAL aqueous extract significantly ( $p < 0.001$ ) increased the activity of both ALT and GGT in the liver with concomitant significant

decrease ( $p < 0.0001$ ) in the serum when compared with the acetaminophen-treated group. Mice pre-administered 100 mg/kg b. w. of the extract exhibited significant ( $p < 0.001$ ) decrease in serum activity of ALT than other treated groups and values obtained in 100 mg/kg treated group were not significantly different from the non-induced control group. Strikingly, the activity in mice pre-treated with 100 mg/Kg bw for liver GGT (146 U/l) was almost twice the range of the non-induced control (76 U/l).



**Figure 1:** Activity of alanine aminotransferase in the liver and serum of APAP-treated mice pre-administered with aqueous extract of *Laportea aestuans* leaf for 7 days

Values are means of six replicates  $\pm$  SEM; Bars values with different superscript letters are significantly different from each other.



**Figure 2:** Activity of gamma-glutamyl transferase (GGT) in the liver and serum of APAP-treated mice pre-administered with aqueous extract of *Laportea aestuans* leaf for 7 days

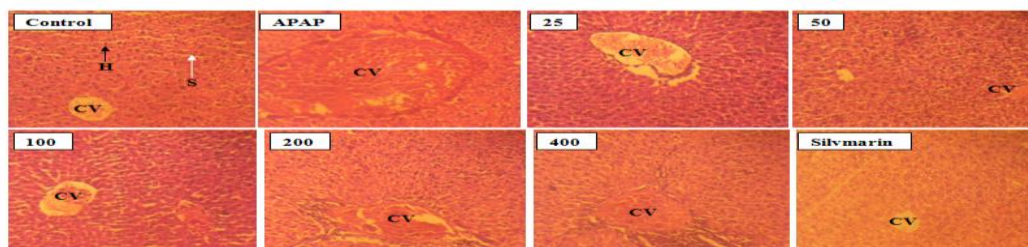
Values are means of six replicates  $\pm$  SEM; bar values with different superscript letters are significantly different from each other.

#### Effect of LAL extract on liver histology

Photomicrograph (x100) of the liver of mice that served as the non-induced control showed normal architecture with the central vein surrounded by large number of hepatocytes with well dilated sinusoids (Figure 3). Photomicrograph of the liver of APAP-treated mice at the same magnification (x100) showed severe degeneration, congested central veins and sinusoids coupled with hepatocyte depletion. Similarly, APAP-treated mice pre-

administered 25 mg/kg b. w aqueous extract *L. aestuans* leaf showed distorted central vein with depletion in number of hepatocyte. However, those pre administered with 50 and 100 mg/Kg b.w. of the aqueous extract showed improvement in the central vein morphology and hepatocyte with no congestion of the sinusoid. Mice pre-administered higher concentration of the aqueous extract (200 and 400 mg/kg b.w.) showed mild hepatocyte degeneration with distorted to severe central vein congestion. The liver morphology of the

reference drug (silymarin) treated mice showed normal architecture of central vein, sinusoid and hepatocyte.



**Figure 3:** Photomicrograph (x100, Haematoxylin and Eosin) of the liver of APAP-treated mice pre-administered with 25– 400 mg/kg b.w. aqueous leaf extract of *Laportea aestuans* for 7 days

CV= central vein; H = Hepatovacoules; S = Sinusoids

*Effect of LAL extract on activities of other marker enzymes of liver function*

Activities of AST and ALP significantly ( $p < 0.0001$ ) decreased in the liver and increased in the serum of APAP-treated mice when compared to the non-induced control group (Table 2).

In mice pre-administered 50 and 100 mg/kg bw of LAL extract, activities of AST and ALP significantly ( $p < 0.0001$ ) decreased in the

serum, whereas activities of both enzymes significantly ( $p < 0.05$ ) increased in the liver, when compared with the APAP-treated group pre-administered only the vehicle. It is noteworthy to mention that mice pre-administered 50 and 100 mg/kg bw of the extract recorded significant ( $p < 0.0001$ ) decrease in activity of ALP in the serum than the silymarin treated group or the non-induced control groups.

