Cameroon Journal of Biological and Biochemical Sciences, 2025, 33, 14-25.
DOI: https://doi.org/10.63342/cjbbs2025.432

CAMEROON JOURNAL OF BIOLOGICAL AND BIOCHEMICAL SCIENCES
Published by the Cameroon Biosciences Society (CBS)

Research Article

Hepatoprotective and Antioxidant Effects of *Tetrapleura tetraptera* Fruits (Fabaceae) Against Indomethacin-Induced Liver Failure in Wistar Rats

OUMAR MAHAMAT[1], MOTIKA DELPHINE KEBOTE[1], MARC AURELE NDASSI SOUFO[2], YOUMBIE DJANCHE DUPLEX BONHEUR[1*]

[1]Departement of Zoology, Faculty of Science, University of Bamenda, Box 39 Bambili, Cameroon.
[2]Departement of Sciences, Faculty of Sciences and Technology, Cameroon Evangelic Institute, Bandjoun, Cameroon.
[*]Corresponding author: youmbidjanche@gmail.com, +237677644983.

Received: 24 Aug 2024, Reviewed: 01 Oct 2024, Revised: 28 Oct 2024, Accepted: 29 Oct 2024, Published: 11 Mar 2025

ABSTRACT

Tetrapleura tetraptera is used in Cameroon in ethnomedicine to cure liver diseases and jaundice. The gas chromatography-mass spectroscopy (GC/MS) spectra of sample *T. tetraptera* showed the presence of 25 peaks and twenty volatile oils some of which are terpenoids, esters, fatty acids, alkanes, and phenols. The identified volatile oils included Benzene, 1-methyl-3-(1-methylethyl), Limonene, 1-Methone, and Cyclohexanone. The aim of this study was to investigate the hepatoprotective and antioxidant effects of the aqueous extract fruits of *T. tetraptera* in rats subjected to indomethacin-induced liver failure. All substances were administrated daily by oral route. Thirty-five rats including males and females were divided into 7 groups of 5 each, namely: Group I, normal rats received distilled water (5mL/kg); Group II control of extract received the highest dose (300 mg/kg); Group III, the negative group received (Indomethacin 4mg/kg + distilled water 5mL/kg); Group IV positive group received (Indomethacin 4mg/kg+ silymarin 50 mg/kg); Group V received (Indomethacin 4mg/kg+ aqueous extract 100mg/kg); Group VI received Indomethacin 4mg/kg+ aqueous extract 200mg/kg) and Group VII received (Indomethacin 4mg/kg+ aqueous extract 300mg/kg). From Group III to VII, indomethacin was administrated (4mg/kg) to induce hepatotoxicity every Tuesday, Thursday, and Saturday and treatment every Wednesday, Friday, and Sunday for 4 weeks. Thereafter, histological sections were examined. The body weights of the negative group decreased significantly ($P<0.001$) throughout the treatment period compared to the normal group. The doses (200 and 300 mg/kg) of the extract significantly ($P<0.05$) increased the liver's index compared to the negative group. Administration of extract (300 mg/kg) significantly ($P<0.001$) decreased the enzyme activity (ALT, ASP, ALP, and T. Bil) compared to the negative group. Administration of extract (300 mg/kg) significantly increased ($P<0.001$) the lipid profile (Total cholesterol, TG, HDL-cholesterol, and LDL-cholesterol) compared to the negative group. Administration of extract significantly ($P<0.001$) alleviated the toxic effect of indomethacin in oxidative parameters (SOD, CAT, and GSH) compared to the negative group. Administration of extract significantly ($P<0.001$) alleviated the toxic effect of indomethacin in oxidative parameters (SOD, CAT, and GSH) compared to the negative group. These results were confirmed by observation of histological sections of the liver. These results demonstrated that the aqueous extract of *T. Tetraptera* fruits has potential hepatoprotective and antioxidant effects. The control group indicated that the aqueous extract (300 mg/kg) is not toxic.

Keywords: Liver, Indomethacin, Antioxidant, *Tetrapleura Tetraptera*.

RÉSUMÉ

Tetrapleura tetraptera est utilisé au Cameroun en ethnomédecine pour soigner les maladies du foie et la jaunisse. Les spectres de chromatographie en phase gazeuse-spectroscopie de masse (GC/MS) de l'échantillon de *T. tetraptera* montrent la présence de 25 pics et d'une vingtaine d'huiles volatiles dont certaines sont des terpénoïdes, des esters, des acides gras, des alcanes et des phénols. Les huiles volatiles identifiées incluent les substances ci-apres : benzène, 1-méthyl-3-(1-méthyléthyl), limonène, 1-méthone, cyclohexanone. Le but de cette étude était d'expérimenter les effets hépatoprotecteurs et antioxydants des extraits aqueux des fruits de *T. tetraptera* chez des rats soumis à une toxicité du foie induite par l'indométacine. Toutes les substances ont été administrées quotidiennement par voie orale. Trente-cinq rats, mâles et femelles, ont été répartis en 7 groupes de 5 rats chacun ainsi qu'il suit. Le Groupe I, rats normaux ont reçu de l'eau distillée (5 mL/kg) ; Le groupe II, contrôle de l'extrait a reçu la dose la plus élevée de l'extrait (300 mg/kg) ; le Groupe III, contrôle négatif a reçu (Indométacine 4 mg/kg + eau distillée 5 mL/kg) ; le Groupe IV contrôle positif a reçu (indométacine 4 mg/kg + silymarine 50 mg/kg) ; le Groupe V a reçu (Indométacine 4 mg/kg + extrait aqueux 100 mg/kg) ; le groupe VI a reçu (Indométacine 4 mg/kg + extrait aqueux 200 mg/kg) et le Groupe VII a reçu (Indométacine 4 mg/kg + extrait aqueux 300 mg/kg). Du Groupe III à VII, l'indométacine a été administrée (4 mg/kg) pour induire l'hépatotoxicité tous les Mardis, Jeudis, Samedis et le traitement tous les Mercredis, Vendredis et Dimanches ceci pendant 4 semaines. Par la suite, les coupes histologiques ont été examinées. Le poids corporel du groupe négatif a diminué de manière significative

(P<0,001) tout au long de la période de traitement par rapport au groupe normal. Les doses (200 et 300 mg/kg) de l'extrait aqueux ont augmenté de manière significative (P<0,05) l'index du foie par rapport au groupe négatif. L'administration de l'extrait aqueux à la dose de 300 mg/kg a diminué de manière significative (P<0,001) l'activité enzymatique (ALT, ASP, ALP et T. Bil) par rapport au groupe négatif. L'administration de l'extrait (300 mg/kg) a augmenté de manière significative (P<0,001) le profil lipidique (cholestérol total, TG, cholestérol HDL et cholestérol LDL) par rapport au groupe négatif. L'administration de l'extrait a atténué de manière significative (P<0,001) l'effet toxique de l'indométacine sur les paramètres oxydatifs (SOD, CAT et GSH) par rapport au groupe négatif. Ces résultats ont été confirmés par l'observation des coupes histologiques du foie. En conclusion, ces résultats ont démontré que l'extrait aqueux des fruits de *T. Tetraptera* possède des effets hépatoprotecteurs et antioxydants. Le groupe contrôle a montré que l'extrait aqueux (300 mg/kg) n'est pas toxique.

