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ORIGINAL ARTICLE

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Therapeutic effects of hydroethanolic extract of *Erythrina senegalensis* in diclofenac sodium-induced hepatotoxicity in male Wistar rat: biochemical, redox potential and histopathological outcomes

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Background: Hepatotoxicity is one of the main side effects associated with Diclofenac sodium (DFS) administration. The present study aimed to examine the therapeutic effects of hydroethanolic leaf extract of *Erythrina senegalensis* (HELEES) on DFS-induced hepatotoxicity. Thirty male Wistar rats, 5 per group, were used in this study. They were randomly divided into 6 experimental groups (A–F) and treated for 21 days. Rats in Group A served as the control group and received distilled water orally; group B was given DFS at 10 mg/kg body weight intraperitoneally (IP). HELEES were given to groups C and D at doses of 200 and 400 mg/kg body weight, respectively. Groups E and F were given DFS at 10 mg/kg + HELEES at 200 and 400 mg/kg respectively.

Results: DFS administration significantly increased the bilirubin concentration and serum transaminases (ALT, AST, GGT, and ALP) and LDH; total protein and albumin were significantly inhibited. There was a significant reduction in hepatic reduced glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPx), and nitric oxide (NO) activity, together with a significant increase in hepatic malondialdehyde (MAD), superoxide dismutase (SOD), and catalase (CAT). However, concurrent treatment with DFS + HELEES ameliorated the DFS-induced hepatotoxicity and oxidative stress. The results suggest that HELEES may offer some therapeutic effects against hepatic damage. In contrast to the control and HELEES-only groups, which had normal hepatic tissue morphology, rats given DFS alone developed hepatic necrosis and periportal inflammation, with the presence of numerous inflammatory cells and Kuppel cells. Examinations of liver samples from the groups given Concurrent treatment with DFS and HELEES revealed patterns that were comparable to those seen in the control group. Combining DFS with HELEES has always reduced the impact of DFS.

Conclusions: Collectively, HELEES enhanced hepatic function in DFS-treated rats by suppressing nitrosative and oxidative stress.

Key words: Diclofenac, erythrina senegalensis, liver, oxidative stress, rats

The clinical relevance of diclofenac sodium (2-[2,6-dichloranilino] phenylacetic acid) cannot be underrated. The drug is a well-known nonsteroidal anti-inflammatory, analgesic, and antipyretic agent (Vohra *et al*., 2016), which has been widely used in the management of several chronic disease conditions, such as degenerative joint disease, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis (Thanagari *et al*., 2012), actinic keratosis (Kavasi *et al*., 2017), among others. The European Medicines Agency (EMVA/CVMP) (2014) noted that although its primary use is in humans, it is also permitted for veterinary use in several European member states. Additionally, from the 1990s to 2006, the Indian subcontinent registered it for use in treating inflammatory conditions in domesticated ungulates (Risebrough, 2006). It exerts its therapeutic effects by blocking the activity of cyclo-oxygenase-2, thereby inhibiting the formation of prostaglandins from arachidonic acid. (Aycan *et al.,* 2018; Gupta *et al*., 2020; Adeyemi *et al*., 2019).

Despite the drug's evident therapeutic effects, diclofenac sodium (DSF) usage is known to injure the different organs in both humans and animals (Huo *et al*., 2020). DF intoxication can lead to a variety of symptoms, including those that are GI tract-related (such as nausea, abdominal pain, gastritis, and vomiting), skin-related (such as urticaria and itching and redness of the skin), renal- as well as liver-related (such as liver injury), in addition to other non-specific symptoms (general weakness) (Boelasterli, 2003; Gor and Saksena, 2011).

