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Hepatoprotective Effects of the Leaves of *Agauria salicifolia* against Acetaminophen-Induced Liver Injury in Mice

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Abstract

Agauria salicifolia (Ericaceae) is a medicinal plant traditionally used for the treatment of liver ailments. The present study investigates the hepatoprotective effect of the residual aqueous fraction (RAF) of *Agauria salicifolia* on acetaminophen (APAP)-induced liver damage. The ethanol extract obtained as maceration of the dried leaves, was fractionated into hexane, ethyl acetate and residual aqueous fractions. Adult mice of both sexes were pre-treated with the reference drug silymarin (50 mg/kg) or RAF (100 and 200 mg/kg) during 6 days followed by a single administration of APAP (500 mg/kg) on day 7. The hepatoprotective effect and the contribution of antioxidant activities were evaluated by determining the level of transaminases in serum samples, the levels of proteins, nitric oxide (NO), malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and catalase in liver homogenates. Histological analyses of liver slides were also performed. RAF at the doses of 100 and 200 mg/kg and silymarin significantly ($p < 0.001$) decreased serum level of alanine and aspartate aminotransferases. RAF showed no effect on liver weight and its SOD content but significantly inhibited the increase in proteins ($p < 0.01$), NO ($p < 0.01$) and MDA ($p < 0.001$) induced by APAP while silymarin significantly reduced all these parameters. RAF and silymarin also significantly ($p < 0.05$) increased the GSH and catalase content in the liver as compared to the APAP treated group. The vascular congestion, leukocyte infiltration and loss of hepatocytes and liver architecture observed in the liver tissue of disease control mice were corrected in animals treated with RAF. Therefore, it can be concluded that RAF possesses hepatoprotective ac-

tivities that might be mediated at least partially by its antioxidant effects.

Keywords

Agauria salicifolia, Hepatoprotection, Antioxidant, Histology

1. Introduction

The liver is the largest organ in human beings, accounting for approximately 2% of his total weight [1]. Its key position in between the digestive tract and the systemic circulation makes it one of the major regulatory organs and one of the most exposed organs to various attacks by xenobiotic, pathogens and exogenous antigens. As such, the liver is subjected to numerous diseases among which, hepatitis is the most prominent. Two categories of hepatitis are described: infectious hepatitis which can be caused by viruses, parasites and bacteria [2], and non-infectious hepatitis which can be caused by excessive and chronic alcohol consumption (alcoholic hepatitis), metabolic diseases (metabolic syndrome, obesity, diabetes, and hypertriglyceridemia) which may lead to non-alcoholic fatty liver disease (NAFLD), autoimmune dysfunction (autoimmune hepatitis), genetic disorder (alpha-1-antitrypsin deficiency, hemochromatosis) or by toxins and medications.

Concerning the medication, acetaminophen (APAP) also called paracetamol, known for its analgesic and antipyretic properties, is of primary concern [3]. During the metabolism of APAP, it is converted by P450 enzymes into a highly reactive intermediate metabolite called N-acetyl-p-benzoquinone imine (NAPQI). Under normal circumstances, NAPQI is rapidly converted and detoxified to a nontoxic metabolite by glutathione (GSH). However, in case of overdose or inappropriate use of APAP, part of NAPQI is detoxified after being conjugated with GSH and the remaining part attacks the hepatocytes and induces cell damages [4]. Pathways leading to these destructions comprise oxidative stress-induced molecules modification and enzymes inactivation, mitochondrial dysfunction and apoptosis, which will ultimately lead to cell death [5]. During the process of oxidative stress, lipids of the cell membranes are peroxidized, leading to membrane disruption. This lipid peroxidation is accompanied by the production of reactive lipid aldehydes, such as malondialdehyde. Oxidative stress is also known to induce inflammation by stimulating the production of inflammatory cytokines and increasing the level of NO synthesis.

Therefore, it can be hypothesized that substances with antioxidant properties could have hepatoprotective effects against APAP-induced hepatocellular injury and cell death. In this prospect, many herbal formulations have been made available to ensure hepatocyte regeneration and protection against drugs-induced damages [6] [7]. The effectiveness of such remedy is underpinned by their content in secondary metabolites such as polyphenols and particularly flavonoids

which represent the most important and potent source of antioxidants. As an example, silymarin, a mixture of flavonoids from the herbal origin, is commercialized for its hepatoprotective activity.

