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Ameliorative effects of *Byrsocarpus coccineus*, *Dialium guineense* and *Newbouldia laevis* leaf extracts on diclofenac-induced hepatorenal injuries in Wistar rats

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ABSTRACT

Background & Aim: Diclofenac sodium (DFS) is a drug used for treatment of pain in humans and animals. Plant extracts have been used by the herbalists for treatment of hepatorenal disorders in humans for many years without scientific evidence. The present study evaluated the effects of *Byrsocarpus coccineus*, *Dialium guineense*, and *Newbouldia laevis* extracts (BCE, DGE and NLE respectively) on DFS-induced hepatorenal damage in rats and compared their efficacies.

Experimental: Rats were divided into 5 groups with 5 rats per group. The rats in group 1 were injected with normal saline while those in group 2 were injected with11 mg/kg DFS intramuscularly for 5 days. The rats in groups 3, 4 and 5 were injected with DFS like group 2 rats but treated with 342 mg/kg BCE, DGE and NLE respectively for 6 days. About 24 hours after treatment, blood was collected from a rat, serum was separated, and used for the estimation of biochemical parameters. The rats' liver and kidneys were removed and histological examinations were performed on the tissues of animals.

Results: There were significant ($P<0.05$) alterations in the levels of biomarkers of hepatorenal injuries in rats treated with DFS when compared with the control. The treatment of DFS-exposed rats with BCE, DGE and NLE significantly ($P<0.05$) reversed the altered markers of hepatorenal injuries when compared with the DFS group. The photomicrographs of hepatorenal tissues showed microstructural features which corroborated our biochemical findings.

Recommended applications/industries: These findings suggest that the three plant extracts have ameliorative effects on DFS-induced hepatorenal injuries in rats but DGE has the greatest efficacy. Therefore, they may be processed and utilized for the treatment of hepatorenal injuries in animals.

1. Introduction

The metabolism of drugs performed by the liver of animals, and to a lesser extent by the kidney makes these organs susceptible to the adverse effects of these foreign compounds (Hussain *et al.*, 2019). These metabolic processes may cause liver and kidney disorders, which are major public health problems worldwide (Eid *et al.*, 2015; Alorabi *et al.*, 2022). Diclofenac sodium (DFS) is a non-steroidal anti-inflammatory drug (NSAID), which is a derivative of phenyl acetic acid (Mei *et al.*, 2011; Bussmann *et al.*, 2022). It is commonly used as analgesic, for the treatment of pain and musculoskeletal diseases in

animals and humans (Owumi *et al.*, 2020). It is a potent inhibitor of cyclooxygenase, which catalyzes the synthesis of prostaglandins from arachidonic acid (Van Swelm *et al.*, 2013). It elevates the levels of malon dialdehyde, nitric oxide and pro-inflammatory cytokines, which suggests that the drug exerts its effect through oxidative stress and inflammatory responses in animal tissues (Izak-Shirian *et al.*, 2022). Thus, any plant extract with an antioxidant activity may be useful in the management of DFS-induced hepatic and renal disorders in animals.

There are many plants which have been used traditionally as herbal medicines by humans since ancient times, with varying degree of biological effectiveness (Eid *et al.*, 2015; Abdel-Baky and Abdel-Rahman, 2020). The three plant leaves used in this study were selected, after the herbalists claimed they can cure liver and kidney disorders but without any scientific evidence. *Byrsocarpus coccineus* Schum. & Thonn. - is a shrub which belongs to the family Connaraceae, and found in the tropical West Africa (Burkill, 1985). It is known by the name "Crimson thyme" (English), "Onyilakwechi" (Idoma), and "Tsaamiyar-kasa" in Hausa language (Agishi, 2010). Parts of the plant are used in the traditional medicine practice to treat toothache, diarrhoea, sores, and other diseases (Ahmadu *et al.*, 2006). Scientific evaluation of the medicinal potentials of this plant showed that the aqueous leaf extract possesses anti-diarrhoeal (Akindele and Adeyemi, 2006), anti-malarial (Kamanzi *et al.*, 2002), and anti-inflammatory (Akindele and Adeyemi, 2007) activities.