Liver injury has been identified as the common cause of the withdrawal of some nonsteroidal anti-inflammatory drugs (NSAIDs) including DFS (Teoh *et al*., 2003; Basavraj *et al*., 2012). Idiosyncratic hepatic injury associated with DFS is usually mild, and gradual, building up over time and ultimately reaching a threshold after which massive injury occurs (Boesterli, 2002a,b,c, 2003; Uetrecht, 1999). The mechanism of hepatotoxicity associated with DFS involves bioactivation to reactive intermediate metabolites 4-OH & 5-OH following its metabolism by CYP2C9 & CYP3A4 enzymes, as a consequence, enhanced superoxide production and increased intracellular calcium ion (Ca_2^+) results in lethal cell injury (Lim, 2006). DFS administration causes a significant increase in the levels of catalase, superoxide dismutase, malondialdehyde, hydrogen peroxide, and malondialdehyde (Alabi and Akomolafe, 2020). It enhances the generation of reactive oxygen and nitrogen species while decreasing the total antioxidant capacity (Aycan *et al*., 2018). The induced oxidative stress due to peroxide-catalyzed reaction (Cantonie, 2003), mitochondrial injury (uncoupling of oxidative phosphorylation and decrease ATP synthesis) (Masubuchi *et al*., 2002, Siu *et al*., 2008), and immune-mediated mechanism (Lim, 2006) have been suspected also to play a role in DFS mediated liver toxicity.

The efficiency of antioxidants found in herbal medicine and their potential to prevent drug-induced liver damage has drawn the attention of researchers worldwide. (Singh *et al*., 2016). There has been a paradigm shift toward the treatment of liver disorders with natural remedies rather than conventional medication in recent years (Singh *et al*., 2016). Natural products rich in triterpenes, flavonoids, or polyphenols, have now been established as powerful hepatoprotective agents (Gupta *et al*., 2002, 2004; King and Cousins, 2006; Upadhyay *et al*., 2007, 2008, 2010a, b).

ES is one of the typical medicinal herbs used to treat chronic liver disorders in Western Africa. The plant's aerial portions are used as emmenagogues, to induce abortion, and to treat digestive issues like diarrhea and stomachaches (Christensen *et al.,* 2015; Larsen *et al*., 2016 The leaves are used to treat a variety of conditions including pain, fever, nausea, secondary sterility, diarrhea, jaundice, and malaria (Togola *et al*., 2008). It has been demonstrated that the stem bark possesses hepatoprotective effects (Donfack *et al*., 2008). Additionally, the plant's extracts are used typically to treat wounds, parasites, and bacterial infections (Kone *et al*., 2012; Ilodigwe *et al.*, 2014).

As a result of the fact that there seems to be a paucity of scientific reports on the ameliorative effect of HELEES against drug-induced oxidative stress and hepatotoxicity in rats, coupled with the claims by traditional medicine practitioners that the plant extract is

effective in the management of liver diseases in humans, there is need for scientific studies to validate this claim. Therefore, this study was initiated to evaluate the therapeutic effects of HELEES against DFS-induced oxidative stress and hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals and kits

The drug DFS is an injectable liquid purchased from the North China Pharmaceutical Co. Ltd, 115 Hainan Road, Shijiazhuang, Hebei, China. . Each 3 ml ampoule contains 75 mg of DFS.

The reagent kits for biochemical assays were purchased from Randox Laboratories Ltd, United Kingdom. All the reagents used are of analytical grade.

Plant Material and Plant Extraction

The ES leaves were harvested from the premises of the College of Agriculture Garkawa, Plateau state. The plant was identified by a taxonomist; a voucher specimen number UAM/FH/242/21 already exists in the College of Forestry herbarium, Federal University of Agriculture, Makurdi, Benue State.

Preparation of HELEES

The leaves were washed under a running tap and air dried for one month in the laboratory at room temperature, the leaves were pulverized using an electric blender and then sieved using a locally made mesh.

Briefly, the solvent mixture was prepared by adding 800 ml of distilled water to 200 ml of absolute ethanol and mixing. Then, 100 g of the pulverized sample was macerated in 1000 ml of aqueous ethanol mixture and allowed to stand for 72 hrs. The mixture was sieved with a white piece of cloth and the liquid obtained was filtered with Whatman no. 1 filter papers. The filtrate was concentrated in a water bath at 45 °C, and the extract obtained was dried to a constant weight in a desiccator. The concentrated HELEES were weighed, and every 100g of the powdered leaves yielded 7g of extracts.

Experimental animals and management

Thirty adult male Wistar rats (*Rattus norvegicus*) weighing 200–250 g, were used for this study. They were purchased as litters at the age of 6 weeks from National Veterinary Research Institute (NVRI), Vom, Plateau state. Then they were kept in plastic cages to grow unto maturity and acclimatized for about 4 weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi, Nigeria. The rats were kept under normal environmental conditions of 12 h dark and 12 h light cycle, with an average temperature of 29°C. They were fed with standard animal feeds, produced by Grand Cereal and Oil Mills Ltd, Jos, Nigeria, and clean water *ad libitum*. The rats were handled with care according to International guidelines for the use of laboratory animals. (NIH, 1978).