**Table 2:** Activities of aspartate aminotransferase and alkaline phosphatase in the liver and serum of APAP-treated mice pre-administered with aqueous extract of *Laportea aestuans* leaf for 7 days

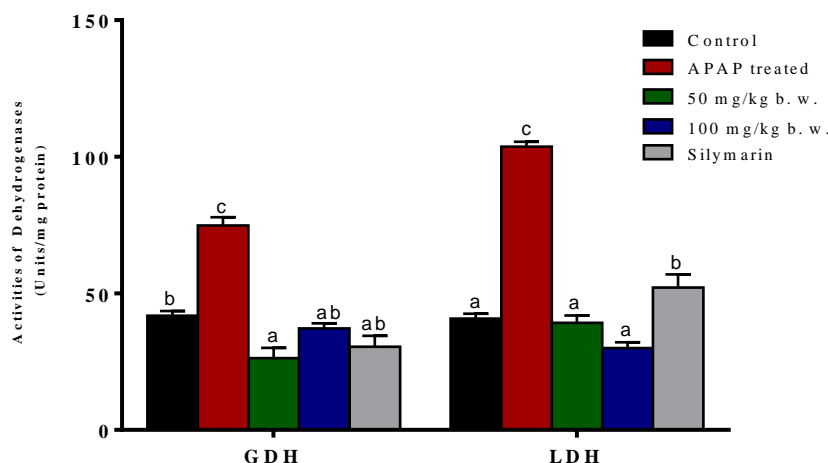
Groups	AST (U/I)		ALP (nM/min/mg protein)	
	Liver	Serum	Liver	Serum
<b>Control</b>	264.75 ± 14.96 <sup>d</sup>	111.25 ± 1.61 <sup>b</sup>	34.38 ± 0.54 <sup>a</sup>	29.03 ± 0.39 <sup>b</sup>
<b>APAP-treated</b>	141.75 ± 3.95 <sup>a</sup>	168.13 ± 8.32 <sup>c</sup>	25.73 ± 1.06 <sup>d</sup>	39.25 ± 0.40 <sup>c</sup>
<b>50mg/kg b. w.</b>	162.75 ± 9.13 <sup>ab</sup>	83.75 ± 2.39 <sup>a</sup>	40.04 ± 1.45 <sup>b</sup>	25.12 ± 0.48 <sup>a</sup>
<b>100mg/kg b. w.</b>	207.00 ± 3.52 <sup>c</sup>	109.38 ± 4.83 <sup>b</sup>	46.16 ± 1.13 <sup>c</sup>	24.35 ± 0.71 <sup>a</sup>
<b>Silymarin</b>	183.00 ± 5.44 <sup>bc</sup>	121.25 ± 6.25 <sup>b</sup>	38.57 ± 0.46 <sup>ab</sup>	28.40 ± 0.18 <sup>b</sup>

Values are means of six replicates ± SEM and those with different superscript letters are significantly different down the column ( $p < 0.05$ )

*Effect of LAL extract on activities of GDH and LDH*

There was significant ( $p < 0.0001$ ) increase in activities of GDH and LDH in the serum of APAP-treated mice when compared with the control (Figure 4). Pre-administration of 50 and 100 mg/kg b. w. of the LAL extract significantly ( $p$

$< 0.0001$ ) decreased the activities of GDH and LDH in the serum compared with the APAP-treated mice. Mice pre-administered both doses of the LAL extract recorded significant ( $p < 0.05$ ) decrease in activity of LDH when compared with the silymarin group but values were not significantly different from the non-induced control group.



**Figure 4:** Activities of glutamate and lactate dehydrogenases in the serum of APAP-treated mice pre-administered with aqueous extract of *Laportea aestuans* leaf for 7 days

Values are means of six replicates ± SEM; Bars with different superscript letters are significantly different (p < 0.05)

*Effects of LAL on activities of antioxidant enzymes*

There was significant (p < 0.0001) decrease in the concentration of protein and activities of antioxidant enzymes (G6PDH, GST, CAT, SOD, GPx and GR) in the liver of APAP-treated mice when compared with the non-induced control (Table 3). Pre-administration of 50 and 100 mg/kg b.w. of the extract significantly (p <

0.001) increased the concentration of the proteins and activities of G6PDH, GST, CAT, SOD, GPx and GR compared with the APAP-treated mice. Mice pre-administered 50 mg/kg bw of the extract recorded the highest increase in activity of G6PDH and those treated with 100 mg/Kg bw had the highest activity of GPx, while activities of CAT and SOD was highest in the group pre-administered with silymarin group.