In West Cameroon Region, *Agauria salicifolia* (Ericaceae) is used in folk medicine for the treatment of various diseases including liver ailments. Our previous studies showed that *Agauria salicifolia* contains phenolic compounds, possesses antioxidant properties and hepatoprotective effects against CCl_4-induced hepatotoxicity, with the aqueous residual fraction (RAF) being the most efficient [8]. However, nothing is known about the effects of *Agauria salicifolia* against drug-induced liver damage. The present study aimed at investigating the protective effects of RAF against APAP-induced hepatotoxicity.

2. Materials and Methods

2.1. Chemicals

Acetaminophen, adrenaline, Na_2CO_3, $NaHCO_3$, Na_2HPO_4, NaH_2PO_4, Tris, 5,5'-Dithiobis (2-nitrobenzoic acid), hydrogen peroxide, acetic acid, potassium dichromate, thiobarbituric acid, trichloroacetic acid, hexane, ethyl acetate, ortho-phosphoric acid, were purchased from Sigma-Aldrich Chemical Co. (Taufkirchen, Germany). The kits for liver biochemical assays of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Immesco industries. All chemicals used were of analytical grade.

2.2. Animals

Mice (25 to 30 g) of both sexes were obtained from the animal house of the Department of Animal Biology, University of Dschang, Cameroon. The animals were housed in plastic cages and maintained at room temperature and in a natural light/dark cycle. They were fed with standard laboratory food and water *ad libitum.* Animals were maintained and used in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines (EEC Directive of 2010; 10/609/EEC).

2.3. Plant Material and Extracts

The fresh leaves of *A. salicifolia* were harvested in the South West Region of Cameroon, in Wabane Sub-division and authenticated at the National Herbarium of Cameroon by comparison with the Voucher specimen number 66695/HNC. The leaves powder (500 g) of *A. salicifolia* was extracted with 2 L of ethanol and the procedure was repeated with 1 L of the same solvent. The filtrates obtained after alcoholic extraction were concentrated using a rotary evaporator. The remaining solvent in the extract was allowed to evaporate at room temperature. The process yielded 96.98 g of the ethanol extracts of which 20 g were suspended in distilled water (150 mL) and further fractioned successively with hexane (100 mL) and ethyl acetate (75 mL). After concentration in rotary a evaporator and

evaporation in an oven, 1.09 g of hexane, 3.2 g of ethyl acetate and 9.8 g of residual aqueous fractions were obtained.

2.4. Acetaminophen-Induced Acute Hepatotoxicity in Mice

For the experimental design, 30 mice were randomly divided into five groups (I - V) of six animals each (3 males and 3 females) and treated orally as follows:

Group I (naive) was used as normal control and they were given distilled water orally for seven days.

Group II (disease control) received daily oral administration of distilled water for six days and acetaminophen on the seventh day.

Group III (reference control) received silymarin (50 mg/kg) orally daily for six days and acetaminophen on the seventh day.

Group IV and V (test groups) received dally administration of RAF (100 and 200 mg/kg) for six days and acetaminophen on the seventh day.

Acetaminophen suspension was administered orally to animals belonging to groups II to V at the dose of 500 mg/kg, b.w. Six hours post-administration of APAP, blood samples were obtained via retro-orbital sinus plexus and the mice were later sacrificed. Blood was left to clot at room temperature and the serum was obtained by centrifugation at 3000 rpm for 10 min and was immediately used for alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) assays. The assays were performed using commercial kits from INMESCO, according to the manufacturer's instructions. The liver was rapidly collected after sacrifice, weighted and divided into two parts. The biggest liver lobe was fixed in 10% buffered formalin while the rest was used for the different biochemical measurements.

2.5. Measurement of Lipid Peroxidation

Lipid peroxidation assay was performed by determining spectrophotometrically the level of malondialdehyde (MDA) in liver homogenates as thiobarbituric acid reactive substances (TBARS) according to the methodology described by Fofie *et al.* [9]. Briefly, 100 µl of a sample, 500 µl of orthophosphoric acid (1%), 500 µl of thiobarbituric acid prepared in 1% trichloroacetic acid were added in a test tube. The mixture was kept at 100˚C in a water bath for 15 minutes and then cooled in iced water. After centrifugation at 3000 rpm for 15 min, the absorbance of the supernatant was read at 532 nm against a blank.

