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Research Paper

Aqueous leaf extract of *Vernonia amygdalina* ameliorates adverse effects of diclofenac-induced hepatotoxicity

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Vernonia amygdalina has many uses in folk medicine which has been authenticated. Diclofenac is a frequently prescribed anti-inflammatory, analgesic drug; its use has been reported to produce severe hepatic reactions. This study was carried out to determine the effect of *Vernonia amygdalina* on diclofenac-induced hepatotoxicity. Twenty five albino rats were randomly assigned into 5 groups of 5 rats each. Rats in groups 2 to 5 were administered 100 mg/Kg body weight diclofenac once intraperitoneally. After 24 h groups 3, 4 and 5 received 200, 400 and 600 mg/kg body weight of the extract respectively for 21 days. Group 1 served as normal control and group 2 as diclofenac control. Liver function indices and histopathology of the liver were evaluated. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, phlobatannins, terpenoids and cardiac glycosides. Diclofenac caused significant ($p<0.05$) increase in the activities of alkaline phosphatase, aspartate and alanine transaminases in the serum and liver homogenates of rats and significantly reduced ($p<0.05$) total protein compared to control. The extract was able to restore the activities of the enzymes towards normal. The liver tissues of animals treated with *Vernonia amygdalina* produced normal histolopathological features while the diclofenac control showed mild vacuolar degeneration. The results have shown the adverse effects of diclofenac administration on the liver of experimental animals while treatment with aqueous leaf extract of *Vernonia amygdalina* was able to ameliorate these changes by restoring the function indices and histopathological lesions towards normal.

Keywords: *Vernonia amygdalina*, diclofenac, hepatotoxicity, histopathology.

INTRODUCTION

Hepatotoxicity is the capacity of drugs, chemicals or other agents to cause injury to or have damaging effects on the liver. This is characterized by impairment in liver function (Sriutta *et al.,* 2018). Many drugs and chemicals have been reported to cause liver injury; one of such drugs is the non-steroidal anti-inflammatory drug (NSAID) diclofenac. It is used for the therapy of chronic forms of arthritis and mild-to-moderate acute pain. Therapy with diclofenac in full doses is frequently associated with mild serum aminotransferase elevations and, in rare instances, can lead to serious clinically apparent, acute or chronic liver disease (Lewis and Stine, 2013). Diclofenac is a phenyl acetic acid derivative belonging to the acetic acid class of NSAIDs that includes indomethacin, etodolac, ketorolac, nabumetone, tolmetin and sulindac. It is available in multiple generic and brand formulations, either alone or in combination with other analgesics or gastointestinal mucosal protective agents (such as misoprostol). Common commercial names for agents containing diclofenac include: Arthrotec, Cataflam, Duravolten, Novo-Difenac, Nu-Diclo, Voltaren and Zorvoflex. Nonsteroidal anti-inflammatory drugs such as diclofenac have been available for over forty years. It is available over-the-counter in many countries where indications include joint and muscle pain from trauma, bursitis, tendonitis, headache and dysmenorrhea. As a

result, diclofenac is one of the most frequently used NSAIDs worldwide (Moore and Derry, 2018). Although diclofenac hepatitis was thought to be rare, there are clinical reports of severe hepatic reactions associated with its use; more than a hundred instances of clinically apparent liver injury due to diclofenac have been reported in the literature and, in most case series, diclofenac ranks high in the causes of drug induced liver injury (Björnsson, 2016). Clinical findings of diclofenac hepatotoxicity appear to be consistent with a direct toxic effect of the drug or a drug metabolite (Scully *et al.,* 1993). The mechanism of diclofenac induced liver injury appears to be multifactorial. Diclofenac was found to generate protein adducts in the livers of treated mice as well as in rat hepatocytes via protein acylation by the drug glucoronide (Pumford *et al.,* 1993; Meunier and Larrey, 2018). An immuno-allergic component is suggested by the rapid and acute recurrence of injury, even many years after initial exposure and injury. Genetic studies have suggested a linkage with allelic variants of UGT 2B7, CYP 2C8 and ABC C2, which are genes involved in the metabolism, conjugation and excretion of diclofenac. The formation of reactive metabolites by drug oxidation, which could be related to drug toxicity, has been reported (Miyamoto *et al.,* 1997).