Dialium guineense Wild. - is a plant which belongs to the family Fabaceae and subfamily Caesalpinioideae. It is a tall, fruit-bearing tree, which has small, grape-sized edible fruits, with dark and hard inedible shells (Hutchinson and Daniel, 1958). It is known by the name "Velvet tamarind" (English), "Agigle" (Idoma), and "Tsamiyar biri" in Hausa language (Agishi, 2010). The leaves and stem bark are used in the traditional medicine practice as remedies for cough, diarrhoea, malaria fever, and jaundice (Bero *et al.*, 2009). The scientific evaluation of this plant's extract has shown that it has antiplasmodial (Bero *et al.*, 2009), antidiarrhoeal (Ogu and Amiebenomo, 2012), and antioxidant (Adeleye *et al.*, 2014) activities. *Newbouldia laevis* P. Beauv - is a medium-sized tree, belonging to the family Bignoniaceae, which can grow to a height of about 10 m. It is known by the name "Fertility tree" (English), "Ogblichi" (Idoma), and "Aduruku" in Hausa language (Agishi, 2010; Kolawole *et al.*, 2013). It is used in traditional medicine practice for the treatment of diabetes, toothache and different bacterial infections (Kolawole *et al.*, 2013). Scientific evaluation showed that its stem bark extract has radical scavenging effect on certain free radicals (Ogunlana *et al.*, 2008).

There is paucity of scientific reports on the ameliorative effects of BCE, DGE and NLE on DFS-induced hepatorenal injuries in rats, and the comparison of these extracts' therapeutic potentials. Therefore, this study was initiated to evaluate the ameliorative effects of these three plant leaf extracts on DFS-induced hepatorenal injuries in Wistar rats, and determine the extract which has the greatest efficacy.

2. Materials and Methods

All chemicals used were of analytical grade.

2.1. Preparation of diclofenac sodium

Diclofenac sodium (DFS) was purchased as an injectable liquid from the North China Pharmaceutical Co. Ltd, 115 Hainan Road, Shijiazhuang, China. Each 3 ml ampoule contains 75 mg of DFS. A single dose of the drug was suspended in 0.2 ml of normal saline (0.9 g/dL sodium chloride solution).

2.2. Preparation of ethanol extracts of B. coccineus, D. guineense and N. laevis leaves

The leaves of *B. coccineus*, *D. guineense* and *N. laevis* plants were harvested from a forest at Otukpa, in Benue state of Nigeria. They were identified and authenticated by Mr. Mark Uleh, a Taxonomist in the Department of Forestry and Forest Products, Federal University of Agriculture, Makurdi, Nigeria. The voucher specimens of the plant leaves were earlier deposited in the College of Forestry herbarium, and given voucher nos. as *B. coccineus* - FH/0138, *D. guineense* - FH/0257, and *N. laevis*- FH/0202. The leaves were dried at room temperature for about three weeks, pulverized to fine particles with mortar and pestle, and sieved with a porcelain sieve. The ethanol extracts of the three plants (BCE, DGE and NLE) were prepared by a modified form of a method previously described by Abotsi *et al.* (2010). A 100 g of each pulverized sample was placed in a white piece of cloth and tied, then placed in 500 ml of 95% ethanol and left

for 48 hours. Thereafter, the liquid mixture was filtered with Whatman no. 1 filter papers. The filtrate was concentrated at room temperature under electric fans and in a water-bath set at 50℃. Then, the extract obtained was dried to a constant weight in a desiccator. The weight of the extract was determined and the percentage yield was calculated by the expression:

$$\% \text{ Yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of dry sample (g)}} \times 100$$

The percentage yields of BCE, DGE and NLE were determined to be 4.48 % $^{w}/_{w}$, 6.18 % $^{w}/_{w}$ and 2.74 %$^{w}/_{w}$ respectively.

2.3. Experimental animals and management

Forty adult albino Wistar rats (*Rattus norvegicus*) of either sex, weighing 150 - 200 g, were purchased from the Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria. Fifteen rats were used for acute toxicity test while twenty-five rats were used for the main study. They were allowed to acclimatize for three weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi; under normal environmental conditions of 12 h dark and 12 h light cycle, with an average temperature of 29 ^{0}C. They were housed in plastic cages, fed with standard animal feeds, and clean water *ad libitum*. The rats were handled with care according to the Institutional and international guidelines for the use of laboratory animals.

The protocols adopted in this study were approved by the Animal Welfare and Ethics Committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria; with an approval reference no: FUAM/CVM/002.

2.4. Phytochemical analyses of B. coccineus, D. guineense and N. laevis leaf extracts

The aqueous leaf extracts of *B. coccineus*, *D. guineense* and *N. laevis* were prepared by maceration of 60 g powdered sample in 200 ml distilled water. After 48 hours, the mixture was filtered with Whatman no. 1 filter papers. The filtrate obtained was tested for phytochemical constituents according to the methods previously described by Trease and Evans (1989).