**Table 3:** Concentration of proteins and activities of antioxidant enzymes in the liver of APAP-treated mice pre-administered with Aqueous Extract of *Laportea aestuans* Leaf for 7 Days

Groups	Protein (g/l)	G-6-PDH (nm/min)	GST (nM/mg protein)	CAT (nmol/min/mg protein)	SOD (nmol/min/mg protein)	GPx (nmol/min/mg protein)	GR (nmol/min/mg protein)
Control	311.79 ± 4.87 <sup>c</sup>	51.71 ± 1.63 <sup>b</sup>	257.8 ± 7.81 <sup>c</sup>	1806.31 ± 2.15 <sup>b</sup>	1712.75 ± 32.50 <sup>c</sup>	103.00 ± 3.00 <sup>c</sup>	367.00 ± 14.00 <sup>a</sup>
APAP-treated	259.82 ± 1.83 <sup>a</sup>	29.01 ± 1.63 <sup>a</sup>	70.31 ± 7.81 <sup>a</sup>	1762.90 ± 11.14 <sup>a</sup>	1196.98 ± 31.03 <sup>b</sup>	50.00 ± 3.00 <sup>a</sup>	151.00 ± 13.00 <sup>c</sup>
50 mg/kg LAL + APAP	297.05 ± 3.17 <sup>bc</sup>	153.90 ± 8.80 <sup>d</sup>	171.90 ± 15.62 <sup>b</sup>	1898.13 ± 9.39 <sup>c</sup>	1575.49 ± 17.38 <sup>b</sup>	70.00 ± 7.00 <sup>ab</sup>	289.00 ± 50.00 <sup>b</sup>
100 mg/kg LAL + APAP	310.98 ± 5.29 <sup>c</sup>	127.41 ± 1.63 <sup>c</sup>	148.40 ± 14.96 <sup>b</sup>	1817.19 ± 13.52 <sup>b</sup>	1537.08 ± 11.14 <sup>c</sup>	105.00 ± 7.00 <sup>c</sup>	293.00 ± 30.00 <sup>b</sup>
Silymarin + APAP	289.05 ± 3.10 <sup>b</sup>	59.29 ± 3.92 <sup>b</sup>	179.70 ± 7.81 <sup>b</sup>	2033.40 ± 8.26 <sup>d</sup>	1787.24 ± 32.50 <sup>b</sup>	84.00 ± 5.00 <sup>bc</sup>	306.00 ± 50.00 <sup>b</sup>

Values are means of six replicates ± SEM and those with different superscripts are significantly different across the column at p<0.05

## Discussion

This study demonstrated protective effect of *Laportea aestuans* leaf extract in acetaminophen induced liver injury in mice. Increase in the serum alanine aminotransferase activity in acetaminophen-treated mice observed in this study were similar to previously reported results by Watkins et al. (2006); Jaeschke et al. (2012); Owumi et al. (2015). Also, Owumi et al. (2015) reported increase in the activities with respect to GGT, ALP and LDH. The consequence of the alteration in the activities of liver enzymes, particularly ALT, its release into the serum may be evidence of a key process of hepatocyte necrosis following cellular swelling and inflammation as earlier reported by Jaeschke et al. (2012).

Similarly, in this study, increase in serum activities of ALT by 40%, AST by 34%, GGT by 50%, ALP by 26%, LDH by 60 %, GDH by 44% were observed in APAP-treated mice when compared to the report of Owumi et al. (2015). Sensitive indicators of hepatocellular damage and dysfunction are increased activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) (Kaplan 1993; Adedara and Faronmbi, 2010). This study revealed that indicators of hepatic damage were higher in the serum of APAP-treated mice than the control which was similar to the report of Qureshi (2016). The interaction between APAP and the liver cell membranes could result in structural damage and leakage of these marker enzymes into blood circulation in the APAP-treated mice. In addition, glutamate dehydrogenase is an injury-dependent and specific biomarker of mitochondria damage and dysfunction (McGill and Jaeschke, 2014). Serum glutamate dehydrogenase activity increases with hepatocellular damage (Giffen et al., 2003). Likewise, lactate dehydrogenase is an established cytoplasmic enzyme that is present in all cells and rapidly released into blood circulation when the plasma membranes of cells are injured (Mubarak et al., 2018).