Vernonia amygdalina Del.*(Astereaceae*) popularly known as bitter leaf is a shrub of 2-5m tall with petiolate green leaves of about 6mm diameter. The leaves are characteristically bitter and are used to make soup while stem and root divested of the bark are used as chew-sticks in Nigeria (Aregheore *et al.,* 1998). All parts of the plant are pharmacologically useful; the leaves have great nutritional (Aregheore *et al.,* 1998) and medicinal value (Idu and Onyibe, 2007). At least, 13 new compounds have been found in *Vernonia amygdalina* following several studies and it has been found to have the following benefits: Anti-malarial (Emmanuel and Moses, 2016), anti-parasitic (Huffman, 2003), anti-diabetic (Atangwho *et al.,* 2016), antioxidant (Adesanoye and Farombi, 2014) and anti-cancer (Wong *et al.,* 2013). It is also effective in preventing indigestion, scurvy, sciatica and rheumatism (Cherepy *et al.,* 1997; Bakowska *et al.,* 2003). Previous studies have reported the effect of carbon tetrachloride on the liver of rats and the hepatoprotective effectsof *Vernonia amygdalina* in ameliorating the effects (Babalola *et al.,* 2001; Adesanoye and Farombi, 2010). This study was therefore designed to investigate if the aqueous extract on *Vernonia amygdalina* leaves can modulate the effects of diclofenac induced hepatotoxicity.

MATERIALS AND METHODS

Plant material

Fresh leaf samples of *Vernonia amygdalina* were collected from the staff quarters, Modibbo Adama University of Technology, Yola, Adamawa State and was authenticated at Biological Science Department, Modibbo Adama University of Technology Yola (MAUTECH) Adamawa State.

Chemicals

Aspartate aminotransferase kit, alanine aminotransferase kit and alkaline phosphatase kit were obtained from Randox laboratories Ltd. Crumlin, Co-Antrim, UK. All other reagents were of analytical grade obtained from Sigma Chemical Company, St. Louis, Mo, USA.

Experimental animals

Twenty five albino rats weighing between 150-200 g were purchased from the Department of Biochemistry, University of Jos Plateau State and were housed in a well-ventilated room in plastic cages on a 12-h light/dark cycle. They were fed with animal feed obtained from vital feeds at Chika vital feeds, along hospital road Jimeta in Adamawa State and given tap water *ad libitum*.

Preparation of extract

The extract was prepared according to the method of Ojiako and Nwanjo, (2006). Fresh leaves were sorted, washed to remove debris and dust particles without squeezing and then dried in the shade for seven days. The dried leaves were milled into a coarse powder from which 25 g was soaked with 250 ml of distilled water in a beaker and the mixture shaken and kept on the laboratory bench for 24 h before filtering. The filtrate was evaporated using a water bath at 40°C to obtain a concentrated extract. The extract was dissolved in distilled water and administered orally to rats.

Phytochemical screening

Standard tests were carried out on the aqueous extracts for the qualitative determination of phytochemical constituents as described by Harborne, (1973) and Sofowora, (1993).

Experimental design

Twenty five adult albino rats (Wistar strain) were used. The rats were housed in well-ventilated plastic cages at room temperature and fed with commercial feed. The rats weighing between 150-200 g were randomly assigned into five groups 1, 2, 3, 4 and 5 of five rats each. Rats in groups 2 to 5 were each administered 100 mg/Kg body

weight of diclofenac once intraperitoneally. After 24 h, rats in groups 3, 4 and 5 received 200, 400 and 600 mg/kg body weight respectively, of the aqueous extract of *Vernonia amygdalina* once daily for twenty one days. Rats in group 1 served as the normal control and received distilled water (which is the same solvent used to dissolve the extract) orally throughout the duration of the experiment while those in group 2 served as diclofenac control and were also administered distilled water for the duration of the study.

Blood and liver tissue collection and preparation

Twenty four hours after the last treatment, animals were anesthetized using diethyl ether and sacrificed. After sacrificing, the blood was collected by cardiac puncture into plain bottles and centrifuged for 10 min at 3000 rpm, after which the clear supernatant was carefully collected using a Pasteur pipette and later used in the analysis of biochemical parameters. The liver of each animal was also quickly removed, cleansed of superficial connective tissue and blood and weighed. They were then homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored frozen overnight to ensure maximum release of enzymes.

Biochemical analyses

Enzyme assays

The activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in the serum and liver homogenates were determined according to the method of Reitman and Frankel, (1957). Alkaline phosphatase activity was determined by the method of Wright *et al.* (1972).