2.5. Acute toxicity tests

The acute toxicity test was done according to the limit dose protocol of the up and down procedure (ASTM, 1987). Five rats were each given a single dose of 5000 mg/kg b. wt. BCE, DGE or NLE at appropriate intervals and separately monitored for 24 hours, then up to two weeks, for visible signs of acute toxicity. All the 15 rats used for acute toxicity tests survived, and there was no visible sign of toxicity, only a reduction in feeding by animals was observed. The median lethal dose (LD_{50}) was determined for the three plant extracts as >5000 mg/kg b. wt., and this value served as a guide to estimate the dose of extract which was used for the treatment of DFS-exposed rats.

2.6. Animal groups and treatments

The rats were randomly divided into 5 groups, consisting of 5 rats per group.

Group 1 rats were injected 0.2 ml of normal saline per day via the intramuscular (i.m.) route for 5 days, and served as normal control.

Group 2 rats were injected with 11.0 mg/kg body weight DFS in 0.2 ml normal saline/day by the i.m. route for 5days, and served as DFS control.

Groups 3, 4, and 5 rats were injected with DFS as in group 2, and further treated with 342 mg/kg body weight/day BCE, DGE and NLE respectively by per os for 6 days.

2.7. Collection and preparation of serum and tissue samples

About 24 hours after treatment, blood was collected from rats by intra-cardiac puncture, under ether anesthesia. The blood was left to clot and centrifuged at 3000 rpm for 10 minutes. Thereafter, serum was separated with clean Pasteur pipettes and used for biochemical assays. The rats were euthanized, and their liver and kidney were immediately excised, rinsed in normal saline, dried and placed in 10 % formalin until when they were processed histologically.

2.8. Biochemical analyses

The biochemical parameters used for assessment of liver and kidney functions in rats were estimated by standard methods earlier described, and according to the procedures in the reagent kits manuals provided by Randox Laboratories Ltd, United Kingdom. The

biochemical parameters include serum aspartate aminotransferase and alanine aminotransferase (Reitman and Frankel, 1957), total proteins (Gornall *et al.*, 1949), albumin (Doumas *et al.*, 1971), creatinine (Bartels *et al.*, 1972), and urea (Fawcett and Scott, 1960). The serum levels of bilirubin, sodium ions, potassium ions, and chloride ions were determined by standard procedures as outlined in the reagent kits manuals manufactured by Agappe Diagnostics Ltd, Switzerland GmbH. After the samples were mixed with the appropriate reagents, they were incubated at the recommended temperatures and duration. The absorbance of each coloured solution was determined with a UV-VIS spectrophotometer at the appropriate wavelength. Globulin levels were determined by taking the difference between the values of total protein and albumin of a sample (He *et al.*, 2017).

2.9. Histological examinations of liver and kidney tissues

Histological examinations of liver and kidney tissues were carried out according to standard procedures. The liver and kidney tissues were fixed in 10% formalin, dehydrated with ascending grades of alcohol and cleared with xylene. Then, they were embedded with paraffin wax, sectioned into appropriate shapes and sizes (5 µm thickness) with a microtome, and stained with haemotoxylin and eosin (H&E) dyes. The glass slides of stained tissue sections were mounted and viewed under a light microscope at x10 and x40 objective lenses. The sections were captured with a microscope camera and presented as photomicrographs.

2.10. Statistical analysis

The data was statistically analyzed by the Statistical Package for Social Sciences (SPSS) version 21.0 software produced by IBM Corp. Ltd, U.S.A. All the data were expressed as Mean ± Standard Error of Mean (SEM), where n = 5, and analyzed by the one-way analysis of variance (ANOVA). The level of significance in the differences between group means was determined by the least significant difference (LSD) in a Post Hoc test. The differences between mean values were considered significant at $P<0.05$.

3. Results and discussion

3.1 Phytochemical compositions of B. coccineus, D. guineense and N. laevis leaf extracts

The phytochemicals in the aqueous leaf extract of *B. coccineus* include saponins, steroids, reducing sugars, phlobatannins, and anthraquinones. The aqueous leaf extract of *D. guineense* contains saponins, anthraquinones, phlobatannins, and alkaloids while the *N. laevis* aqueous leaf extract contains saponins, phlobatannins, and anthraquinones.