Preparation of DFS

The method of Hassan *et al.* (2021) was adopted for the preparation of DFS to be administered with some modification. The dose to be administered in volume to groups B, E, and F rats were calculated using the 10 mg/kg body weight dosage, and a single dose of the drug was suspended in 0.2 ml of normal saline (0.9 g/dL NaCl) solution.

Experimental Procedure

Thirty (30) male albino rats weighing (100-118g) were used. The rats were assigned to six experimental groups of five rats each.

- Group A received 0.2 ml normal saline intraperitoneally (ip)
- Group B received 10mg/kg of DFS ip.
- Group C received 200mg/kg of HELEES orally.
- Group D received 400mg/kg of HELEES orally.
- Group E received 10mg/kg of DFS ip and 200mg/kg of HELEES orally.
- Group F received 10mg/kg of DFS ip and 400mg/kg of HELEES orally.

All treatments with DS and HELEES were done concurrently for 21 days. During this treatment weekly and observed for signs of toxicity and death daily of toxicity and death on daily basis.

Collection and Preparation of Blood and Tissues

On day 22, the rats were sacrificed by cervical dislocation, and blood from the orbital sinus into plain sample bottles for serum biochemistry. The blood samples in plain bottles were centrifuged at 3000 rpm

for 5 minutes to obtain serum and stored at 40ºC until used for analysis. A midventral abdominal incision was made on each male rat. The liver samples were excised, attaching connective tissues were removed, rinsed in normal saline, and weighed using Mettler Balance (C282001, China) according to Abu and Uchendu (2011).

Hepatic tissue preparation for biochemical assays

The rats were sacrificed by cervical dislocation 24 hours after the last treatment. The liver was removed and weighed and a portion of it was rinsed in 1.15% potassium chloride (KCl) and homogenized in potassium phosphate buffer (0.1 M, pH 7.4) and centrifuged at 12, 000 g for 15 minutes to obtain the post mitochondrial fraction (PMF)/cytosolic fractions. The PMF of the liver was obtained and subsequently stored at -20°C until the time of use.

Antioxidant Assays

A spectrophotometer was used to examine the biochemical parameters: the reduced glutathione (GSH) level was evaluated using the liver homogenate according to the technique of Jollow *et al* (1974). According to the approach described by Wright *et al* (1981), lipid peroxidation was measured by measuring the quantities of Malondialdehyde formed as a result of lipid peroxidation. According to Mohandas et al., the activity of the enzyme glutathione peroxidase (GPx) was determined (1985). Catalase (CAT) activity was determined according to the method of Claiborne (1985). The level of SOD activity was determined by the method of Misra and Fridovich (1972). Glutathione-S-transferase (GST) was determined by the method of Habig *et al*. (1974). The level of H_2O_2 was assessed by H_2O_2-mediated horseradish peroxidase-dependent oxidation of phenol red by the method of Pick and Keisari (1981). Nitrite assay was done using Griess reagent with some modifications of the method of Green *et al.* (1982).

Biochemical Assays

Alanine aminotransferase (ALT) was estimated calorimetrically using the method described by Thefeld *et al*., (1974). Aspartate aminotransferase (AST) was estimated calorimetrically using the method described by Thefeld *et al.* (1974). Alkaline phosphatase (ALP) was estimated calorimetrically using the method described by Thomas (1998). The serum total protein was assayed by the Biuret reaction (Thomas, 1998). The procedure is based on the binding reaction between albumin in the serum and the bromocresol-green dye (Doumas *et al*., 1975). Serum Globulin was estimated using the biuret reaction method of serum globulin determination (Alberto *et al*., 1966).

Histopathological Analysis

The fixed tissues were dehydrated in ascending series of ethanol, cleared in two changes of xylene, infiltrated in three changes of molten paraffin wax (melting point 58–60 ℃), and embedded in molten paraffin. Sections of 4 microns thickness were cut by using a rotary microtome and stained with Ehrlich's hematoxylin and counterstained with eosin (Lillie & Fulmer, 1976)

Statistical analysis

All the data were expressed as the mean ± standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA followed by Dunnett's test using a computer-based fitting program (Prism, Graph pad 8.01). Values of $P<0.05$ were considered significant.