Determination of liver function indices

The concentration of serum protein was determined using the Biuret method as reported by Gornall *et al.* (1949). Bilirubin was determined by the method of Walter and Gerard, (1970).

Histopathological assessment

The method described by Krause, (2001) was used to assess liver tissue from all the groups of rats. Livers from different groups of animals were fixed in 10% formalin solution and dehydrated through ascending grades of ethanol to absolute alcohol. They were cleaned in xylene, impregnated and embedded in paraffin wax. Sections were cut at 5 µm on a rotatory microtome. The cut sections were fixed on clean microscope slides and counter stained with hematoxylin–eosin (H&E). The slides were then observed using Leitz, DIALUX research microscope at x 400 magnification and the photomicrographs were taken in a bright field.

Statistical analysis

The results presented as mean ± SEM. Statistical analyses were performed using student's *t*-test and Duncan's multiple range tests for the difference. $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical constituents

Preliminary phytochemical screening of the aqueous extract of *Vernonia amygdalina* leaf revealed the presence of alkaloids, tannins, flavonoids, phlobatannins, terpenoids, saponins and cardiac glycosides.

Enzymes

Table 1 shows the effects of aqueous extract of *Vernonia amygdalina* on the activities of ALT, AST and ALP in the serum of albino rats. Treatment with diclofenac resulted in significant increase ($p<0.05$) in the activities of alkaline phosphatase, aspartate and alanine transaminases in the serum of the animals compared to control. These were restored towards normal by treatment with aqueous extract of *Vernonia amygdalina*. This effect was dose dependent.

Table 2 shows the effect of aqueous extract of *Vernonia amygdalina* on the activities of ALT, AST and ALP in the liver of albino rats. Treatment with diclofenac also resulted in significant increase ($p<0.05$) in the activities of alkaline phosphatase, aspartate and alanine transaminases in the liver of the animals compared to control. Treatment with aqueous extract of *Vernonia amygdalina* also caused a dose-dependent significant decrease ($p<0.05$) in the activity of these enzymes restoring them towards normal.

Liver function indices

Table 3 shows the effects of aqueous extract of *Vernonia amygdalina* on liver function indices in albino rats. Treatment with diclofenac resulted in a significant decrease ($p<0.05$) in the concentration of total protein compared to control; and although treatment with aqueous extract of *Vernonia amygdalina* was able to increase the protein concentration slightly at the doses of

Table 1. Effects of aqueous extract of *Vernonia amygdalina* on the activities of ALT, AST and ALP in the serum of albino rats.

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	50.50± 7.39[a]	21.50± 3.17 [a]	45.58± 2.05 [a]
Diclofenac control	88.50± 5.50[b]	89.00± 0.00 [b]	170.45± 52.45 [b]
200 mg/kg b.w.t	85.50± 8.50 [b]	89.00± 0.00 [b]	165.00± 96.60 [b]
400 mg/kg b.w.t	69.00± 9.60[c]	45.00± 15.13[c]	89.53± 23.31[c]
600 mg/kg b.w.t	46.50± 2.17[a]	30.75± 7.77[a]	76.50± 15.90[d]

Values are means ± SEM for five rats in each group. Values in each column with different alphabets are significantly different ($p<0.05$).

Table 2. Effect of aqueous extract of *Vernonia amygdalina* on the activities of ALT, AST and ALP in the liver of albino rats.

Treatment group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	50.50± 7.39[a]	21.50± 3.17 [a]	45.58± 2.05 [a]
Diclofenac control	88.50± 5.50[b]	89.00± 0.00[b]	170.45± 52.45[b]
200 mg/kg b.w.t	85.50± 8.50[b]	89.00± 0.00 [b]	165.00± 96.60[b]
400 mg/kg b.w.t	69.00± 9.60[c]	45.00± 15.13[c]	89.53± 23.31[c]
600 mg/kg b.w.t	46.50± 2.17 [a]	30.75± 7.77 [ac]	76.50± 15.90[d]

Values are means ± SEM for five rats in each group. Values in each column with different alphabets are significantly different ($p<0.05$).

Table 3. Effects of aqueous extract of *Vernonia amygdalina* on liver function indices in albino rats.