The experimental studies of drug-induced hepatorenal toxicity in rats and the ameliorative effects of plant extracts have been extensively studied, in order to evaluate their pharmacological effects on animal tissues (Kadir *et al.*, 2013; Hussain *et al.*, 2019; Alorabi *et al.*, 2022). To compare the efficacies of plant extracts with ameliorative effects on drug-induced hepatorenal injuries in animals is important, so as to determine which extract has the greatest biological activity (Ogbe *et al.*, 2020). The medicinal value of plants has been attributed to the pharmacological activities of their phytochemical constituents such as flavonoids, alkaloids, and other phenolic compounds (Kadir *et al.*, 2013; Rajakrishnan *et al.*, 2017). The plant extracts; BCE, DGE and NLE used in the present study contain phytochemicals such as saponins, phlobatannins and anthraquinones but only DGE contains alkaloids. These secondary metabolites of plants may be responsible for the ameliorative effects of these extracts on DFS-induced hepatorenal injuries in rats. However, as shown by this study, DGE contains alkaloid, which is the most biologically active phytochemical found in these three plant extracts, as commonly reported in literature.

3.2 Effect of B. coccineus, D. guineense and N. laevis extracts on the biomarkers of liver and kidney injuries in DFS-injected rats

There was a significant ($P<0.05$) increase in the levels of serum ALT, AST, bilirubin, total protein and globulin, but a significant ($P<0.05$) decrease in the levels of albumin and A/G in the group of rats injected with 11.0 mg/kg b. wt. DFS when compared with the normal control. The treatment of DFS-injected rats with 342 mg/kg b. wt. BCE and DGE significantly

(P<0.05) reduced the levels of ALT, AST, bilirubin, total protein and globulin but significantly (P<0.05) increased the A/G, while the DGE significantly (P<0.05) increased the albumin levels when compared with DFS-injected group. The treatment of DFS-injected rats with 342 mg/kg b. wt. NLE significantly (P<0.05) reduced the levels of ALT, AST, total protein, and globulin but significantly (P<0.05) increased the A/G when compared with the DFS-injected group. There was no significant (P>0.05) difference in the levels of ALT, total bilirubin, total protein, globulin and A/G in DFS-injected rats treated with the 342 mg/kg b. wt. BCE and DGE when compared with the normal control (Table 1 and 2).

Table 1. Effect of *B. coccineus*, *D. guineense* and *N. laevis* extracts on serum markers of liver injury in DFS-treated rats.

Treatment groups	Levels of serum markers of hepatic damage in rats				
	ALT (U/L)	AST (U/L)	T-bilirubin (mg/dL)	D-bilirubin (mg/dL)	Unconjugated bilirubin (mg/dL)
1. Control	73.52±4.44	83.16±3.42	0.62±0.10	0.16±0.04	0.46±0.06
2. DFS	185.06±1.27^{a}	274.8±7.71^{a}	2.10±0.43^{a}	1.09±0.22^{a}	1.01±0.35^{a}
3. DFS + BCE	90.68±8.44^{b}	143.1±5.46^{ab}	1.10±0.13^{b}	0.66±0.09^{ab}	0.44±0.06^{b}
4. DFS + DGE	79.80±6.00^{b}	162.1±18.76^{ab}	1.20±0.18^{b}	0.30±0.06^{b}	0.90±0.12
5. DFS + NLE	74.10±3.13^{b}	185.6±5.54^{ab}	1.71±0.19^{a}	0.82±0.27^{a}	0.89±0.16

Values are Mean±SEM, n=5; DFS - Diclofenac sodium, T - Total, D - Direct, BCE – *Byrsocarpus coccineus* extract, DGE – *Dialium guineense* extract, NLE –*Newbouldia laevis* extract. a: significantly different from control group (P<0.05); b: significantly different from DFS-treated group (P<0.05)

Table 2. Effect of *B. coccineus*, *D. guineense* and *N. laevis* extracts on the serum protein levels in DFS-treated rats.

Treatment groups	Levels of serum proteins in rats			
	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	A/G
1.Control	68.53±0.86	32.08±0.17	36.51±0.69	0.87±0.01
2.DFS	200.42±8.90^{a}	22.24±2.54^{a}	178.18±6.43^{a}	0.12±0.01^{a}
3.DFS + BCE	65.83±2.65^{b}	25.56±0.70^{a}	39.53±1.96^{b}	0.64±0.02^{b}
4.DFS + DGE	62.84±1.64^{b}	30.92±0.75^{b}	31.92±1.26^{b}	0.97±0.04^{b}
5. DFS + NLE	48.34±1.19^{ab}	24.62±1.49^{a}	23.72±0.52^{ab}	1.00±0.09^{b}

Values are Mean±SEM, n=5; DFS - Diclofenac sodium, BCE –*Byrsocarpus coccineus* extract, DGE – *Dialium guineense* extract, NLE – *Newbouldia laevis* extract. a: significantly different from the control group (P<0.05); b: significantly different from DFS-treated group (P<0.05).