RESULTS

HELEES administration abrogated DFS-induced hepatocellular toxicity in Wistar rats

The activities of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and alanine aminotransferase (AST) together with total bilirubin level increased significantly ($p<0.05$) in DFS-only treated rats compared to the control (Table 1). The therapeutic effects of HELEES were demonstrated with a significant ($p < 0.05$) reduction in the serum ALT, ALP, AST, and total bilirubin of rats co-treated with DFS and HELEES plus (200 and 400 mg/kg) (Table 1). There was a significant reduction in the levels of total protein and the globulin was significantly ($p<0.05$) reduced in the group administered DFS alone when compared to the control group and the groups administered HELEES only, however, concurrent treatments resulted in the ameliorative effects.

Table 2. Shows that the injection of DFS led to a remarkable increase ($p < 0.05$) in hepatic CAT and SOD activities in the DIC-alone treated group relative to the control animals. However, treatment with HELEES at doses of 200 and 400 mg/kg remarkably elevated liver SOD and CAT activities relative to the DIC-alone treated group. Also, there was a noticeable ($p < 0.05$) decline in liver GPx activity in the DSF-alone treated group compared to the control animals. However, the administration of HELEES at doses of 200 and 400 mg/kg caused a remarkable increase ($p < 0.05$) in liver GPx activity in comparison with the DSF-alone injected group. Also, injection of DSF caused a noticeable decline ($p < 0.05$) in liver GSH and GST compared to the control group (Table 3). However, in groups given a combined treatment of HELEES at varying doses and DFS (groups E and F), liver GSH and GST were noticeably elevated ($p < 0.05$) compared to the second group (DIC-alone injected group).

Histopathological findings

The histological observations show that rats in the control group (A) showed normal hepatic architecture. Also, the rats in the groups administered HELEES at 200 and 400 mg/kg (C & D) showed normal hepatic morphology as the rats in the control groups. However, the liver rats that were administered DFS alone (B) showed some areas of necrosis, numerous kupffer cells, the presence of ferritin, and evidence of periportal inflammation with numerous infiltration of inflammatory cells. The photomicrograph of the co-treatment of DFS with 200 and 400mg/kg HELEES (E & F) shows a great extent of lesion resolution.

Table 1 Ameliorative effects of HELEES on the serum biochemical parameters in DFS-induced toxicity in male albino rats for 21 Days

PARAMETERS	Group A	Group B	Group C	Group D	Group E	Group F
ALT (u/l)	21.00±0.58	38.00±5.03[a]	26.33±3.48	30.00±2.65	21.00±1.53[b]	22.00±1.53[b]
AST (u/l)	26.00±3.06	73.00±2.08[a]	30.67±1.33	25.33±4.41	38.67±1.76[b]	44.00±2.00[b]
ALP (u/l)	31.03±0.95	52.10±4.36[a]	42.40±6.65	33.50±1.99	38.77±0.66	36.84±1.53
T Prot (g/l)	69.57±0.44	45.90±1.06[a]	68.13±2.46	66.63±2.26	65.70±2.01[b]	68.47±3.50[b]
ALB (g/l)	42.00±1.16	42.93±0.52	40.57±2.74	43.70±2.33	41.03±0.69	40.43±1.68
GLO (g/l)	27.57±1.36	15.30±2.19[a]	21.80±0.76	22.93±2.07	24.10±2.80	28.50±3.97[b]
BIL (mg/dl)	0.4±0.19	5.47±0.76[a]	0.87±0.27	0.87±0.43	2.03±0.37[b]	0.87±0.23[b]

Data are presented as mean ± standard deviation (n = 5).. [a] Significant difference at (p< 0.05) when groups B, C, D, E, and F are compared with group A. [b] Significant difference at (p< 0.05) when groups E and F are compared with group B. Alanine aminotransferase: ALT; Aspartate aminotransferase: AST; Alkaline phosphatase: ALP; Total protein: T Prot; Albumin: ALB; Globulin: GLO; Bilirubin: BIL.