Mots clés : Foie, Indométacine, Antioxydant, *Tetrapleura tetraptera*.

1. INTRODUCTION

Liver failure is a serious health condition characterised by the impairment of liver function and has various causes including drug-induced liver injury (Andréia *et al.*, 2013). According to (Adewusi *et al.*, (2019), hepatic diseases continue to be a principal threat to public health, and remain a problem worldwide. Despite significant developments in modern medicine, no fully effective medications exist to promote hepatic function, provide total organ protection, or assist in the regulation of hepatocytes (Salawu *et al*. 2018). Therefore, much research has been conducted targeting the development of an appropriate drug in the treatment of liver failure, amongst which is the use of medicinal plants.

Indomethacin is a potent non-steroidal anti-inflammatory drug (NSAID) typically used for chronic inflammatory arthritis (Nemat *et al*. 2015). Indomethacin is recognised as a drug and toxin-induced liver injury, it is a frequent cause of liver failure, and it represents a health problem in our society (Entedhar Rifaat Sarhat *et al*. 2019). At overdose, Indomethacin acts on COX 1 which is present in almost all tissues including the liver; instead of COX 2 inductive, necessary to reduce inflammation (Wilmana & Gunawan 2012). Drug-induced hepatotoxicity leads to abnormalities in liver tests or liver dysfunction. An elevation of alanine aminotransferase (ALT), alkaline phosphatase (ALP) or conjugated bilirubin was confirmed in several studies(Olatosin *et al*.2013). Unfortunately, fewer than a dozen cases of indomethacin-related toxic hepatitis have been reported in the literature (Hilal *et al*. 2018). However, chronic indomethacin intake also brings about severe damage to the liver (Ornbjerg *et al*. 2008).

In traditional medicine, there is a record number of plants with hepatoprotective properties. Ethnopharmacological studies on such medicinal plants continue to interest investigators throughout the world nowadays (Kuate 2015). *Tetrapleura tetraptera* fruits are a key plant products used in the management of liver complaints, stomach disorders, diabetes, intestinal worms, malaria and fever in many countries (Kuate 2015). *Tetrapleura tetraptera* is used for its gastroprotective (Salawu *et al*. 2018), anti-inflammatory and antioxidant (Sonibare *et al*. 2018) activities. The fruits of *T. tetraptera* commonly called four corners are one of the best-known domestic remedies. The gas chromatography-mass spectroscopy (GC-MS) spectra of sample *Tetrapteura tetraptera* shows the presence of 25 peaks and twenty volatile oils some are terpenoids, esters, fatty acids, alkanes, and phenols. The identified volatile oils include Benzene, 1-methyl-3-(1-methylethyl), Limonene, 1-Methone and Cyclohexanone. Various phytochemical characteristics of plants such as tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids are attributed to their prospective curative properties (Sonibare *et al*. 2018). In spite of the widespread use of this plant in traditional therapy, there is a scarcity of evidence related to its protective value in treating indomethacin-induced liver failure. The current research aimed to investigate the effectiveness of aqueous extract of *T. tetraptera* fruits as a hepatoprotective and antioxidant agent in experimental rats.

2. MATERIALS AND METHODS

2.1. Collection and preparation of plant material.

Fresh fruits of *Tetrapleura tetraptera* were collected in Ndengnieup village, Bangou Subdivision in Upper plateau Division, West region Cameroon (5° 14' 5" North and 10° 25' 57" East) on December 10th 2022. The fruits were identified with the help of local herbalists. The information gathered included vernacular name (*Tchetcho'o* or Four Corners), parts used (root, leaves and fruits), quantity used (one coffee spon in a cup of water), and the ailment treated (jaundice, epilepsy, infertility, and liver diseases). The samples were collected with acceptable bio-conservative methods and were properly sorted out, cleaned, and transported at the University of Bamenda (North West region, Cameroon). The plant samples were provided to an acknowledged

taxonomist Dr. Walter Ndam Tacham for botanical authentication and a voucher specimen deposited at the University of Bamenda. The fruits of *T. tetraptera* were chopped into small pieces and air dried away from solar radiation (temperature 22-25°C) for two months until proper dried. The dried fruits of *T. tetraptera* were crushed into fine homogenous powder using electrical mill followed by sieving through mesh sieve and stored at room temperature until extraction.

2.2. Extraction of aqueous extract of *Tetrapleura tetraptera* fruits.

According to Youmbie *et al* (2021) with some modifications, the dried powder (800g) was macerated in 7 litters of distilled water for 48 hours to extract the active compounds. The successive extract was filtered using Wattman's No1 filter papers. The obtained filtrate was evaporated at 40°C to dryness for 72 hours in a thermostat oven (DHG-9101-15APEC) in biochemistry laboratory, University of Bamenda. A dark brown solid extract was obtained, weighted and 307.43g recorded representing an extraction yield of 37.80%. It was stored in tight labelled bottle in a refrigerator at 4°C until further use. Based on the information from the local herbalists, the doses of 100, 200, and 300mg/kg body weight (bw) were selected. Extract was dissolved in distilled water and administrated by oral route to rats.

2.3. Experimental animals

Thirty-five (35) healthy male and female Wistar rats (*Rattus norvegicus*) (6 weeks old, average weight 120-140g) were obtained from breeder at mile 4 Nkwen Bamenda, North-west region Cameroon. The experimental rats were raised in the animal house and kept under standard laboratory conditions (12 hours light and dark sequence, temperature 25 ±3°C, and 35-60% humidity). The animals were caged in plastic cages of dimension (45.5 cm of diameter and 22.5 cm of high), with an appropriate diet (corn flour, soya beans and wheat brown), and water tap ad libitum. They were handled by considering to the ethical guidelines of Cameroon national veterinary laboratory as referenced by the approval and head central No 001/17CCS/MINPIA/RD-NW/DD-MELSSV. All the animals were acclimatized for one week before the beginning of the experiment and food was withdrawn 12 hours before initiation of experiments.

2.4. Experimental design and treatment of experimental animals.

According to Youmbie *et al*, (2021) with some modifications, after the acclimatization period of one week, 35 male and female Wistar rats were weighed and divided into 7 different groups. The groups were named I-VII and designed as follows: Group I, normal rats received distilled water (5mL/kg) ; Group II control of extract received highest dose (300 mg/kg) to control possible hepatotoxicity by extract; Group III, negative group received (Indomethacin 4mg/kg + distilled water 5mL/kg); Group IV positive group received (Indomethacin 4mg/kg+ silymarin 50 mg/kg); Group V received (Indomethacin 4mg/kg + aqueous extract 100mg/kg); Group VI received Indomethacin 4mg/kg+ aqueous extract 200mg/kg); Group VII received (Indomethacin 4mg/kg+ aqueous extract 300mg/kg). From Group III to VII, indomethacin was administered (4mg/kg) to induce hepatotoxicity every Tuesday, Thursday, Saturday and treatment was applied every Wednesday, Friday and Sunday for 4 weeks. At the end of the experiment on the twenty ninth day, the animals were sacrificed by decapitation. Blood samples were collected, centrifuged at 3000rpm for 15 minutes to obtain clear serum. The serum obtained was stored at -20°C and used for liver functional parameter analyses. Thereafter, animals were dissected, and liver tissues were removed and weighed to calculate liver's index (liver weiht/rat weight). One part was used to assess oxidative stress parameters and the other part was trimmed down for histological analysis.