Treatment	Total Protein (g/dl)	Direct Bilirubin (mg/dl)	Total Bilirubin (mg/dl)
Control	8.10± 0.22 [a]	3.43± 0.05 [a]	2.15± 0.13 [a]
Diclofenac control	6.95± 0.45[b]	3.00± 0.00 [a]	2.15± 0.05 [a]
200 mg/kg b.w.t	7.75± 1.85[b]	2.50± 0.80 [a]	1.50± 0.10[b]
400 mg/kg b.w.t	7.23± 0.55[b]	2.27± 0.12[ab]	1.37± 0.12[b]
600 mg/kg b.w.t	6.23± 0.95[b]	2.23± 0.36[b]	1.98± 0.39 [a]

Values are means ± SEM for five rats in each group. Values in each column with different alphabets are significantly different ($p<0.05$).

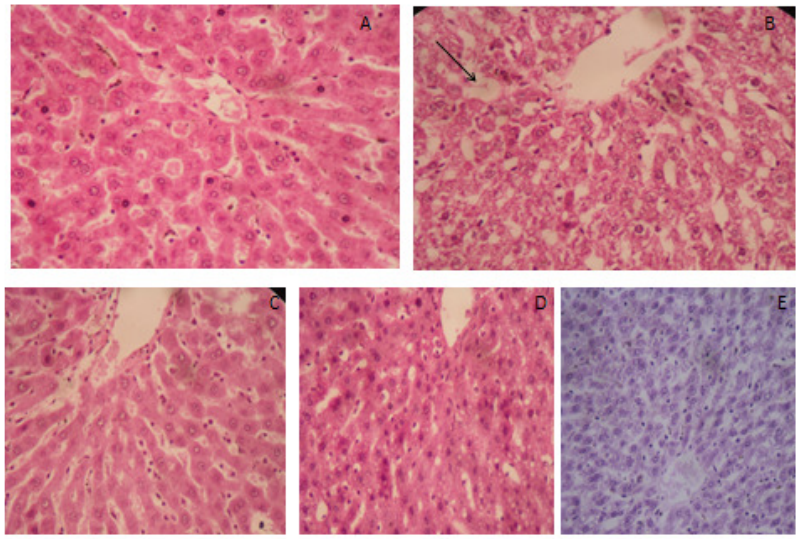

Plate 1. Photomicrograph of the liver of rats administered various doses of aqueous extract of *Vernonia amygdalina*. A-E: Control, Diclofenac control, 200, 400 and 600 mg/Kg b.wt respectively (H and E x400).

200 and 400 mg/kg body weight, the increase was not significantly different ($p<0.05$) from the diclofenac control group. Diclofenac did not significantly alter ($p<0.05$) the direct and total bilirubin concentration compared to normal but treatment with aqueous extract of *Vernonia amygdalina* caused a significant decrease ($p<0.05$) in these parameters at some doses.

Histopathology of the liver

Histopathological examination of the liver sections of normal rats showed normal architecture of the liver tissue (Plate 1). The liver from the diclofenac control group showed mild vacuolar degeneration compared to normal animals (Plate 1). However, all the groups treated with aqueous extract of *Vernonia amygdalina* had essentially normal liver architectures too; there was no visible change in the architecture of the tissues of treated animals compared to normal control (Plate 1).

DISCUSSION

This study has demonstrated that injecting rats with 100mg/kg body weight of diclofenac intraperitoneally caused hepatic injury by causing changes in some liver function indices, enzymes and presence of mild vacuolar degeneration in the liver tissue itself. The serum enzymes ALT and AST were significantly increased in the animals treated with diclofenac. The assay of enzymes in serum and tissue plays a significant role in the diagnosis of diseases, investigation and assessment of drugs or plant extract for safety/ toxicity. Increase in these serum enzymes has been linked to cytotoxic and cholestatic hepatic injuries (Adesanoye and Farombi, 2010). Increased AST activity is an index of hepatocellular injury while increased ALT activity indicates a necrotic state (Navarro and Senior, 2006). Serum ALP activity which is used in assessing obstructive liver injury (Kaplan, 1986) was also found to be significantly elevated in diclofenac rats. However, the observation that these enzyme activities were significantly increased in both the serum and liver of rats treated with diclofenac suggests other reasons for the elevation in addition to hepatocellular damage. It may be that diclofenac caused increased *de novo* synthesis of the enzyme molecules, as an adaptation by the liver to offset the stress imposed on it by the drug leading to activity higher than control (Malomo *et al.,* 1995). The administration of aqueous extract of *Vernonia amygdalina* however, ameliorated the adverse effects of diclofenac by inducing a dose dependent reduction in the activities of these enzymes. The protective effects of *Vernonia amygdalina* against sodium arsenite-induced genotoxicity in rats has previously been reported by Adetutu *et al.* (2013). Adefisayo *et al.* (2017) also reported that the methanolic extract of *Vernonia amygdalina* significantly reverted aspirin induced gastric damage. Both studies attributed these protective/ameliorative effects of *Vernonia amygdalina* to the phytochemicals it contains some of which have antioxidant properties.