Liver and kidney tissue injuries in animals may be induced by the adverse effects of DFS overdose (Owumi *et al.*, 2020). Therefore, the marked increase in the levels of serum AST, ALT, bilirubin, total protein and globulin, and the decrease in the levels of albumin and albumin-globulin ratio, after the injection of rats with DFS may indicate hepatocellular and hepatobiliary impairments in rats, which may be attributed to the adverse effects of the drug. These findings are in agreement with earlier researchers who reported that alterations in the levels of serum aminotransferases, total protein, bilirubin, albumin, globulin, and A-G ratio are evidences of patho-biochemical changes in rats (Eid *et al.*, 2015; Jeyadevi *et al.*, 2019; Al-Asmari *et al.*, 2020). Thus, these biochemical parameters are commonly used as biomarkers of liver tissue injury in animals. The activities of aminotransferases in the blood of rat sare normally low but when there is hepatocellular damage, they leak out into the general circulation, resulting in their elevated values in the blood plasma (Eid *et al.*, 2015). The elevated levels of bilirubin may be due to hepatobiliary impairment in rats. Serum bilirubin may be elevated when there is an abnormal increase in erythrocyte breakdown (haemolysis), and a decreased capacity of the liver to conjugate and excrete this metabolic product (Jeyadevi *et al.*, 2019). The elevated levels of serum total protein, and the reduced levels of albumin, with a marked increase in the levels of globulin, following the injection of rats with DFS may indicate hepatocellular impairments, attributed to the deleterious effect of the drug in the animal tissues (Iseghohi and Orhue, 2017; Hussain *et al.*, 2019). Previous studies showed that the alteration in the levels

of serum proteins may vary depending on the degree of liver damage (Eid *et al.*, 2015). The elevation in globulin level has been found to be associated with chronic inflammation (Wang *et al.*, 2016). The plasma albumin participates in physiological activities of an animal's body such as the transport of cations, drugs, and bilirubin (He *et al.*, 2017), thus its reduced level may affect these functions in rats.

The findings of several studies have shown that herbal extracts have pharmacological effects on drug-induced liver injury in experimental animals, and some may even reverse the altered levels of the biomarkers of liver injury to near normal values (Eid *et al.*, 2015; Hussain *et al.*, 2019; Al-Asmari *et al.*, 2020). Thus, the marked reduction in serum AST and ALT activities, following treatment of DFS-exposed rats with BCE, DGE and NLE may suggest that these extracts have ameliorative effects on DFS-induced hepatocellular injury in rats. The reduction in serum bilirubin by the treatment of DFS-exposed rats with BCE and DGE may indicate that the plant extracts have the potential to ameliorate hepatobiliary impairments in rats. The elevation in the levels of albumin and A-G ratio, with a concomitant reduction in the levels of total protein and globulin, following the treatment of DFS-exposed rats with BCE, DGE and NLE may suggest that these plant extracts have ameliorative effects on hepatocellular impairments in rats. These findings may imply that the treatment of DFS-injected rats with the three plant extracts could enhance the capacity of the liver to synthesize albumin, promote the conjugation and excretion of excess bilirubin from the body. The present findings have also shown that the treatment of DFS-injected rats with these plant extracts may inhibit the synthesis of globulin and its release into the blood, thereby mitigating the inflammation of hepatic tissues in the animals. These findings are in agreement with Eid *et al.* (2015) who found that the treatment of drug-intoxicated rats with plant extracts remarkably reduced the levels of serum ALT, AST, and total protein, and Al-Asmari *et al.* (2020) who reported that the treatment of drug-injected rats with plant extracts restored the altered levels of serum bilirubin and albumin to near normal values. The restoration of the levels of direct bilirubin, albumin and A-G ratio by the DGE was closest to the normal control values than that of NLE and BCE.

There was a significant ($P<0.05$) increase in the levels of serum creatinine, urea, and sodium ions in the DFS-injected rats when compared with the normal control. The treatment of DFS-injected rats with 342 mg/kg b. wt. BCE and DGE significantly ($P<0.05$) reduced the serum levels of creatinine, urea and sodium ions when compared with the DFS-injected group. However, there was no significant ($P>0.05$) difference between the levels of creatinine, urea and sodium ions in DFS-injected rats treated with DGE and the control. There was a significant ($P<0.05$) reduction in the levels of sodium ions but there was no significant ($P>0.05$) difference in the levels of creatinine, urea, potassium and chloride ions in DFS-injected rats, treated with 342 mg/kg b. wt. NLE, when compared with the DFS-injected group (Table 3).

Table 3. Effect of *B. coccineus*, *D. guineense* and *N. laevis* extracts on serum markers of kidney injury and electrolytes in DFS-treated rats.