2.5. Analysis of liver enzymes

The activity of transaminases alanine amino transferase (ALT) and aspartate amino transferase (AST) was assayed by the methods of Randox Laboratories (India) Pvt Ltd plot kit. Serum alkaline phosphatase (ALP) concentration was determined by the method of Randox Laboratories (India) Pvt Ltd plot kit. T Bil (total bilirubin) is considered as an indicator of cholestasis and was determined by colorimetric method as described by Randox Laboratories (India) Pvt Ltd plot kit.

2.6. Analysis of lipid profile

Total cholesterol, triglyceride (TG), and HDL- cholesterol were determined according to the method described by Randox Laboratories (India) Pvt Ltd plot kit. The concentration of LDL- cholesterol in samples was determined by the following equation described by Youmbie *et al* (2021) as follow:

$$\text{LDL cholesterol} = Total\ cholesterol - \frac{triglyceride}{5} - HDL\ cholesterol$$

2.7. Antioxidant activity

Measurement of some biomarkers of oxidative stress was as follows: The procedure to estimate the reduced glutathione (GSH) level was done following the method described by Ellman in 1959. The activity of catalase (CAT) in tissues was evaluated by the method of (Sinha, 1972). The superoxide dismutase (SOD) activity was determined in supernatant of homogenate by the method of (Misra and Fridovich, 1972). The protein content in the homogenate was measured using the method described by Gornall *et al*, (1949).

2.8 Histological studies

To study the liver sections under the microscope, 10% formalin was used for the fixation of the collected liver sample (Rashmi Ka & K Bhasker Shenoy 2020). Later, samples were passed via many proceedings of fixation, sectioning and staining. In fixation, liver samples of all experimental groups were kept in 10 % neutral formalin. After fixation, tissues were dehydrated in different percentages of alcohol (75 %, 95 % and 100% absolute), embedded in paraffin block and serially sectioned (5µm size) using a microtome. Liver sections were stained with Mayer hematoxylin and eosin. Indomethacin-induced liver damage was observed using a microscope (Zeiss, Hallherbermoos, Germany).

2.9. Statistical analysis

All data were presented as mean ± S.E.M of five rats (n=5). The difference between means was assessed by one-way and two-way Analysis of Variance (ANOVA), followed by Bonferroni post-test using GraphPad Prism 8.0.1.244.

3. RESULTS

3.1. Effect of the aqueous extract of *T. tetraptera* fruits on the body weight of rats in indomethacin-induced liver failure.

There was an overall, general significant ($p<0.001$) increase in body weights of normal group throughout experimental period. Indomethacin decreased significantly ($p<0.001$) the body weight of rats compared to the normal group and no significant change at the 4th week compared to 1st week. The three doses of the aqueous extract increased the body weight of the rats compared to the negative group with significant ($p<0.001$) change on body weight at the 4th week compared to the 1st week. Hence, co-treatment of rats with indomethacin and the three different doses of the aqueous extract fruits decreased the negative effect of indomethacin by causing a general increase in body weight of animals with the highest level of significance ($p<0.001$) observed at 200 and 300 mg/kg of extract compared to the group receiving only indomethacin (Figure 1).

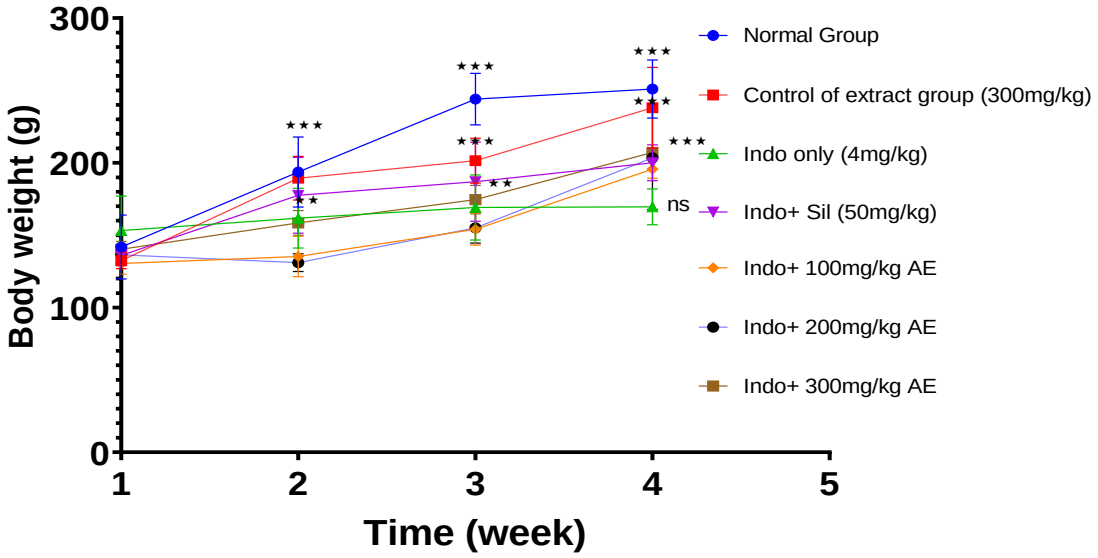

Figure 1: Effect of the aqueous extract of *T. tetraptera* fruits on body weight of indomethacin-induced liver failure rats. The values represent means ± S.E.M of 5 rats per group. ***p<0.001, significantly different compared to week 1. ns, not significantly different compared to week 1. Indo: Indomethacin; Sil: Silymarin; AE: Aqueous Extract.

3.2. Effect of the aqueous extract of *T. tetraptera* fruits on the liver index (liver weight/rat weight) in indomethacin-induced liver failure.

As shown in Figure 2 below, Indomethacin led to liver weight loss of the rats compared to the normal group. Liver index (liver weight/rat weight) in the negative group or indomethacin only was significantly decreased ($p<0.001$) when compared with the normal group. The three doses of aqueous extract increased the liver index. The significances observed at 200 ($p<0.05$) and 300 ($p<0.01$) mg/kg when compared to negative group are shown in (Figure 2).