2.6. Measurement of Nitric Oxide (NO) Production and Proteins

The NO level was determined in the supernatant of liver tissue homogenates by the method of Griess as described by Nguelefack-Mbuyo *et al.* [10]. Briefly, 250 µl of the tissue sample was mixed with 250 µl of 1% sulfanilamide prepared in 5% orthophosphoric acid. After 5 minutes incubation in the dark, 250 µl of 0.1% naphthyl ethylenediamine was added, and then all incubated in the dark for an additional 5 minutes. The optical density was read at 530 nm. The quantity of nitric oxide was calculated from sodium nitrite's standard curve. Tissue protein

contents were determined according to the method described by [11].

2.7. Catalase Activity Determination

Catalase activity was measured in liver homogenates according to the following protocol: to 50 µl of a sample, was added 500 µl phosphate buffer (200 mM) and 100 µl of H_2O_2 (100 mM). The obtained solution was mixed with vortex and incubated at room temperature for 3 minutes. Then, 1000 µl of potassium dichromate solution (prepared by mixing one volume of 5% potassium dichromate with 3 volumes of glacial acetic acid) was added to the mixture. After that, tubes were incubated for 10 minutes in boiling water, cooled with tape water and centrifuged at 2000 rpm for 5 minutes to remove precipitated proteins. The supernatant was read at 570 nm against the reagent blank.

2.8. Superoxide Dismutase (SOD) Activity Determination

Superoxide dismutase (SOD) activity was measured in the liver homogenates as described by Wandji *et al.* [12]. The sample (70 µl) was mixed with 830 µl of carbonate buffer (pH 10.2). The reaction was initiated by introducing 100 µl of epinephrine (0.3 mM). The absorbance was read at 60 and 120 seconds after epinephrine was introduced, at 480 nm using a spectrophotometer (Helios Epsilon). The SOD activity was expressed as units/mg of liver protein. One unit was defined as the enzyme activity that inhibited the auto-oxidation of adrenaline by 50%.

2.9. Determination of Glutathione

The determination of GSH in liver samples was done using the Ellman's reagent and according to the method of [13]. One milliliter of $Na_2HPO_4{\cdot}_2H_2O$ (0.3 M) and 1 mL of dithiobisnitrobenzoate (0.4 mg/mL prepare in 1% of sodium citrate) were successively added to 250 µl of a sample. The absorbance of the mixture was measured spectrophotometrically at 412 nm.

2.10. Liver Histopathological Analysis

Fragments of liver tissues in each group were collected in 10% buffered formalin for fixation at room temperature. These tissues were further embedded in paraffin. Sections of 5 - 6 µm in thickness were made and stained with hematoxylin and eosin (H&E). These sections were microscopically examined for morphology and necrosis.

2.11. Statistical Analysis

Data are expressed as mean ± SEM for each group. Results were statistically analyzed using one-way analysis of the variance (ANOVA) followed by Tukey's post-test. Differences between groups were considered significant when the probability p was less than 0.05. Statistical analyses were performed using GraphPad Prism software (version 5.0).

3. Results

3.1. Effects of the Residual Aqueous Fraction of *A. salicifolia* on Liver Transaminases

The oral administration of APAP resulted in a significant ($p < 0.001$) increase in ALAT and ASAT activities in the serum of control animals as compared to the naive mice. Serum ALT activity was increased by 91% in APAP treated mice as compared to the control. In mice pre-treated with RAF at the doses of 100 and 200 mg/kg, this increase was significantly ($p < 0.001$) prevented by 86% as compared to control (Figure 1(a)). The ASAT activity increased in APAP-treated mice by 69% as compared to the naive mice. RAF administration at both doses (100 and 200 mg/kg) significantly ($p < 0.01$) reduced the ASAT level by 77%, as compared to the control group (Figure 1(a)).

3.2. Effect of the Residual Aqueous Fraction of *A. salicifolia* on the Body and Liver Relative Weights

The bodyweight of mice treated solely with APAP was not significantly different from that of healthy mice; none of the treatment administered also affected this parameter (Figure 2(a)). A significant ($p < 0.01$) increase of 26% of the liver weight was observed in disease mice when compared to the naive group. Only silymarin was able to significantly ($p < 0.01$) prevent this liver overweight by 25% (Figure 2(b)).