Table 2 Effects of HELEES and DFS on the liver tissue antioxidant capacity, nitrite content, and MDA of experimental rats

PARAMETERS	Group A	Group B	Group C	Group D	Group E	Group F
GSH (mg/mg tissue)	48.53±1,17	27.60±0.58[a]	50.77±0.45	52.45±1.46	33.16±0.37[b]	36.62±0.62[b]
GST (mg/mg tissue)	0.08±0.00	0.03±0.00[a]	0.09±0.00	0.08±0.00	0.05± 0.02[b]	0.47±0.01[b]
GPx (mg/mg tissue)	110.00±0.67	67.1±1.13[a]	112±0.44	116.1±1.01	72.4±0.59[b]	78.6±0.48[b]
SOD (m/mg tissue)	2.00±0.20	2.50±0.25[a]	2.00±0.21	2.01±0.27[b]	2.10±0.33[b]	2.10±0.42[b]
CAT (mM/mg tissue)	2.89± 1.43[b]	4.87±0.02	2.65±2.00[b]	2.62±2.13	3.23 ±0.033[b]	3.42±0.03[b]
MDA (nM/mg tissue)	6.93±2.04	19.01±5.20[a]	7.03±3.10	7.00±3.00	14.87±0.18[b]	14.45±0.39[b]
NO (U/mg protein)	0.24±0.00	0.05±0.01[a]	0.35±0.00	0.34±0.00	0.23±0.00[b]	0.28±0.01[b]

Data are presented as mean ± standard deviation (n = 5).
[a] Significant difference at (p< 0.05) when groups B, C, D, E, and F are compared with group A.
[b] Significant difference at (p< 0.05) when groups E and F are compared with group B.
GST: glutathione-s-transferase, GPx: glutathione peroxidase, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde, NO: nitric oxide