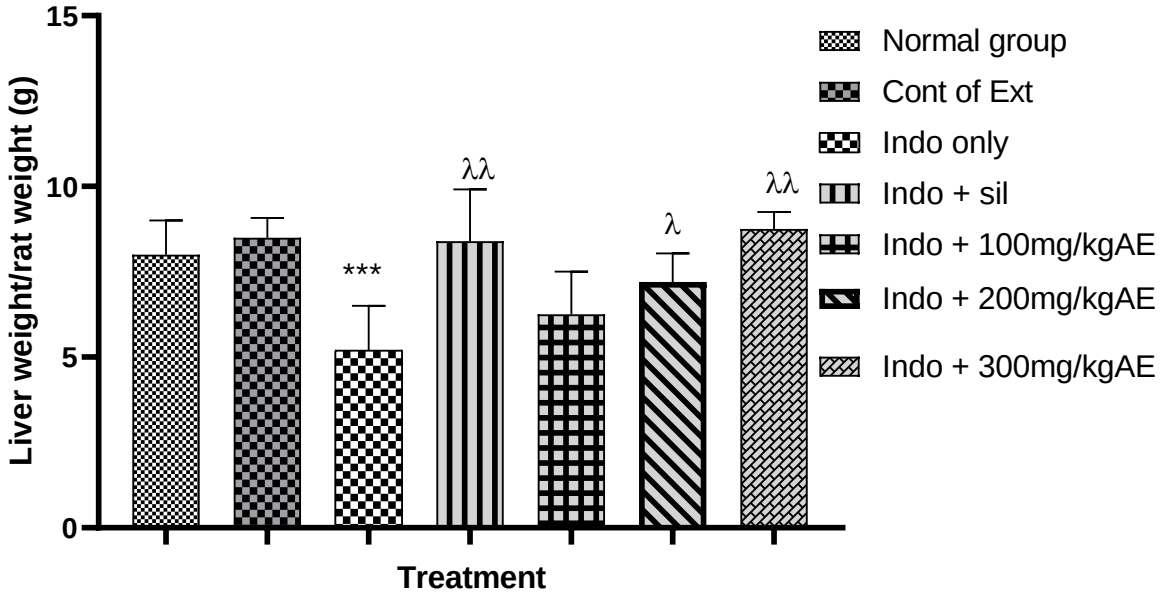

Figure 2: Effect of the aqueous extract of *T. tetraptera* fruits on liver index in indomethacin-induced liver failure. The values represent means ± SD of 5 rats per group. ***p<0.001, significantly different compared to normal group. $^{\lambda}$p<0.05; $^{\lambda\lambda}$p<0.01 significantly different compared to negative group. AE: Aqueous Extract; Cont of Ext: Control of Extract (300 mg/kg AE); Indo: Indomethacin (4 mg/kg) which is the negative group; Sil: silymarin (50 mg/kg) which is the positive group.

3.3. Effect of the aqueous extract of *T. tetraptera* fruits on enzyme parameters in indomethacin exposed Wistar rats.

Table 1: Effect of the aqueous extract of *T. tetraptera* fruits on AST, ALT, ALP and T. Bil. in the serum of indomethacin exposed Wistar rats.

Experimental groups	AST (Units/L)	ALT (Units/L)	T. Bil. (mg/dL)	ALP (Units/L)
Normal group (Distilled water, 5ml/kg)	68.97±1.61	84.26±9.96	1.49±0.19	22.74±1.02
Control of extract at 300mg/kg	67.18±1.07	58.40±1.65	1.49±0.22	26.36±1.17
Negative group (indomethacin only ,4mg/kg)	161.90±1.00***	181.10±1.91***	2.12±0.36*	44.35±1.08***
Indomethacin (4mg/kg) + Sylimarine(50mg/kg), positive group	67.01±1.83	85.55±1.52	1.77±0.36	34.20±0.95
Indomethacin (4mg/kg) + Extract (100mg/kg)	67.01±1.83	85.55±1.52	1.77±0.36	34.20±0.95
Indomethacin (4mg/kg) + Extract (200mg/kg)	132.20±1.63 $^{\lambda\lambda\lambda}$	137.20±1.65 $^{\lambda\lambda\lambda}$	1.66±0.14	47.45±1.44 $^{\lambda\lambda\lambda}$
Indomethacin (4mg/kg) + Extract (300mg/kg)	69.97±1.94 $^{\lambda\lambda\lambda}$	83.28±1.12 $^{\lambda\lambda\lambda}$	1.67±0.18	26.03±1.71 $^{\lambda\lambda\lambda}$

Results are expressed as mean ± S.E.M; n=5. *p<0.05, and ***p<0.001, significantly different compared to Normal group; λλλp<0.001 significantly different compared to negative group (Intoxicated rats, treated with distilled water); AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; T BIL: Total bilirubin.

As shown in table1, indomethacin-induced hepatotoxicity significantly increased ($p<0.001$) the level of serum's enzymes (ALT, AST and ALP) as well as total bilirubin ($p<0.05$) in negative group (rats treated with indomethacin and distilled water) when compared to normal group. Whereas rats of control extract group did not show any significant difference compared to the normal group. However, administration of aqueous extract at different doses significantly ($p<0.001$) lowered ALT and AST levels in serum compared to negative group. In addition, the fruits aqueous extract significantly decreases ($p<0.001$) the effect of indomethacin in the serum's ALP activity compared to the negative group. Furthermore, the level of ALP decreases with changes in concentration of aqueous extract fruits, it was more reduced ($p<0.001$) at 100 and 300 mg/kg when compared to the negative group. The three doses of aqueous extract fruits did not show any significant change in the level of total bilirubin. However, there was a slight decrease at 200 mg/kg (1.65±0.14) when compared to the negative group.

3.4. Effect of the aqueous extract of *T. tetraptera* fruits on lipid profiles in the serum of indomethacin exposed Wistar rats

Table 2 below shows the results of the lipid profiles. It is shown that Indomethacin has induced liver injury and significantly increased the level of the serum's total cholesterol, triglyceride and HDL-cholesterol ($p<0.001$) as well as LDL- cholesterol ($p<0.01$) in negative group when compared to normal group. Moreover, there is a significant decrease ($p<0.001$) in serum's total cholesterol in group treated with aqueous extract fruits at the doses of 100, 200 and 300 mg/kg when compared to negative group. Treating rats with different doses of aqueous extract fruits significantly decreases the effect of indomethacin on the serum's triglyceride level compared to negative group. Amongst all tested groups, serum's triglyceride at the dose of 300 mg/kg of *T. tetraptera* extract was quite reduced ($p<0.001$) compared to the negative group. Rats administration with the three different doses of the aqueous extract fruits has increased the level of HDL- cholesterol when compared to negative group. Depending on the dose, aqueous extract fruits (300mg/kg) significantly increased ($p<0.001$) the level of HDL-cholesterol when compared to the negative group. Treatment with the various doses of the aqueous extract fruits of *T. tetraptera* did not show any statistically significant change in the level of LDL-cholesterol. Whereas, depending on the dose, there was a decrease at 300 mg/kg (20.65±3.21) compared to the negative group.

3.5. Effect of the aqueous extract of *T. tetraptera* fruits on tissue's protein content concentration in indomethacin-induced liver injury.

Table 2: Effect of aqueous extract fruits of *T. tetraptera* fruits on total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in the serum of indomethacin exposed Wistar rats.