3.3. Effect of the Residual Aqueous Fraction of *A. salicifolia* on the Levels of Proteins and Nitric Oxide

Treatment of mice with APAP increased the liver's protein content by 36% as compared to the naive mice (Figure 3(a)). Administration of silymarin or RAF at both doses significantly reduced the increased protein content induced by APAP. APAP administration also significantly ($p < 0.01$) increased the nitric oxide level by 44% in the mice liver. Silymarin and ARF at the dose of 100 mg/kg, significantly reversed the NO increase (Figure 3(b)).

3.4. Effect of the Residual Aqueous Fraction of *A. salicifolia* on Catalase, Superoxide Dismutase, Glutathione and Lipid Peroxidation Levels in APAP Treated Mice

Although the APAP administration did not significantly reduce catalase activity in the liver, RAF at the dose of 100 mg/kg significantly ($p < 0.05$) increased it by 71.60% as compared to the control group (Figure 4(a)). None of the treatments administered significantly affected SOD activity (Figure 4(b)). APAP significantly ($p < 0.001$) and drastically reduced (85.5%) the level of GSH in the liver. Extract or silymarin administrations significantly reversed this reduction. Silymarin antagonized the reduction by 54% while extract prevented it by 39% and 43% at respective doses of 100 and 200 mg/kg (Figure 4(c)). As shown in Figure 4(d), the level of MDA (an end product of lipid peroxidation) significantly ($p < 0.001$) and drastically increased by 178% in APAP treated mice as compared to

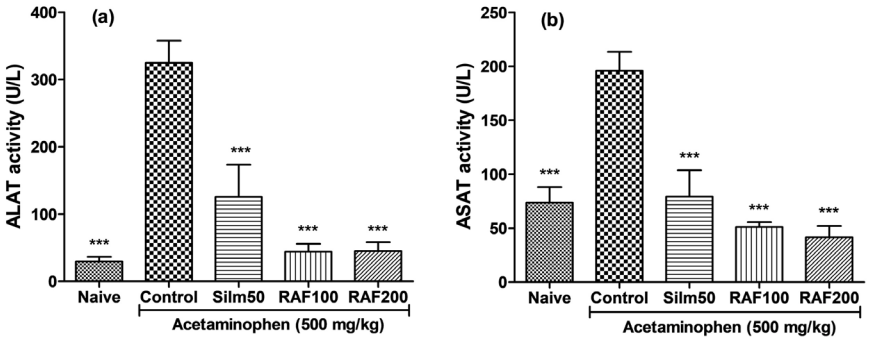

Figure 1. Effect of the residual aqueous fraction of *A. salicifolia* on the levels of serum ALAT and ASAT in APAP-induced hepatotoxicity. Each bar represents the mean ± SEM from 6 mice. ***$p < 0.001$ significantly different with respect to the disease control group (control). Silm 50: silymarin at the dose of 50 mg/kg, RAF 100: residual aqueous fraction of *A. salicifolia* at 100 mg/kg, RAF 200: residual aqueous fraction of *A. salicifolia* at 200 mg/kg.

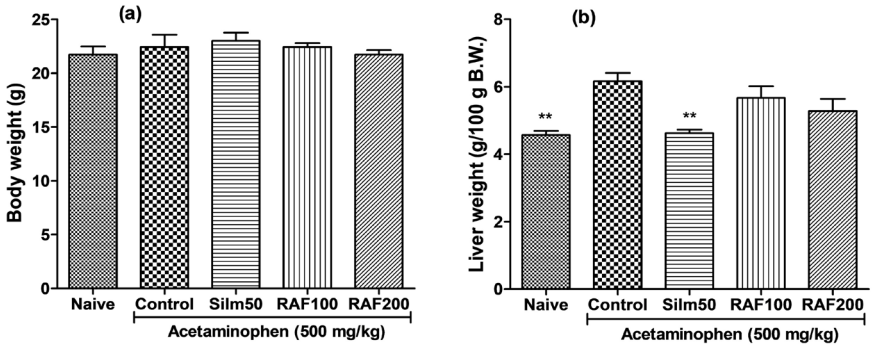

Figure 2. Effect of the residual aqueous fraction of *A. salicifolia* on body weight (a) and liver relative weight (b) in APAP-induced hepatotoxicity. Each bar represents the mean ± SEM from 6 mice. **$p < 0.01$ significantly different with respect to the disease control group (control). Silm 50: silymarin at the dose of 50 mg/kg, RAF 100: residual aqueous fraction of *A. salicifolia* at 100 mg/kg, RAF 200: residual aqueous fraction of *A. salicifolia* at 200 mg/kg.