Silymarin is the utmost clinically common medicine for patients and is known to have hepatotherapeutic properties out of the drugs used in the management of liver damage (Kim et al., 2013). However, many authors consider it to have low bioavailability (Giacomelli et al., 2002). Pre-administration of aqueous extract of *Laportea aestuans* leaf before APAP-treatment was aimed at protecting the hepatocytes so as to prevent degeneration of

parenchymal cells, thus protecting against membrane fragility and preventing the leakage of marker enzymes into the circulation. Protection was achieved because the extract at 100 mg/kg brought about decrease in the activities of ALT by 40%, AST by 34%, GGT by 68%, LDH by 71% and GDH by 50%; this might be due to the presence of flavonoid in the extract as reported by Vertuani et al. (2004).

Furthermore, the histopathological alteration with respect to the liver of APAP-treated mice was prevented by pre-administration with aqueous extract of *Laportea aestuans* leaf at 100 mg/kg, which was remarkably indicated by the absence of degeneration, congestion and hepatovacuolar depletion. Alteration in the normal hepatic physiology is prevented based on the effectiveness of a hepatoprotective medication (Kyung et al., 2018). Husain et al. (1998) reported that congestion of the central vein might be attributed to the direct irritant effect of drugs, acetaminophen inclusive on the wall of the blood vessels. Likewise, Halliwell and Gutteridge (1999) reported that degeneration of the hepatocytes is attributed to reactive oxygen species. Ability of the aqueous extract of *Laportea aestuans* leaf to preserve the liver histopathology might be due to redox properties of the phenolic compounds present in extract which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Parr and Bolwell, 2000) for the exhibited protection.

Antioxidant defense systems both enzymatic (SOD, CAT, GPx, GPr, GST) and non-enzymatic antioxidants are known to scavenge free radicals and prevent damage due to oxidation (Adedara and Farombi, 2010). The decrease in activities of G6PD observed in APAP-treated mice was prevented by pre-administration of aqueous extract and might suggest that the extract could help in the prevention of depletion of NADP<sup>+</sup> that may have caused by acetaminophen metabolism. Reduction in the activity of glutathione-S-transferase of APAP-treated mice was prevented upon pre-administration of the aqueous extract of *Laportea aestuans* leaf; this might be due to the antioxidant property of secondary metabolites especially flavonoid in the extract which may be enough to conjugate N-acetyl-p-benzoquinoneimine (NAPQI) to form soluble glutathione-NAPQI conjugate expelled out of the body.

The principal line of defense against free radicals is Superoxide dismutase (SOD) and catalyzes the reductive dismutation of superoxide anion radical into hydrogen

peroxide, which is acted upon by catalase (CAT) or glutathione peroxidase (GPx) and converted into water and molecular oxygen (Halliwell, 2007). Increase in the activities of activities of antioxidant namely SOD, CAT, GPx and glutathione reductase (GR) as a result of pre-administration of aqueous extract of *Laportea aestuans* leaf may be attributed to the flavonoids identified in the extracts which prevented the oxidative assault causing clear decrease in the activities of these enzymes in the liver of acetaminophen-treated mice.

Presence of secondary metabolites in various plant extracts has been and continue to be the source of remedy for the treatment and management of various diseases like diabetes, liver diseases, asthma among others (Bhandary et al., 2012). Flavonoid which was identified to be predominant among the metabolites in the aqueous extract of *Laportea aestuans* leaf has also been demonstrated to be the bioactives responsible for the treatment of hepatitis induced by chemicals (Shin et al., 2005). Free radical-scavenging properties, anti-platelet aggregation and inhibition of vascular smooth muscle cell proliferation of the polyphenols may be connected to the protective properties of the extract. Phenol group compound and flavonoid have been reported to possess hepatoprotective property (Thamizh et al., 2015). Hence, the availability of these secondary metabolites amongst others in the extract may contribute to the hepatoprotective action as demonstrated in this study.