	Lipid parameters			
Experimental groups	**Total cholesterol (mg/dL)**	**Triglyceride (mg/dL)**	**HDL-cholesterol (mg/dL)**	**LDL-cholesterol (mg/dL)**
Normal group (Distilled water, 5ml/kg)	59.84± 1.11	70.6±1.82	29.64±1.39	16.89±3.82
Control of extract at 300mg/kg	57.28±1.23	70.67±1.92	31.69±1.47	11.29±3.71
Negative group (indomethacin only, 4mg/kg)	82.59±1.49***	107.3±2.08***	21.66±0.77***	39.48±1.61**
Indomethacin (4mg/kg) + Sylimarine(50mg/kg), positive group	55.38±1.30	74.55±1.74	29.54±1.53	14.97±3.20
Indomethacin (4mg/kg) + Extract (100mg/kg)	79.28±1.64 λλλ	83.72±2.25 λλ	24.44±0.88	38.09±3.57
Indomethacin (4mg/kg) + Extract (200mg/kg)	74.9±1.027 λλλ	84.62±2.22 λλ	27.28±1.07	26.7±3.13
Indomethacin (4mg/kg) + Extract (300mg/kg)	66.93±1.32 λλλ	73.58±1.97 λλλ	31.56±1.00 λλλ	20.65±3.21

Results are expressed as mean ± S.E.M; n=5. **p<0.01 and ***p<0.001 significantly different compared to Normal group, ΛΛp<0.01 and ΛΛΛp<0.001 significantly different compared to negative group (Intoxicated rats, treated with distilled water).

Administration of indomethacin significantly decreased ($p<0.001$) liver's protein content concentration as compared to the normal group. Regarding co-administration of rats with indomethacin and plant extract, figure 3 shows that, aqueous extract at the doses of 100, 200 and 300 mg/kg normalized the protein activity by decreasing the effect of indomethacin and significantly increasing ($p<0.01$) the level of protein concentration when compared to negative group (Figure 3).

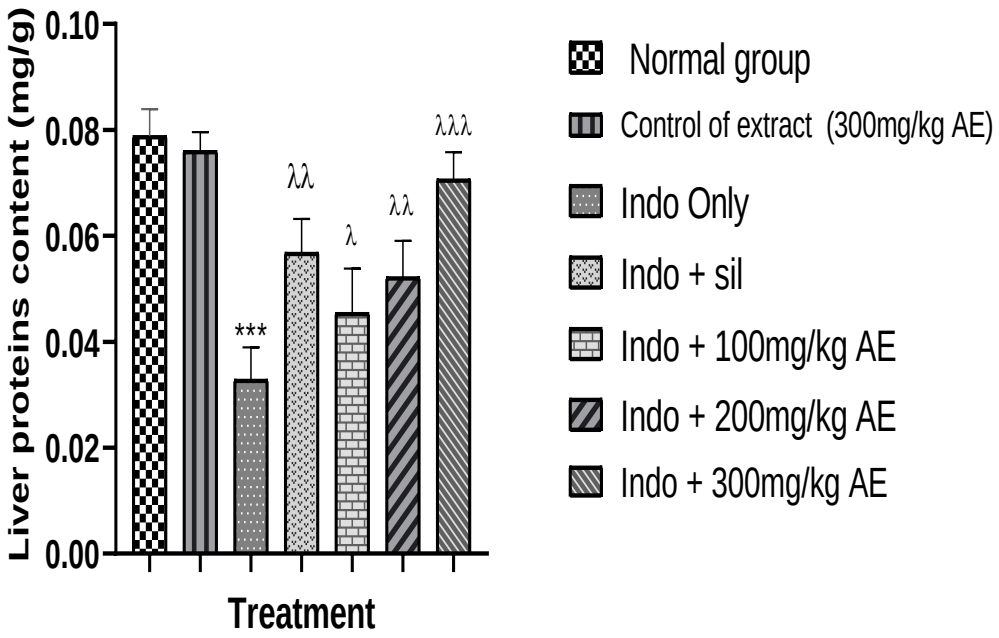

Figure 3. Effect of *T. tetraptera* fruits aqueous extract on proteins concentration on indomethacin liver injury. The values represent means ± S.E.M of 5 rats per group. ***p<0.001, significantly different compared to normal group. λp<0.05, λλp<0.01, λλλp<0.001 significantly different compared to negative group. AE: Aqueous Extract; Pharma group: pharmacological group (300 mg/kg AE); Indo: Indomethacin (4 mg/kg); Indo only: Negative group (Indo 4mg/kg + distilled water); Sil: Silymarin (50 mg/kg).

3.6. Effect of the aqueous extract of *T. tetraptera* fruits on oxidative stress in indomethacin induced liver injury.

Table 3: Effect of the aqueous extract of *T. tetraptera* fruits on oxidative stress in indomethacin induced liver injury.

	Stress parameters		
Experimental groups	**GSH (mmole/mg of proteins)**	**CAT activity (IU/mg of proteins)**	**SOD (IU/mg of proteins)**
Normal group (Distilled water, 5mL/kg)	0.51±0.03	0.09±0.01	0.04±0.04
Control of extract at 300mg/kg	0.51±0.02	0.07±0.03	0.05±0.05
Negative group (indomethacin only ,4mg/kg)	0.31±0.06***	0.06±0.01**	0.02±0.04*
Indomethacin (4mg/kg) + Sylimarine(50mg/kg), positive group	0.35±0.01	0.09±0.03	0.04±0.03
Indomethacin (4mg/kg) + Extract (100mg/kg)	0.31±0.02	0.05±0.07	0.01±0.02
Indomethacin (4mg/kg) + Extract (200mg/kg)	0.41±0.05$^{\lambda}$	0.07±0.03$^{\lambda}$	0.02±0.01
Indomethacin (4mg/kg) + Extract (300mg/kg)	0.51±0.01$^{\lambda\lambda\lambda}$	0.08±0.09$^{\lambda\lambda}$	0.05±0.06 $^{\lambda\lambda\lambda}$

Results are expressed as mean ± S.E.M; n=5. *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to Normal rats. $^{\lambda}$p<0.05, and $^{\lambda\lambda\lambda}$p<0.001 significantly different compared to negative (Intoxicated rats, treated with distilled water). GSH: Reduced glutathione; CAT: Catalase; SOD: Superoxide dismutase.

Administration of indomethacin alone or in combination with the aqueous extract fruits of *T. tetraptera* or silymarin has affected oxidative stress markers of the liver's rats (Table 3). Oral route administration of indomethacin significantly decreased liver's reduced glutathione (GSH) (p<0.001), catalase (CAT) activity (p<0.01), and superoxide dismutase (SOD) (p<0.05) when compared to normal group. The doses of 200 and 300mg/kg of aqueous extract fruits counteracted the effect of indomethacin, but also normalized the oxidative stress parameters as compared to the negative group. Treatment with the three doses of aqueous extract fruits, normalized CAT activity with significant increase at 200 and 300 mg/kg (p<0.05); p<0.01) respectively when

compared to the negative group. Co-treatment of rats with indomethacin and highest dose (300mg/kg) of aqueous extract fruit significantly increased ($p<0.001$) the activity of SOD as compared to the negative group (table 3).