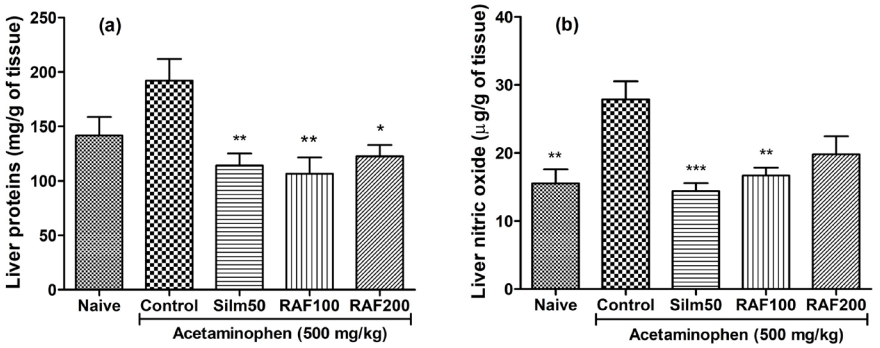

Figure 3. Effect of the residual aqueous fraction of *A. salicifolia* on protein level (a) and nitric oxide levels (b) in APAP-induced hepatotoxicity. Each bar represents the mean ± SEM from 6 mice. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$ significantly different with respect to the disease control group (control). Silm 50: silymarin at the dose of 50 mg/kg, RAF 100: residual aqueous fraction of *A. salicifolia* at 100 mg/kg, RAF 200: residual aqueous fraction of *A. salicifolia* at 200 mg/kg.

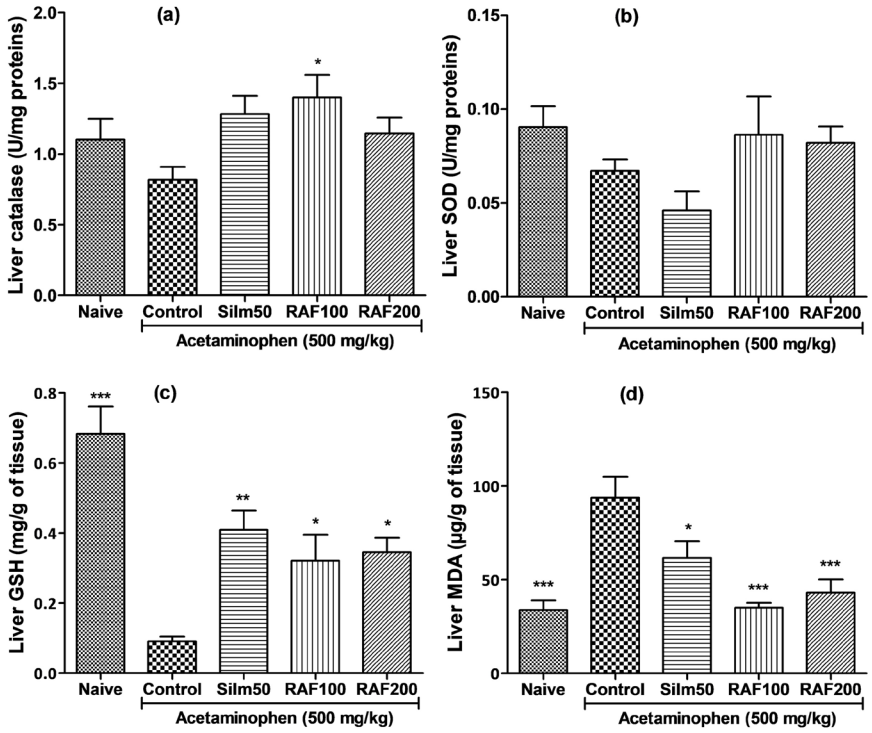

Figure 4. Effect of residual aqueous fraction of *A. salicifolia* leaves on the levels of CAT, SOD, GSH and LPO in the liver of APAP-induced hepatotoxicity mice. Each bar represents the mean ± SEM from 6 mice. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ significantly different with respect to the disease control group (control). Silm 50: silymarin at the dose of 50 mg/kg, RAF 100: residual aqueous fraction of *A. salicifolia* at 100 mg/kg, RAF 200: residual aqueous fraction of *A. salicifolia* at 200 mg/kg.

control. Silymarin administered at the dose of 50 mg/kg inhibited the MDA production by 54% while RAF showed the best effect with inhibition percentages of 98% and 85% when administered at the respective doses of 100 and 200 mg/kg.