### Conclusion

This study showed that aqueous extract of *Laportea aestuans* leaf has hepatoprotective properties which may be attributed to presence of secondary metabolites in the extract thereby supporting its acclaimed use for the prevention and treatment of liver diseases.

### Declaration of Interest

The authors report that there is no conflict of interest.

### References

Adedara, I. A., Farombi, E. O. (2010). Induction of oxidative damage in the testes and sperm and hematotoxicity in rats exposed to multiple doses of ethylene glycol monoethyl ether. *Hum. and Exp. Toxicol (UK)*, 29(10), 801-812.

Adeniyi, S.A., Orjiekwe, C. L., Ehiagbonane, J.E. (2009). Determination of alkaloid and oxalates in some selected food samples in Nigeria. *Afri. J. of Biotech.*, 8, 110 -112.

Asrani, S. K., Devarbhavi, H., Eaton, J., Kamath, P. S. (2019). Burden of liver diseases in the world. *J. of hepatol.*, 70(1), 151-171.

Bafor, E. E., Nwogu, J. K., Elvis-Offiah, U. B., Amaechina, F., Ofeimun, J., Ayinde, B., Edrada-Ebel, R. (2018). Modulation of ex-vivo uterine contraction by the methanol leaf extract of *Alchornea laxiflora* Benth. (Euphorbiaceae) and preliminary spectrometric identification of associated secondary metabolites. *J. of Med. Plants for Econ. Develop.*, 2(1), 13.

Bhandary, S. K., Kumari, S., Bhat, V. S., Sharmila, K. P., Bekal, M. P. (2012). Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *J. of Heal Sci.*, 2(4), 35-38.

Christensen, C. B., Soelberg, J., Jäger, A. K. (2015). Antacid activity of *Laportea aestuans* (L.) Chew. *J. of ethnopharmacol.*, 171, 1-3.  
Federico, A., Dallio, M., Loguercio, C. (2017). Silymarin/silybin and chronic liver disease: a marriage of many years. *Mole.*, 22(2), 191.

Ding, W. X., & Yang, L. (2019). Alcohol and drug-induced liver injury: Metabolism, mechanisms, pathogenesis and potential therapies. *Liv. res.*, 3(3-4), 129

Giacomelli, S., Gallo, D., Apollonio, P., Ferlini, C., Distefano, M., Morazzoni, P., Riva, A., Bombardelli, E., Mancuso, S., Scambia, G. (2002). Silybin and its bioavailable phospholipid complex (IdB 1016) potentiate *in vitro* and *in vivo* the activity of cisplatin. *Life Sci.*, 70(12), 1447-1459.

Giffen, P. S., Turton, J., Andrews, C. M., Barrett, P., Clarke, C. J., Fung, K.W., Munday, M. R., Roman, I. F., Smyth, R., Walshe, K., York, M. J. (2003). Markers of experimental acute inflammation in the Wistar Han rat with particular reference to haptoglobin and Creactive protein. *Arch. Toxicol.* 77, 392–402.

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochem. Soc. Transac.*, 35(5), 1147-1150.

Halliwell, B., Gutteridge, J. N. C. (1999). Mechanism of damage of cellular targets by oxidative stress: lipid peroxidation. *Free Rad. in Bio. and Med.*, 284, 313.