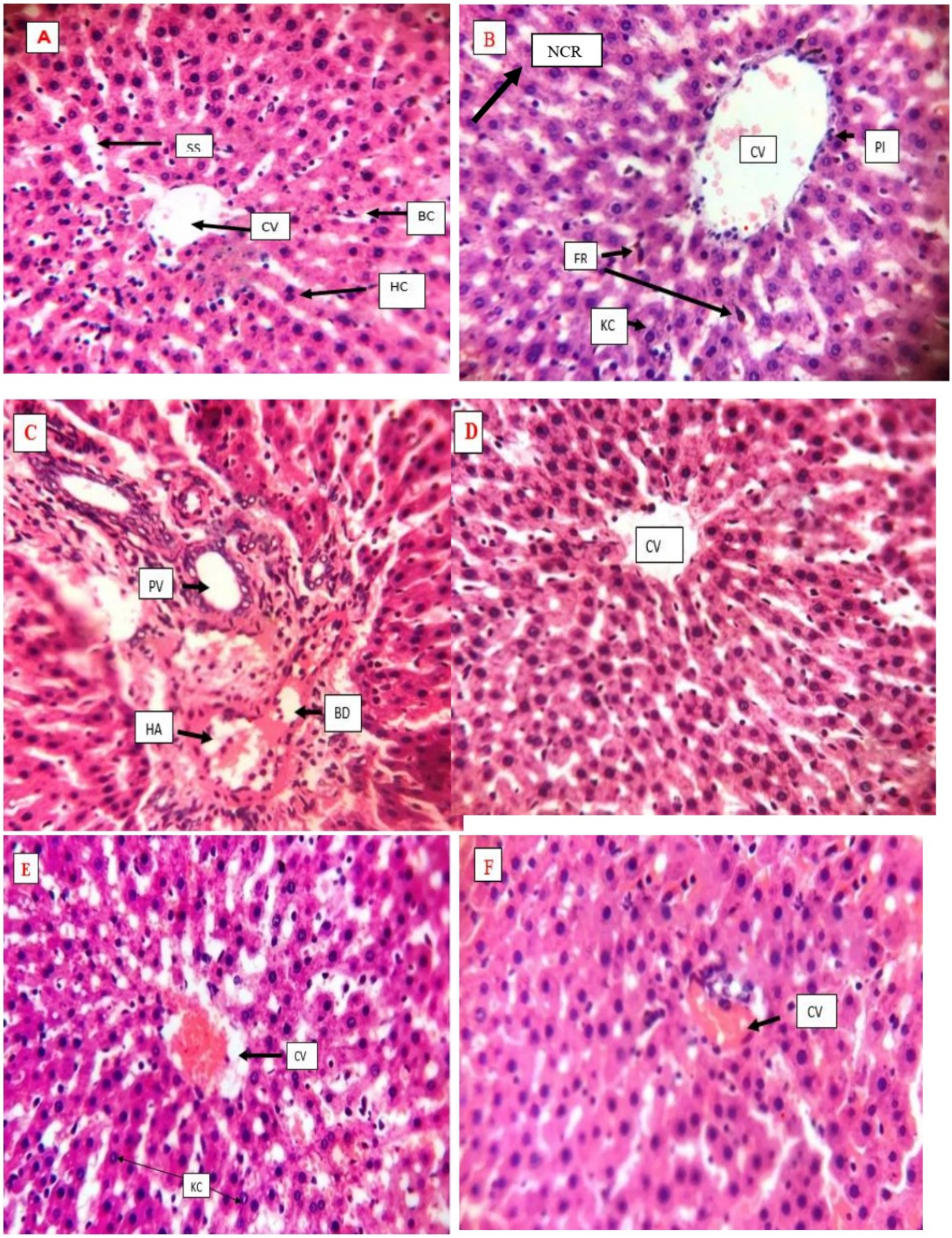

Figure 1 Representative photomicrographs of liver tissues sections (×400) in male Wistar rats treated with DFS and HELEES (A): control rats show normal liver architecture with the central vein (CV), bile canaliculi (BC), hepatocytes (HC), and sinusoids (SS). DFS alone-treated rats (B)showed areas of necrosis (NCR), periportal inflammation (PI) area with numerous inflammatory cells, presence of ferritin (FR) and numerous kupffer cells (KC), HELEES alone treated rats at the dose of 200 and 400mg/kg (Group C & D) showed normal hepatic morphology similar to that of the group (A) with the liver of those of (C) showing the portal vein (PV), bile duct (BD), and hepatic artery (HA), rats treated with DFS + HELEES at 200 and 400mg/kg (group E & F) are the recovery groups and showed normal hepatic architecture.

DISCUSSION

The therapeutic dosages of DFS are usually safe, however high doses of the drug can be hazardous to both human and animal tissues. The toxicity effects are caused by the reactive metabolites of DCF: 4Õ hydroxy 3 diclofenac, 5Õ hydroxy 4 diclofenac, and 5Õ hydroxy 6 diclofenac (Boerma *et al.,* 2012; den Braver *et al*., 2016; Lazarska *et al*., 2018). The conjugation of DFS with reduced GSH and inactivated by GST (Dragovic *et al*., 2013; Vredenburg *et al*., 2014) enhances the excretion of DCF-metabolite from the body system (Daly, 2017) with subsequent reduction, developing disposition enzyme and enhancing cellular antioxidant status will therefore reduce DFS-associated toxicities. Our research on the combined administration of HELEES and DFS reinforces the hypothesis that plants can mitigate the toxicity caused by pharmaceuticals.

Hepatic transaminases (ALT, AST, GGT, ALP) and LDH are widely used as biomarkers for hepatic injury, and in this present study, the serum levels of transaminases were elevated ($p < 0.05$) in rats treated with DCF, and as previously reported (Alabi *et al*., 2017; Aycan *et al*., 2018; Peter *et al*., 2017), leading to release of enzymes into the bloodstream. Markers of liver damage and hepatocyte death are due to membrane breakdown of the liver cell and ultimate leakage of several contents from the intracellular to extracellular milieu (Wang *et al.,* 2015; Kobylinska *et al*., 2015; Nagai *et al*., 2016). According to our results, treatment of DFS in tandem with HELEES at varying doses abrogated the DFS-induced hepatotoxicity with the consequent reduction in the activities of the hepatic transaminases and LDP in a dose-dependent fashion. . The toxicity associated with DFS might be due to the toxic metabolite following the metabolism of DFS after its administration (Boerma *et al*., 2012). Furthermore, medicinal foods that contain antioxidants might be of potential benefit to patients with liver damage.

Research has established that DFS treatment could result in ROS generation which leads to increased oxidative stress as a result of the decline in the antioxidant system activities (Galati *et al*., 2002). In this very research, the administration of DFS caused a significant ($p<0.05$) reduction in hepatic NO and reduced glutathione content together with a significant increase in hepatic MDA content. Therefore, we propose from this study that DFS administration enhanced both hepatic oxidative and nitrosative stress. The observed reduction in the NO content following DFS might be related to the superoxide radical anion that can combine with NO, thereby facilitating its reduction. The combination of NO and superoxide radical anion forms peroxynitrite (Wen *et al*., 2015), which is a cytotoxic molecule that can damage important macromolecules like proteins, DNA, and ribonucleic acid (RNA). Peroxynitrite has also been reported to participate in nitrosative stress (Lee *et al*., 2016). Conversely, it was observed that concurrent treatment enhanced an enhancement in the levels of NO in the rats which shows the abrogative effects of HELEES against nitrosative stress.