3.5. Effects of the Residual Aqueous Fraction of *A. salicifolia* on the Histology of the Liver of APAP-Treated Mice

Liver slices were examined at the level of the central lobular vein and the portal hepatic artery. APAP administration induced severe injuries when compared to the healthy liver from the naive mice (**Figure 5(a)** and **Figure 6(a)**). These damages were characterized by features typical of drug-induced diseases, including alteration of hepatic architecture with trabecular collapse and disorder, infiltration of leucocytes, presence of ballooned cells and important necrosis as depicted by the hepatocellular degeneration (**Figure 5(b)** and **Figure 6(b)**). Besides, in the liver of disease mice, a vascular congestion was also observed (**Figure 6(b)**). These harmful effects of APAP were significantly attenuated by pre-treating the mice with silymarin (**Figure 5(c)** and **Figure 6(c)**) or RAF at the dose of 100 (**Figure 5(d)** and **Figure 6(d)**) and 200 mg/kg (**Figure 5(e)** and **Figure 6(e)**). However, a mild leucocytes infiltration and strong vascular congestion were still

observable in extract-treated groups.

4. Discussion

Acetaminophen (APAP) commonly called paracetamol is a well-known analgesic and antipyretic drug widely used, and often without medical prescription. Meanwhile, overdose or improper use can cause significant morbidity and mortality. Indeed, APAP toxicity remains the most common cause of drug-induced liver failure [14]. In accordance with the literature, results from the present study showed an increase in serum transaminases, in liver weight, MDA and NO content, and reduction in GHS level that matched the histological alterations in APAP treated mice. These altered parameters were significantly corrected by the pre-treatment with RAF.

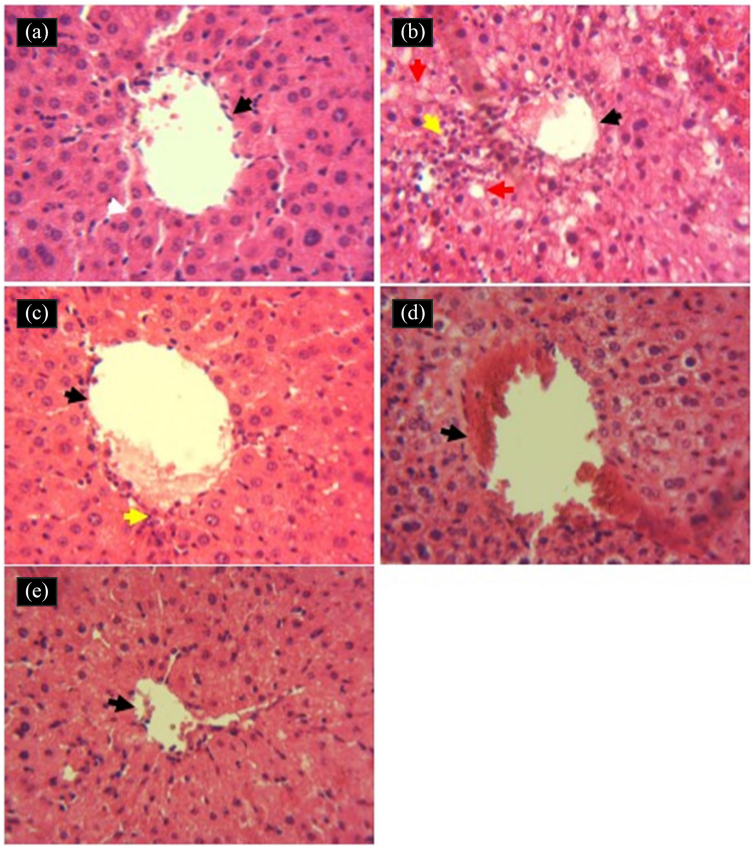

Figure 5. Microphotographs presenting the central lobular vein (black arrow) of liver tissues. (a) Liver of naive mice showing normal hepatocytes (white arrow) with a normal arrangement and no leucocyte infiltration; (b) Control mice treated with acetaminophen, tissue shows the loss of hepatocytes, disorganized architecture with trabecular collapse and disorder, presence of ballooned cells (red arrow) and leucocyte infiltration (yellow arrow) around the central vein; (c) Silymarin + acetaminophen treated group showing the absence of necrosis, normal hepatocytes and very low infiltration of leucocyte; (d) RAF 100 mg/kg + acetaminophen treated group showing mild degree necrosis and disorganized architecture and microvascular congestion; (e) Mice treated with RAF 200 mg/kg + acetaminophen, showing normal hepatocyte and normal central zone although apparent reduced number of hepatocytes.