Treatment groups	Levels of serum electrolytes and markers of renal damage in rats				
	Creatinine (µmol/L)	Urea (mmol/L)	Na^+ (mmol/L)	K^+(mmol/L)	Cl^- (mmol/L)
1.Control	63.64±1.77	9.03±0.17	161.38±1.38	10.40±1.18	82.70±0.69
2.DFS	71.14±2.08^a	44.86±0.64^a	572.65±24.1^a	8.28±0.70	82.00±0.50
3.DFS + BCE	52.45±2.86^{ab}	8.01±0.06^b	146.18±6.77^{ab}	6.66±0.27^a	92.24±1.27^{ab}
4.DFS + DGE	57.46±3.42^b	9.76±0.75^b	151.90±8.74^b	11.10±1.55	82.60±0.88
5. DFS + NLE	71.32±0.38^a	41.21±1.74^a	299.12±47.1^{ab}	8.85±1.32	81.70±0.99

Values are Mean±SEM, n=5; DFS - Diclofenac sodium, BCE – *Byrsocarpus coccineus* extract, DGE – *Dialium guineense* extract, NLE – *Newbouldia laevis* extract. a: significantly different from the control group ($P<0.05$). b: significantly different from DFS-treated group ($P<0.05$).

The elevation in the levels of serum creatinine and urea are commonly used as markers of renal injury in animals (Hussain *et al.*, 2019; Al-Asmari *et al.*, 2020; Alorabi *et al.*, 2022). Thus, the marked elevation in the levels of serum creatinine and urea, after the injection of rats with DFS may indicate an acute renal injury, which may be attributed to the adverse effects of the drug. The urea and creatinine are excretory products of

the catabolism of proteins and creatine respectively, which are removed from the blood of animals through the glomerular filtration and excretion by the kidney (Elsawy *et al.*, 2021). The impairment in renal function, as a result of tissue damage and reduced glomerular filtration rate (GFR), may cause increase in the levels of these markers of renal damage in the blood of rats (Ogbe *et al.*, 2019). Serum creatinine and urea levels were elevated in rats, following their injection with high doses of DFS in this study. The DFS causes a decrease in GFR, resulting in reduced excretion of urea, which may lead to an increase in its level in the blood (Dhanvijay *et al.*, 2013). It is an inhibitor of cyclooxygenases that catalyze the production of prostaglandins from arachidonic acid, which plays an important role in maintaining the GFR of the kidneys (Owumi *et al.*, 2020). Previous study has shown that an increase in serum creatinine may represent a marked decrease in GFR of the kidneys of rats (Alorabi *et al.*, 2022). Thus, DFS may alter the renal functions through its effect on renal prostaglandins, resulting in reduced GFR, and the accumulation of urea and creatinine in the blood (Izak-Shirian *et al.*, 2022). The hypernatremia found in this study is in agreement with Dhanvijay *et al*. (2013) who reported an elevation in the levels of sodium ions in the serum of a patient who was exposed to DFS, and Ogbe *et al*. (2020) who found elevated levels of serum sodium ions in DFS-injected rats.

Some plant extracts have been reported to produce ameliorative effects on drug-induced renal injury in experimental animals (Iseghohi and Orhue, 2017; Hussain *et al.*, 2019; Al-Asmari *et al.*, 2020). Thus, the marked reduction in the levels of serum creatinine and urea, following the treatment of DFS-intoxicated rats with BCE and DGE may suggest that the plant extracts have ameliorative effects on the nephrotoxic injury induced by this drug, and might promote the rapid healing of renal tissue injury. The present findings are in agreement with Kadir *et al*. (2013) and Al-Asmari *et al*. (2020) who demonstrated that treatment of drug-exposed rats with plant extracts markedly reduced the levels of serum creatinine and urea to near normal values, indicating that the extracts have ameliorative effects on drug-induced renal damage in rats. The marked reduction in the levels of sodium ions, following the treatment of DFS-exposed rats with BCE, DGE and NLE may suggest that the extracts have the potential to restore electrolytes balance in drug-exposed animals. The present finding agreed with Al-Asmari *et al*. (2020) who showed that treatment of drug-exposed rats with a plant extract can reduce the levels of serum Na^+ to near normal values, indicating that the extract has the potential to restore electrolyte balance in animals. The reductions of serum creatinine, urea and Na^+ levels by DGE were closest to the normal control values than that of BCE and NLE.

3.3 Effect of B. coccineus, D. guineense and N. laevis extracts on histological features of DFS-injected rats

The photomicrograph of the liver tissue section of group 1 rats injected with only normal saline showed intact hepatocyte nuclei, surrounded by intact cytoplasm, with few kupffer cells and clear sinusoids, without any sign of tissue degeneration. The tissue section of group 2 rats injected with DFS showed degenerative changes, evident by pyknotic changes in hepatocytes, infiltration of intercellular spaces by inflammatory cells and vascular congestions (Figure 1).