On the other hand, the activities of the antioxidant defense system were altered differently. The hepatic glutathione-S-transferase (GST) and glutathione peroxidase (GPx) were significantly inhibited following DFS administration, by contrast, co-treatment of the rats with DFS and HELEES restored the activities of the antioxidant defense. The hepatic superoxide dismutase (SOD) and catalase (CAT) activities in the DFS-treated group increased significantly ($p<0.05$) relative to the control group and the groups administered HELEES only. The SOD partakes in the first line of defense during oxidative stress by converting superoxide anion radical (O_2-) to H_2O_2 while GPx and CAT quench H_2O_2 to water and oxygen (O_2), respectively. Furthermore, GST detoxifies toxic electrophiles including DFS metabolites with the help of GSH as a cofactor to more soluble and less toxic metabolites that can easily be excreted by the kidney (Chen *et al*., 2015; Beyerle *et al*., 2015). Also, the improvement in hepatic GSH, GST, and GPx by the varying doses of HELEES when co-administered with DFS suggests that HELEES is not pro-oxidant in nature. Hence, it could be taken in the use of phytochemicals as antioxidants for chemoprevention. The observed reduction in the activities of GST and GPx might suggest the production/generation of ROS/free radicals by DFS, thus aggravating the buildup of toxic metabolites of DFS, which further exacerbates the hepatotoxicity effect of DFS. On the contrary, the increase in the activity of SOD and CAT could be said to be an adaptive response of

hepatic tissue to DFS toxicity. The mechanism through which DFS induces antioxidant enzyme activity might be via up-regulating SOD and CAT messenger ribonucleic acid (mRNA) and activation of the Nrf_2-ARE pathway. Hence, the therapeutic effect and the antioxidant activities of HELEES were demonstrated by reducing the hepatic transaminases, and hepatic markers of oxidative stress, and improving the antioxidant defense system in a dose-dependent manner. In addition, excessive production of NO was observed in a similar work, which was indicative of nitrosative stress (Wanyong *et al*., 2015). HELEES administration with DFS may be potentially beneficial to liver damage patients by modulating, ameliorating, or reversing hepatotoxicity. The ameliorative effect of HELEES in the present study could be ascribed to its antioxidant and anti-inflammatory properties. The results suggest that HELEES may confer protection against hepatic drug-induced damage. However, the major limitation of this study was the lack of funding.

The histopathological examination of the liver strongly corroborates the biochemical results. Examination of the control liver revealed intact hepatic architecture, distinguished by well-organized hepatic lobules, sinusoids, and bile canaliculi with hepatic strands of radially regular hepatocytes enclosed in the central vein (Fig. 1A). In rats that were administered DFS, several pathological alterations were observed, including dilation and congestion in the blood sinusoids and central vein, infiltration of the inflammatory cells, numerous kupffer cells, ferritin and areas of necrosis which were observed in the liver sections of DFS-administered rats (Fig. 1B). This divulges the devastating effects of DFS toxicity and idiosyncratic reaction and underscores the need for caution when such a drug is used for therapy. This infiltration is leucocyte migration, which indicates an inflammatory response stimulated by tissue damage (Esmaeilzadeh *et al*., 2020) Rats administered 200 and 400mg/kg of HELEES (Fig. 1 C & D) only showed intact hepatic architecture similar to what is seen in the liver of the group A rats. Thus, the ability of HELEES to maintain the structural and functional integrity of both the liver in DSF-treated groups almost to the same extent as that of the control groups was evident of its chemoprotective potential in the liver of the rats. Also, it is interesting to note that the concurrent administration of HELEES with DFS ameliorated the DFS-induced hepatic architectural damage in the experimental rats.

CONCLUSIONS

Oral administration of HELEES to rats reduced the harmful effects of DFS on the liver by rebalancing redox potential, preventing cell death, and offering cytoprotection. These results are extremely important because they open doors for the application of HELEES in addressing DFS-related problems and break new ground for examining its applicability in addressing additional harmful DFS side effects.

CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

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