Changes in the liver function indices can indicate the state of the liver (Ganong, 2015). Treatment with diclofenac resulted in a significant decrease in the concentration of total protein. This might be due to a diminished biosynthetic function of the liver. In hepatocellular dysfunction, the liver is unable to synthesize total protein together with albumin at the normal rate while catabolism proceeds as usual resulting in reduction of total protein concentration (Yakubu *et al.,* 2007).

However, the results of this study shows that the aqueous extract of *Vernonia amygdalina* did not significantly increase the concentration of protein at the doses used compared to diclofenac control. Diclofenac did not significantly alter the direct and total bilirubin concentration but treatment with aqueous extract of *Vernonia amygdalina* caused a significant decrease in these parameters at some doses. Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells. These changes in the bilirubin concentration at some doses may indicate the adverse effect of some phytochemical components of *Vernonia amygdalina* at higher doses as seen in its effect on direct bilirubin. Despite their beneficial effects, phytochemical constituents of plants are also known to elicit adverse effects; the consumption of aqueous extract of *Vernonia amygdalina* leaves at high doses have been shown to cause adverse effects (Ojiako and Nwanjo, 2006). Additionally, Igile *et al.* (1995) have reported the toxicity of saponins extracted from *Vernonia amygdalina* on mice. The mild changes in the normal histological architecture of the liver seen in the diclofenac control group suggest that the drug had adverse effects on the function and structure of the liver. The absence of histopathological lesions in the liver of the groups treated with various doses of *Vernonia amygdalina* suggests that the extract was able to ameliorate the effects of the drug on the liver.

Conclusion

The results of our study have clearly demonstrated the adverse effects of diclofenac administration on the liver of experimental animals as evidenced by perturbations in the liver function indices and mild histopathologic changes. Treatment with aqueous leaf extract of *Vernonia amygdalina* was able to ameliorate these changes by restoring the function indices and histopathological lesions towards normal.