3.7. Effect of the aqueous extract of *T. tetraptera* fruits on histology in indomethacin induced liver injury.

Histological findings supported the results obtained from biochemical estimation. As shown in Figure 4, the liver sections of normal group Figure 4A and Control extract group Figure 4B showed normal hepatic parenchyma architecture with hepatocytes arranged in cobblestones around the central lobular vein and regular liver histology. Whereas, negative group 4C showed liver alterations characterized by degenerating cells or hepatocellular confluent necrosis, mild portal inflammation or bile plugs or obstruction of bile ducts, and sinusoid dilation. Interestingly, the rats treated with highest dose of aqueous extract fruits (300 mg/kg) of T. tetraptera Figure 4G showed an improvement in liver structures similar to the normal group.

4. DISCUSSION

Antibiotics, antineoplastic agents and anti-tuberculosis drugs are groups of drugs identified as most likely to cause hepatotoxicity (Hayashi & Fontana 2014). This study aimed to evaluate the beneficial effect of *T. tetraptera* fruits in the treatment of indomethacin induced liver failure in Wistar rats. Indomethacin is considered to be safe at therapeutic doses and is a widely used antipyretic and analgesic drug in clinical practice. However, high dose of indomethacin in both humans and animals causes severe hepatoxicity and necrosis (Entedhar *et al*. 2017). The body weight is considered as a morphological parameter to test the effect of drugs in animals and humans. The results of this study showed that, the body weight and liver index (liver weight/rat weight) of rats treated with indomethacin (negative group) decreased significantly compared to normal rats, which suggests that cumulative dose of indomethacin has reached certain values in the body and caused hepatotoxicity, necrosis and other diseases leading to reduced body weight and liver weight.

The side effects of indomethacin overdose include: gastrointestinal bleeding, progressive anaemia, raised blood urea levels, and development of proteinuria, neutropenia and thrombocytopenia (Silva *et al*. 2012). Chougule suggested that, inhibition of protective prostaglandins PGE1, PGE2 and prostacyclin (PG12) may be one of the mechanisms by which indomethacin induces liver injury (Chougule *et al* 2018). In this study, administration of aqueous extract significantly decreased ($p<0.001$) body weight and liver index at the dose of 300 mg/kg when compared to the negative group; suggesting that the aqueous extract fruits might protect the general healthy conditions of animals suffering from liver dysfunction and stimulate protective prostaglandins. In addition, some components of plants may inhibit or reduce the side effects of indomethacin.

Serum aminotransferase assays are the most common laboratory tests for the detection of liver diseases. Commonly available tests include serum Alanine amino transferase (ALT) which is relatively specific, affected early by hepatotoxicity and is considered an excellent marker of cellular necrosis. Furthermore, bilirubin is of less importance, but can give additional information on liver damage. In this study, indomethacin caused a significant ($p<0.05$) increase of alanine amino transferase (ALT) and aspartate amino transferase (AST), total bilirubin (T Bil), alkaline phosphatase (ALP) in negative group compared to normal rats because liver marker enzymes might reflect cell rupture, a major permeability, cellular leakage, loss of functional integrity of cell membrane and release of these enzymes from the damaged parenchymal cells (Hanaa *et al*. 2015). Administration of the aqueous extract improved these parameters by significantly decreasing ($p<0.001$) the levels of ALT, AST, ALP and T.Bil; suggesting that the extract could contain substances capable to inhibit hepatic injuries and cholestasis. The decline in these parameters could be due to the presence of phytoconstituents such as tanins, capable of counteracting the toxic effects of indomethacin on hepatocyte membranes, flavonoids, capable ofprotecting the mitochondria by inhibiting cytochromes P450 2E1 and other enzymes responsible for necrosis and cholestasis. This result is consistent with the study of blessing *et al*. (2020) who showed that daily administration of the aqueous extract of *Telfaira occidentalis* to rats for 28 days induced significant decrease in transaminases.

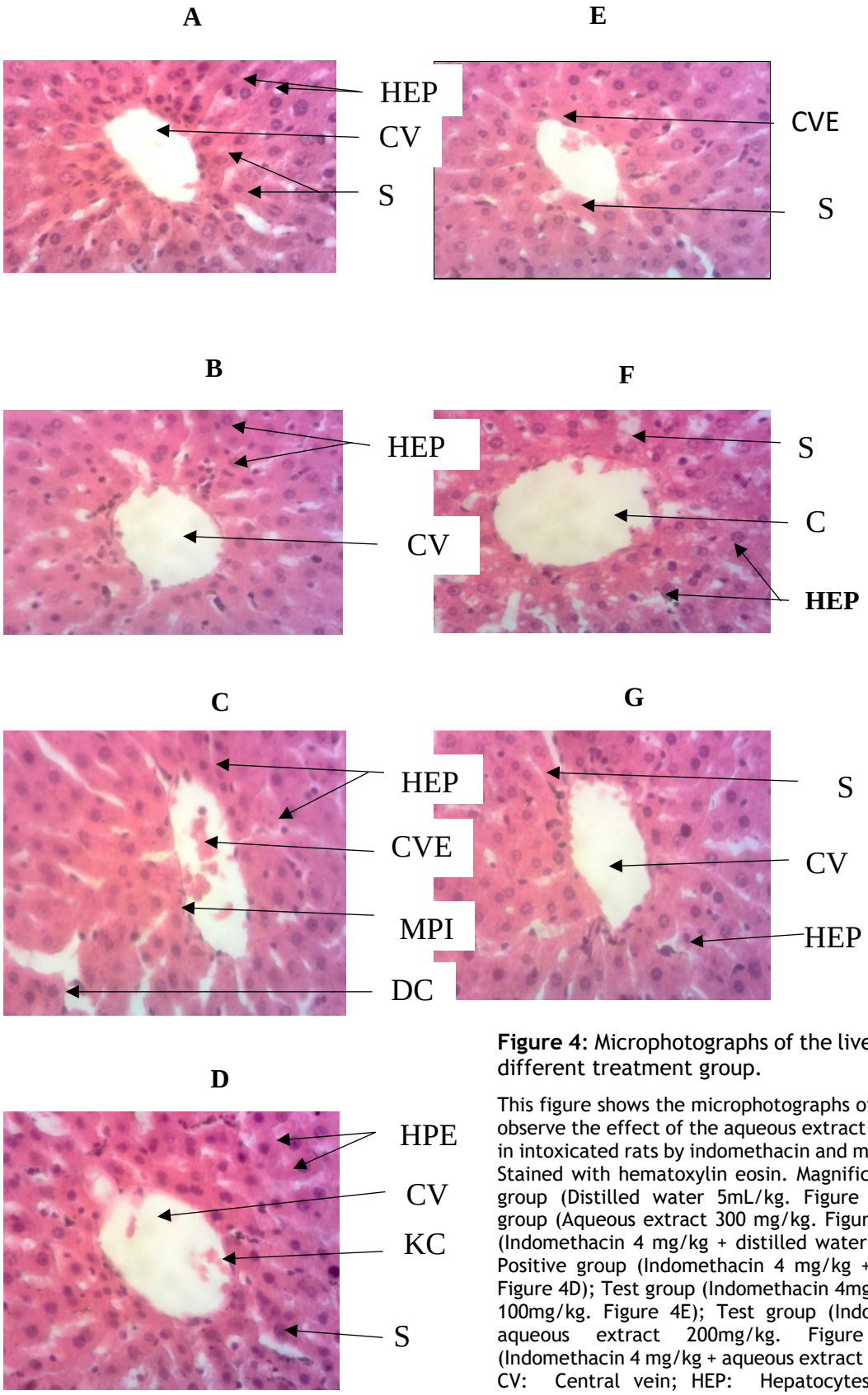

Figure 4: Microphotographs of the liver of Wistar rats with different treatment group.