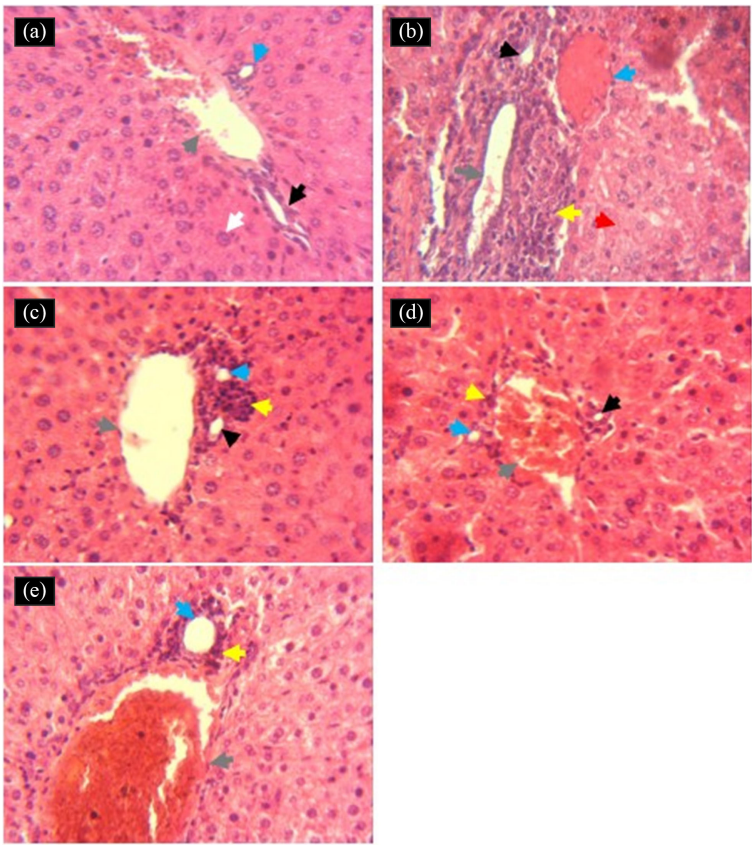

Figure 6. Microphotographs presenting the portal zone of liver tissues. (a) Liver of naive mice showing normal hepatocytes (white arrow), portal vein (green arrow), bile duct (black arrow) and artery (blue arrow), with a normal arrangement and no leucocyte infiltration; (b) Control mice treated with acetaminophen, tissue shows the loss of hepatocytes and architecture disorder (red arrow), macrovascular congestion and considerable leucocyte infiltration (yellow arrow) in the portal zone; (c) Silymarin + acetaminophen treated group showing the absence of hepatocyte necrosis but leucocyte infiltration; (d) RAF 100 mg/kg + acetaminophen treated group showing mild degree necrosis, microvascular congestion and little leucocytes infiltration; (e) Mice treated with RAF 200 mg/kg + acetaminophen, showing normal hepatocyte and liver structure, vascular congestion and little leucocyte infiltration.

APAP is generally well metabolized in the liver. However, in the case of overdose, it is highly converted through cytochrome P_{450} system into the toxic free-radical product N-acetyl-p-benzoquinone imine (NAPQI), which produces necrosis of hepatocytes located in central lobules [15]. AST and ALT enzymes then leak out from the liver into the bloodstream when these hepatocytes are disrupted [16] [17]. Serum levels of these transaminases reflect the degree of liver damage. It is, therefore, an important marker of the liver, routinely used in clinical diagnosis and experiments to appraise liver injury [18] [19]. The significant increase in serum level of AST and ALT observed after the administration of APAP confirmed the presence of a liver injury in our experimental model. Pre-treatment of mice with RAF for 6 days significantly prevented liver injury as depicted by an important decrease in the level of serum AST and ALAT, sug-

gesting the hepatoprotective activity of RAF.