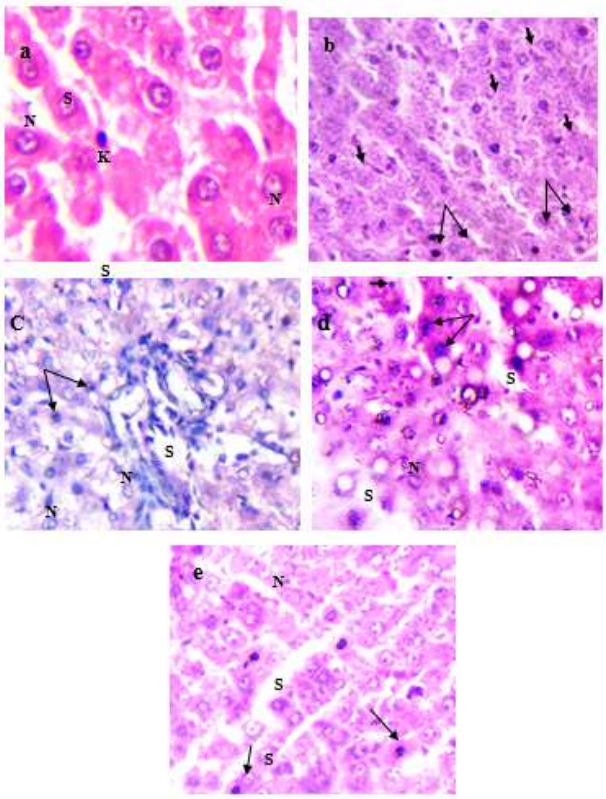

Figure 1. Photomicrographs of liver sections from DFS-exposed rats treated with *B. coccineus*, *D. guineense*, and *N. laevis* extracts (x 400, H&E)
(a) Control (b) DFS only (c) DFS + *B. coccineus* leaf extract (d) DFS + *D. guineense* leaf extract (e) DFS + *N. laevis* leaf extract

DFS - Diclofenac sodium, Thick black arrows - Inflammatory cells, Thin black arrows - Pyknotic hepatocytes, N - Hepatocyte nuclei, K - Kupffer cell, S - Hepatic sinosoids.

The photomicrograph of the kidney tissue section of group 1 rats injected with only normal saline showed normal renal tubules, with their tubular epithelial cell nuclei, and no sign of degenerative changes. The kidney tissue section of group 2 rats injected with DFS showed degenerative changes in the medulla and renal tubules, with infiltration of the renal and interstitial area by inflammatory cells, indicating interstitial nephritis and tissue necrosis (Figure 2).

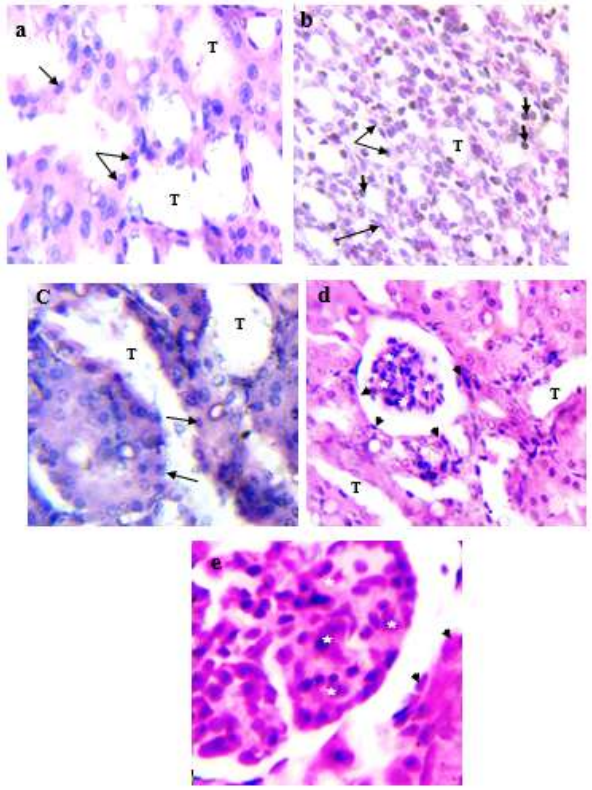

Figure 2. Photomicrographs of kidney sections from DFS-exposed rats treated with *B. coccineus*, *D. guineense* and *N. laevis* extracts (x 400, H&E)
(a) Control (b) DFS only (c) DFS + *B. coccineus* leaf extract (d) DFS + *D. guineense* leaf extract (e) DFS + *N. laevis* leaf extract
DFS - Diclofenac sodium, White stars - Glomerulus, Black arrow heads - Bowman's capsule, T - Renal tubules, Thick black arrows - Inflammatory cells, thin black arrows - tubular epithelial cell nuclei.