This figure shows the microphotographs of the liver structure to observe the effect of the aqueous extract of *T. tetraptera* fruits in intoxicated rats by indomethacin and morphological analysis.? Stained with hematoxylin eosin. Magnification (x 400). Normal group (Distilled water 5mL/kg. Figure 4A); Pharmacological group (Aqueous extract 300 mg/kg. Figure 4B); Negative group (Indomethacin 4 mg/kg + distilled water 5 mL/kg. Figure 4C); Positive group (Indomethacin 4 mg/kg + silymarin 50 mg/kg. Figure 4D); Test group (Indomethacin 4mg/kg + aqueous extract 100mg/kg. Figure 4E); Test group (Indomethacin 4 mg/kg + aqueous extract 200mg/kg. Figure 4F); Test group (Indomethacin 4 mg/kg + aqueous extract 300mg/kg. Figure 4G). CV: Central vein; HEP: Hepatocytes; S: Sinusoids; DC: Degenerating cells; MPI: Mild portal inflammation; KC: Kupffer cells; CVE: Central vein enlargement; SD: Sinusoid dilatation.

The tissue protein level may indicate the state of the liver and the type of damage (Sabiu 2014). Tissue protein levels decreased significantly ($p<0.001$) in the indomethacin-treated rats (negative group) which may reflect decreased protein synthesis or increase protein loss, given that, nearly all proteins are synthesized in the liver.

Hence, hepatic failure is a cause of decreased tissue protein (Dogara, 2018). Administration of aqueous extract significantly increased tissue protein level which may be probably by stimulating DNA gene expression followed by protein synthesis.

Induction of liver failure by indomethacin resulted in a significant increase ($p<0.001$) of total cholesterol, triglyceride, and LDL-cholesterol in negative group when compared to normal group. In the literature, hypercholestemia and hyperglyceridemia are independent risk factors that alone or together could accelerate the development of coronary heart disease and the progression of atherosclerotic lesion of liver (Umamaheswari & Chatterjee 2009) thus, provoking the accumulation of triglyceride in the serum and increasing the bioavailability of free fatty acids (glycerophosphates) (Umamaheswari & Chatterjee 2009). Moreover, increased total cholesterol level is due to an increased activity of enzyme ß-hydroxymethylglutaryl CoA (HMGCoA), which led to an increase in the synthesis of cholesterol and its release in bloodstream (Umamaheswari & Chatterjee 2009). In the present study, the dose of 300mg/kg of aqueous extract fruits showed a significant decrease ($p<0.001$) of total cholesterol, triglyceride and HDL-cholesterol when compared to negative group. These results suggest that the aqueous extract fruits contain bioactive compounds with lipid-lowering properties. These bioactive compounds could inhibit the intestinal absorption of dietary cholesterol or synthesis of cholesterol by liver and stimulate the biliary secretion of cholesterol and then its excretion in the faeces to reduce the level of cholesterol. These results are similar to those of Krzeminski *et al* (2003) who studied the effect of different olive oils on bile secretion.

Liver injury is an inflammation. Moreover, inflammation and oxidative stress are two closely related events. Liver stress by indomethacin caused free radicals and reactive oxygen species (ROS) which are continuously generated inside the body because of exposure to many exogenous drugs (Hessah, 2017). The direct effect of the aqueous extract fruits of *T. tetraptera* as an antioxidant was evaluated via reduced glutathione (GSH),catalase (CAT) and superoxide dismutase (SOD). Administration of indomethacin significantly decreased CAT and SOD ($p<0.01$) as well as GSH ($p<0.001$) in negative group when compared to normal group. The therapeutic activities of indomethacin may derive from COX-2 blockage (COX-2 or inductive cyclooxygenase) (Wilmana & Gunawan 2012). Indeed, after oral administration, 92.99 % of the absorbed indomethacin drug will be bound to plasma proteins (Wilmana & Gunawan 2012). Indomethacin side effects are dose-dependent and the incidence is quite high. Unwanted side effects such as gastrointestinal ulceration, bleeding and plasma membrane damage of cells are due to inhibition of COX-1 (COX-1 or constitutive cyclooxygenase) (Kwiecien 2014). Indeed, indomethacin could induce the activation of inducible NO synthase for production of nitric oxide. NO is an inflammatory mediator that induces the synthesis of prostaglandins, cytokines and ROS which may justify significant decrease (table 3) of GSH, CAT, and SOD in negative group (Hessah, 2017). The aqueous extract fruits of *T. tetraptera* (300mg/kg) have significantly increased the levels of GSH and SOD ($p<0.001$) as well as CAT ($p<0.01$) compared to the negative group. This ability of the extract to restore the level of GSH and activities of enzymes (CAT and SOD) could be due to the ability of the bioactive metabolites contained in the extract to inhibit the synthesis of NO and limit the synthesis of ROS to reduce oxidative stress and pro-inflammatory mediators.

In other hand, histological observations (Figure 4) have shown that, oral administration of overdose of indomethacin induced hepatocyte degeneration, kuffer cells, sinusoidal dilation and mild portal inflammation in negative group (Fig 4C) when compared to normal group. These results indicate that overdose of indomethacin inducing intoxication of liver might cause hepatocyte destruction which could explain observed the liver abnormalities (Youmbie *et al* 2021). The dose of 300mg/kg of aqueous extract fruits of *T. tetraptera* repairs damaged hepatocytes, suggesting that the aqueous extract fruits of *T. tetraptera* might contain components capable to regenerate and/or repair hepatocytes (Figure 4G).

5. CONCLUSION

The findings of the present investigation demonstrated that aqueous extract of fruits of *T. tetraptera* is beneficial for patients suffering hepatic problem. Aqueous extract of fruits of *T. tetraptera* decreased serum levels (ALT and AST) transaminases, ALP, total bilirubin, triglyceride, HDL-cholesterol, LDL-cholesterol and total cholesterol. In addition, it increased GSH, CAT and SOD levels. Furthermore, it has hepatoprotective effect.

ACKNOWLEDGEMENTS. The authors wish to thank Mr. Takala Jean Claude, laboratory technician at the faculty of medicine and biomedical science of University of Yaoundé 1 for his implication in histological realisation.

Authors also thank Dr. Calvin Bogning Zangueu of the Department of Animal Organisms and Biology, Faculty of Science, University of Douala for its contribution by providing sylimarin.

AUTHOR CONTRIBUTIONS. Oumar M, Motika D K and Youmbie D D B conceived and designed the study. Motika D K and Marc A N S performed the experiments. Motika D K and Youmbie D D B analysed and interpreted the data. Oumar M and Youmbie D D B contributed to the writing of the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST. The authors declare that they have no conflicts of interest regarding the publication of this paper.