To evidence this fact, histological analysis was performed. Results showed, in contrary to APAP treated mice, that liver slices from RAF or silymarin treated groups presented very low cell loss and leucocyte infiltration, as well as more or less normal liver architecture, confirming therefore, the hepatoprotective effect of RAF.

Besides the increase of ASAT and ALAT caused by APAP administration, an increase in liver weight and protein level was noted. It has been demonstrated that APAP intoxication is accompanied with hepatic congestion in humans and rodents [20] [21] that occurs early and before the appearance of necrosis. In mice, the congestion results from the accumulation of red blood cells within endocytic vacuoles and the Space of Disse with a collapse of the sinusoidal lumens [22]. This leads to an increase in liver weight and even liver proteins, reaching the maximum at about 6 h [23]. In the present study, RAF was unable to prevent the increase in liver weight. Besides, it was also noticed that mice treated with RAF still presented vascular congestion, strengthening the direct relationship between the increase in liver weight and liver congestion. Ito *et al.* [24] have attributed this liver congestion to the dysfunction of the sinusoidal endothelial cells. It could then be hypothesized that although RAF potently protects hepatocytes, it does not have the same effect on the function of endothelial cells.

APAP-induced liver disease is an inflammatory process and as such, many inflammatory mediators contribute to its development. One of these mediators is nitric oxide (NO) [25]. An increase in liver NO content in APAP treated animals is an obvious typical observation and the reduction in iNOS has been correlated with reduced serum ALT [26], although not very efficient for complete hepatoprotection. In this study, a significant increase in liver NO was indeed observed in APAP-treated mice and RAF pre-treatment significantly prevented it. This result suggests the inhibition of NO production as one of the mechanisms of RAF and further indicates its anti-inflammatory and/or antioxidant effect.

Oxidative stress in APAP liver intoxication highly involves NO which reacts with superoxide anion to form peroxynitrite, an oxidizing and nitrating agent. Peroxynitrite is normally detoxified by glutathione; but the latter is depleted by NAPQI in acetaminophen-induced hepatotoxicity [27] and therefore, enhancing the peroxidation. Results obtained in this study are in accordance with this mechanism and show that overdose of APAP in mice drastically depleted glutathione. Pre-treatment with RAF significantly prevented this depletion and may justify the hepatoprotective effect of this extract. It could then be speculated that antioxidant activity is one of the main mechanisms of action of RAF.

To further understand the RAF action mechanism, we focused on oxidative stress, given the paramount importance of this parameter in the development of liver injuries. We first evaluated if the extract could prevent the oxidation of biomolecules by measuring malondialdehyde (MDA), an end product of lipids peroxidation, in the liver. MDA was significantly increased in APAP intoxicated mice and RAF significantly reduced it to almost the normal level. This result

clearly shows that the extract was able to inhibit lipid peroxidation and thus prevent cell necrosis and the transaminases leakage into the bloodstream. It was then clear that RAF can prevent *in vivo*, the oxidation of biomolecules. This can occur either by the direct antioxidant effect of the extract or by its capacity of enhancing endogenous antioxidant enzymes [28]. We further evaluated the activities of superoxide (SOD) and catalase, two endogenous antioxidant enzymes. APAP administration did not significantly affect the activity of both enzymes in the liver. Nevertheless, RAF significantly increased the catalase activity but did not significantly affect the SOD activity. These findings suggest that RAF has antioxidant properties either through a direct scavenging effect or by enhancing the endogenous antioxidant such as catalase and GSH.

5. Conclusion

The residual aqueous fraction of the ethanol extract of the leaves of *Agauria salicifolia* possesses hepatoprotective activities in acetaminophen treated mice. This hepatoprotective effect evidenced by histological and biochemical parameters is at least partially mediated by the antioxidant effects of the extract. This antioxidant effect of RAF might combine direct scavenging effect and enhancement of the activity of endogenous antioxidant enzymes.

Availability of Data and Materials

The data used and analyzed in this study are available from the corresponding author on reasonable request.

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Authors' Contributions

TBN, EPN-M and ALF conceived the work. MKT, CLKB and CKF collected the data. TBN and MKT analyzed the results. MKT, CLKB and TBN drafted the manuscript. All the authors revised the manuscript for its intellectual content and approved the final version.

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Ethics Approval

Experimental protocols used herein were approved by the laboratory committee, Faculty of Science, University of Dschang and conformed to the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines 2010/63/EU.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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