- Husain, K., Morris, C., Whitworth, C., Trammell, G. L., Rybak, L.P. (1998). Protection by ebselen against cisplatin-induced nephrotoxicity: antioxidant system, *Mol. Cell Biochem.*,178, 127-133.
- Jaeschke, H., Williams, C. D., Ramachandran, A., Bajt, M. L. (2012). Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liv. Int.*, 32, 8–20.
- Kaplan, M. M. (1993). Laboratory tests. Diseases of the Liver, 7th Edn. Philadelphia: JB Lippincott, Pp. 108-144.
- Kim, H. G., Han, J. M., Lee, H. W., Lee, J. S., Son, S. W., Choi, M. K., Son, C. G. CGX, (2013). A multiple herbal drug, improves cholestatic liver fibrosis in a bile duct ligation-induced rat model. *J. of Ethnopharmacol.*, 145(2), 653-662.
- Kyung, E. J., Kim, H. B., Hwang, E. S., Lee, S., Choi, B. K., Kim, J. W., ... & Woo, E. J. (2018). Evaluation of hepatoprotective effect of curcumin on liver cirrhosis using a combination of biochemical analysis and magnetic resonance-based electrical conductivity imaging. *Med. of inflamm.*
- Licata, A., Zerbo, M., Como, S., Cammilleri, M., Soresi, M., Montalto, G., & Giannitrapani, L. (2021). The Role of Vitamin Deficiency in Liver Disease: To Supplement or Not Supplement? *Nutr.*, 13(11), 4014.
- McGill, M. R., Jaeschke, H. (2014). Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. *Exp. Opin. Dru. Met. Toxicol.* 10, 1005–1017.
- Mubarak, M., Mohamed, M. A., Isyaku, U. Y., Jibril, M. N., Ibrahim, Y., Usman, A. D. (2018). Effect of caffeine on serum tumour necrosis factor alpha and lactate dehydrogenase in Wistar rats exposed to cerebral ischaemia-reperfusion injury. *Nig. J. of Phy. Sciences*, 33(1), 1-8.
- Obiajulu, F. J., Badmos, K. B., Awolola, N. A., Dawodu, O. O., Adebayo, L. A., Elesha, S. O., Abdulkareem, F. B. (2019). Autopsy Study of the Liver in Adult Nigerians at Lagos University Teaching Hospital. *W. Afri. J. of med.*, 36(1), 18-24.
- Oloyede, G. K., Oyelola, M. S. (2013). Chrysen-2-ol derivative from West Indian wood nettle *Laportea aestuans* (L.) chew inhibits oxidation and microbial growth *in vitro*. 12, 894.
- Olufunke, M. D., Adebayo, O. I., Ajani, O. I., Ose, O. (2008). Comparison of the chemical compositions of the essential oils from the aerial parts, fruits and roots of nigerian *Laportea aestuans* (L.) Chew (urticaceae). *Natural Pro. Comm.*, 3(11),1934578X0800301126.
- Owumi, S. E., Andrus, J. P., Herzenberg, L. A., Herzenberg, L. A. (2015). Co-administration of N-Acetylcysteine and Acetaminophen Efficiently Blocks Acetaminophen Toxicity. *Dru. Develop. Res.*, 7(5), 251-258.
- Parr, A., Bolwell, G. P. (2000). Phenols in plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenol content or profile. *J. of Sci. Food Agr.* 1,80, 985–1012.
- Qureshi, A. A. (2016). Evaluation of antioxidant and hepatoprotective potential of *Cinnamomum tamala* leaves in rats. *Saud. J. for Heal. Sci.*, 4, 197-211.
- Satishchandran, A., Ambade, A., Rao, S., Hsueh, Y. C., Iracheta-Vellve, A., Tornai, D., Zhong, L. (2018). MicroRNA 122, regulated by GRLH2, protects livers of mice and patients from ethanol-induced liver disease. *Gastroenterol.*, 154(1), 238-252.
- Shin, M. S., Kang, E. H., Lee, Y. I. (2005). A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes. *Antivir. Res.*, 67(3), 163-168.
- Suk, K. T., Kim, D. J., Kim, C. H., Park, S. H., Yoon, J. H., Kim, Y. S., Kim, S. H. A (2012). prospective nationwide study of drug-induced liver injury in Korea. *The Amer. J. of Gastroenterol.*, 107(9), 1380.
- Thamizh, S. N., Santhi, P. S., Sanjayakumar, Y. R., Venugopalan, T. N., Vasanthakumar, K. G., Swamy, G. (2015). Hepatoprotective activity of Averrhoa bilimbi fruit in acetaminophen induced hepatotoxicity in wistar albino rats. *J. of Chem. Pharmacol. Res.*, 7(1), 535-40.
- Vertuani, S., Angusti, A., Manfredini, S. (2004). The antioxidants and pro-antioxidants network:

an overview. *Cur. Pharma. Des.*, 10(14), 1677-1694.

Watkins, P. B., Kaplowitz, N., Slattery, J. T., Colonese, C. R., Colucci, S. V., Stewart, P. W. (2006). Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. *J. of Ame. Med. Ass.*, 296, 87–93.