REFERENCES

Adewusi E, Afolayan A J (2019). A review of natural products with hepatoprotective activity. *Journal of Medicine and Plant Research*, 9(2):1518-1334.

Andréia A S, Anacharis B S, Adelar B, Sandra M G, Eloá A K, Cristina G M, Rosane M P (2013). Hepatoprotective Effects of Mushrooms. *Molecular and cell bioloigy,* 18(3): 63-76.

Blessing C E, Emmanuel C O, Damian N E, Ifeoma P E, Rosemary A A, Ifeoma J O, Akuma K A, Ifeakandu O O, Chikaodili N O E, Francis O E (2020). Hepatoprotective potential of the aqueous leaf extract of *Telfairia occidentalis* on the Liver function parameters in Adult Wistar Rats. *Annals of Geriatric Education and Medical Sciences*, 7(1):39–42

Chougule N B, Nitve S A, Koumaravelou K (2018). Phytochemical Investigation and Screening for Inflammatory Bowel Disease Activity of Ethanolic Extract Kariyat. *Pharmacology Journal*, 10(3): 602-610.

Dogara M A, Sarkiyayi S, Sheriff H G (2018). Effectsof *Canarium schweinfurthii* Oil Extract on Some Biochemical Indices on Indomethacin Induced Hepatotoxicity in Rats. *American Journal of Biochemistry*, 1: 7-12.

Ellman G L (1959). Tissue sulfhydryl groups. *Biochemistry and Biophysics,* 82: 70-77.

Entedhar R S, Siham A W, Ban I S, Thuraia R S (2019). Biochemical and Histological Evaluation of Indomethacin-induced Hepatotoxicity in Rats. *Science Translational Medicine,* 12(109): 1-6.

Gornall A G, Bardawil G S, David M M (1949). Determination of serum proteins by the mean of the Biuret reactions. *Journal of Biology and Chemistry* 177: 751-766.

Hanaa A, Hassan, Wafaa M, EL-Kholy, Nadine A, Galal (2015). Comparative Protective Effect of Moringa and Dandelion Extracts Against Hepatic Disorders and Oxidative Stress Associated with Prolonged Use of Brufen Drug in Rats. *The Egyptian Journal of Hospital Medicine*, 60; 336-346.

Hayashi P, Fontana R (2014). Clinical feature, diagnosis, and natural history of drug-induced liver injury. *Seminar of Liver Diseases* 2: 134-44.

Hessah M A (2017). Effect of *Moringa oleifera* leaves extract on the oxidative stress and gastric mucosal ulcer induced by indomethacin in rats. *African Journal of Biotechnology*.3: 51 -56

Hilal A M, Fatima M, Hossain M M, Chandra M A (2018). Determination of potential oxidative damage, hepatotoxicity, and cytogenotoxicity in male Wistar rats: Role of indomethacin. *J Biochem Mol Toxicol.,* 32(12): 222-226.

Kuate D, Kengne A P N, Biapa C P N, Azantsa B G K, Wan-Muda A M B (2015). *Tetrapleura tetraptera* spice attenuates high-carbohydrate, high-fat diet-induced obese and type 2 diabetic rats with metabolic syndrome features, *Lipids in Health and Disease*, 14: 41-50.

Krzeminski R, Gorinstein S, Leontowicz H, Leontowicz M, Gralak M, Czerwinski J (2003). Effect of different olive oils on bile excretion in rats fed cholesterol containing and cholesterol-free diets. *Journal of Agriculture, Food and Chemistry*, 51: 5774– 5779.

Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, Mach T, Wojcik D, Brzozowski T (2014). Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress–induced gastric injury. *J. Physiol. Pharmacol.*, 65(5):613-22.

Misra H P, Fridovic I (1993). The role of superoxide anion in the autoxidation of epinephrine and a sample assay for superoxide dismutase, *Journal of Biology and Chemistry*, 247: 170-174.

Nemat Z Y, Siham M, Rehab F A R, Mostafa Y, Mohamed H, Sherine H (2015). Effect of a topical copper indomethacin gel on inflammatory parameters in a rat model of osteoarthritis. *Drug Design, Development and Therapy*. 9: 1491–1498

Olatosin TM, Akinduko DS, Uche CZ (2013). Evaluation of the Hepatoprotective Efficacy of Moringa oleifera Seed Oil on Ccl4-Induced Liver Damage in Wistar Albino Rats. *The International Journal of Engineering and Science*, 2(11): 13-18.

Ornbjerg L M, Andersen H B, Kryger P, Cleal B (2008). What do patients in rheumatologic care know about the risks of NSAIDs? *Journal of Clinical Rheumatology*: practical reports on rheumatic musculoskeletal diseases 2: 3–7.

Sabiu S, Wudil A M, Sunmonu T O (2014). Combined administration of *Telfaira occidentalis* and *Vernonia amygdalina* leaf powders ameliorates garlic-induced hepatotoxicity in Wistar rats. *Pharmacologia*, 5: 191–198.

Salawu M O, Olugbode A E, Oloyede H OB (2018). Gastroprotective Activity of Fruit Ethanolic Extract of *Tetrapleura tetraptera* on Indomethacin-Induced Ulcer in Rats. *Al-Hikmah Journal of Pure & Applied Sciences,* 6: 20-29.

Silva M A, Rao V S, Souza, C M, Neves J C S, Menezes D B, Santos F A, Andrade G M (2012). Evaluation of thalidomide against indomethacin-induced small intestinal damage and systemic toxicity in rats. *Biomedical Research,* 1:1676-1683

Sinha A K, (1972). Colorimetric essay of catalase. *Analyses and Biochemistry*, 47: 389-394.

Sonibare M A, Onda E E, Ajayi A M, Umukoro S (2017). Anti-Inflammatory and Antioxidant Effects of *Tetrapleura tetraptera* (Schumach & Thonn.) Taub. Fruit Extract in Carrageenan/Kaolin-induced Acute Monoarthritis in Rats. *Nig. J. Pharm. Res*, 13(2):157-166.

Umamaheswari M, Chatterjee T K (2009). Effect of the fractions of *Ciccinia grandis* on ethanol induced cerebral oxidative stress in rats, *Pharmacological researches*, 1: 25-34.

United States Library of Medicine (2016).*Liver Tox Clinical and Research Information on Drug-Induced Liver Injury*. http://livertox.nih.gov.

Wilmana P F, & Gunawan S G (2012). Analgesik- Antipiretik Analgesik Anti -Inflamasi Nonsteroid dan Obat Gangguan Sendi Lainnya. In S. G. Gunawan (5Ed.), Farmakologi dan Terapi Edisi *Jakarta, Indonesia: Badan Penerbit FKUI*. 4: 230-246

Youmbie D D B, Kada S A, Fosting D, Essama M D S (2021). Hepatoprotective and Antioxidant Effects of Aqueous Extract of *Rauwolfia vomitoria* (Apocynaceae) Stem Bark in Wistar Rats. *Journal of Pharmaceutical and Medicinal Research*, 6(1): 112–116.