However, the photomicrograph of liver tissue section of group 3 rats injected with DFS and treated with BCE showed normal hepatocyte nuclei, hepatic sinusoids, vascular congestion and few hepatocyte nuclei with pyknotic changes. The liver tissue section of group 4 rats injected with DFS and treated with DGE showed few inflammatory cells and mild degenerative or pyknotic changes in the hepatocytes. The liver tissue section of group 5 rats injected with DFS and treated with NLE generally showed normal hepatocyte nuclei but there were few pyknotic hepatocytes. The degenerative changes in all the treated groups of rats are less severe when compared with the group injected with DFS but left untreated (Figure 1). The kidney section of group 3 rats injected with DFS and treated with BCE showed a normal renal tissue, with intact tubules, without any evidence of tissue necrosis. The kidney section of group 4 rats injected with DFS and treated with DGE showed renal tissue with intact but shrunken glomerulus, Bowman's capsule with enlarged capsular space and intact renal tubules, without any evidence of lesion or tissue necrosis. The kidney section of group 5 rats injected with DFS and treated with NLE showed normal renal tissues with intact glomerulus, renal tubules and Bowman's capsule (Figure 2).

Several reports have shown that the photomicrographs of animal tissue sections may reveal the microstructural features of organs such as the liver and kidney, which could provide supportive evidence for the biochemical findings of a particular study (Eid *et al.*, 2015; Hussain *et al.*, 2019; Alorabi *et al.*, 2022). Thus, the degenerative changes in liver and kidney tissues of DFS-exposed rats such as pyknotic changes in hepatocytes, the infiltration of intercellular spaces by inflammatory cells, and renal tubular disruptions are indicators of tissue injuries in the animals. These findings are attributed to the adverse effect of DFS, which was earlier shown to cause tissue damage by oxidative stress, inflammatory responses, and the induction of hepatocyte apoptosis or necrosis (Alorabi *et al.*, 2022). Scientific reports from laboratory animal studies showed that nephrotoxicity may be preceded by DNA fragmentation and oxidative stress, with the appearance of several histopathological lesions, and increase in the markers of tissue damage in animals (Ahmed *et al.*, 2017; Izak-Shirian *et al.*, 2022). These findings are in agreement with Eid *et al.* (2015) who reported degenerative changes in hepatic tissues of rats, following their exposure to carbon tetrachloride, and Alorabi *et al.* (2022) who found necrotic changes in renal tissues of rats after their exposure to DFS.

The treatment of drug-induced hepatorenal damage with certain plant extracts may have ameliorative effects on the hepatorenal tissues, leading to the early recovery from tissue damage, prevention of severe tissue injury and death of the animals (Iseghohi and Orhue, 2017). The reduced necrotic changes in hepatic and renal tissues, marked decrease in the number of inflammatory cells and other signs of inflammation in the tissues, by treatment of DFS-injected rats with BCE, DGE and NLE corroborated our biochemical findings; that the plant extracts have ameliorative effects on hepatorenal damage induced by the adverse effects of this drug. These findings are in agreement with Eid *et al*. (2015) who found that treatment of carbon tetrachloride-injected rats with plant extract has protective effect against hepatic tissue damage, with evidence of reduced pathological changes in the animal tissues. Our findings also agreed with Al-Asmari *et al*. (2020) who reported that the treatment of rats with paracetamol and plant extract has protective effect against the drug-induced hepatorenal damage in rats, with evidence of reduced necrotic changes in the tissues of rats. The protective effects of the plant extracts were attributed to the presence of phytochemicals which have anti-inflammatory and anti-oxidative activities in the tissues of animals.

4. Conclusion

The present study has shown that BCE, DGE and NLE may have ameliorative effects on DFS-induced liver and kidney injuries in rats but DGE is the most effective. The biological and pharmacological activities of these plant extracts may be attributed to their phytochemicals, which have anti-inflammatory and immuno-modulatory activities in animals. Thus, the findings of this study have given credence to the use of these plant extracts by African traditional medicine practitioners for the management of liver and kidney diseases in humans. However, there is need to conduct bioassay-guided isolation and characterizations of the bioactive compounds present in the DGE, which may be responsible for the bioactivity of this plant extract, and decipher the mechanism of action of its bioactive compounds.

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