REFERENCES

Adefisayo MA, Akomolafe RO, Akinsomisoye SO, Alabi QK, Ogundipe

LL, Omole JG, Olamilosoye KP, (2017). Gastro-protective effect of methanol extract of *Vernonia amygdalina* (del.) leaf on aspirin-induced gastric ulcer in Wistar rats. Toxicology Reports. 4:625-633.
Adesanoye OM, Farombi EO (2010). Hepatoprotective effects of *Vernonia amygdalina* (*astereaceae*) in rats treated with carbon tetrachloride. Experimental and Toxicologic Pathology 62:197–206.
Adesanoye OA, Farombi EO (2014). In vitro antioxidant properties of methanolic leaf extract of *Vernonia amygdalina Del*. Nigerian Journal of Physiological Sciences. 29:091-101.
Adetutu A, Oyewo EB, Adesokan AA (2013). Protective effects of *Vernonia amygdalina* against sodium arsenite-induced genetoxicity in rats. Pharmacognosy Research 5(3):207-211.
Aregheore EMK, Makkar HPS Becker K (1998). Feed value for some browse plant from the central zone of Delta state. Nig. Trop. Sci. 38(2):97–104.
Atangwho JI, Yin KB, Umar MI, Ahmad M, Asmawi MZ (2014). *Vernonia amygdalina* simultaneously suppresses gluconeogenesis and potentiates glucose oxidation via the pentose phosphate pathwayin streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine. Doi:10.1186/1472-6882-14-426.
Babalola OO, Anetor JI, Adeniyi FA (2001). Amelioration of carbon tetrachloride-induced hepatotoxicity by terpenoid extract from leaves of *Vernonia amygdalina*. African Journal of Medicine and Medical Sciences; 30(1-2): 91-93.
Bakowska A, Kucharska AZ, Oszmianski J (2003). The effects of heating, UV irradiation and storage on stability of the anthocyanin-polyphenol co-pigment complex. Food Chemistry, 81: 349-355.
Boelsterli UA (2003). Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. Toxicology and Applied Pharmacology 192:307-322.
Björnsson ES (2016). Hepatotoxicity by Drugs: The most common implicated agents. International Journal of Molecular Science. 17(2), 224-232.
Cherepy NJ, Smestad GP, Gratzel M Zang JZ (1997).Ultrafast electron injection: Implications for a photoelectrochemical cell utilizing an anthocyanin dye-sensitized TiO2 nanocrystalline electrode. Journal of Physical Chemistry B 101: 9342-9351.
Emmanuel ED, Moses EU (2016). Antiplasmodial/Antimalarial effect of ethanol extracts of leaves of *Vernonia amygdalina* and *Gongronema latifolium* on the activity of catalase in *Plasmodium berghei*-parasitized mice. International Journal of Biochemistry Research. 10(4):1-9.
Ganong WF, (2015). Review of Medical physiology. 25th edition, Lange medical books; Mc Graw-Hill medical publishing division, London.
Gornall AG, Bardawill CT, David MM (1949). Determination of serum protein by means of Biuret reaction. Journal of Biological Chemistry.Pp.177:751.
Harborne JB (1973). Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. Chapman and Hall Ltd, London. p. 279.
Huffman MA (2003). Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutrition Society; 62(2):371–381.
Idu M, Onyibe HI (2007). Medical plants of Edo State, Nigeria. Research Journal of Medicinal Plant, 1: 32-41.
Igile GO, Oleszek W, Burda S, Jurzysta M (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. Journal of Agricultural and Food Chemistry; 43:2162–2166.
Kaplan MM (1986). Serum alkaline phosphatase—another piece is added to the puzzle. Hepatology; 6:526–528.
Krause WJ (2001). The art of examining and interpreting histologic preparations. A student handbook. Partheton Publishing group, U.K. Pp. 9-10.
Lewis JH, Stine JG (2013). Non-steroidal anti-inflammatory drugs and leukotriene receptor antagonists: pathology and clinical presentation of hepatotoxicity. In, Kaplowitz N, DeLeve LD eds. Drug-induced liver disease, 3rd Edition. Amsterdam: Elsevier Pp. 370-402.
Malomo SO, Daramola AS, Balogun EA (1995). Some serum and tissue enzyme changes in mice infected with *Plasmodium yeolii nigeriensis* before and after administration of halofantrine hydrochloride. Nigerian Journal of Biochemistry and Molecular Biology 10:71-77.
Meeunier l, Larre D (2018). Recent advances in hepatotoxicity of non-steroidal anti-inflammatory drugs. Annals of Hepatology, 17(2)187-191.
Miyamoto G, Zahid N, Uetrecht JP (1997). Oxidation of diclofenac to reactive intermediates by neutrophils, myeloperoxidase and hypochlorous acid. Chemical Research in Toxicology 10:414-419.
Moore RA, Derry S (2018). Diclofenac potassium in acute postoperative pain and dysmenorrhoea: Results from comparative clinical trial reports. Pain Research and Management. https://doi.org/10.1155/2018/9493413.
Navarro VJ, Senior JR (2006). Drug related hepatotoxicity. New England Journal of Medicine 354:731–739.
Ojiako OA, Nwanjo HU (2006). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. African Journal of Biotechnology; 5(18):1648–1651.
Pumford NR, Myers TG, Davilla JC, Highet RJ, Pohl LR (1993).Immunochemical detection of liver protein adducts of the non-steroidal anti-inflammatory drug diclofenac. Chemical Research in Toxicology 6:147-150.
Purcell P, Henry D, Melville G (1991). Diclofenac hepatitis. Gut. 32:1381-1385.
Reitman S, Frankel SA (1957). Colorimeter method for the determination of serum glutamate–oxaloacetate and pyruvate transaminases. American Journal of Clinical Pathology;28:56–63.
Scully LJ, Clarke D, Barr RJ, (1993). Diclofenac induced hepatitis: three cases with features of autoimmune chronic active hepatitis. Digestive Diseases and Sciences 38:744-751.
Sofowora A (1993). Medical plant and traditional medicine in Africa. Spectrum book Pp. 40-46, 120-137.
Sriutta P, Sirichanchuen B, Permsuwan U (2018). Hepatotoxicity of non-steroidal anti-inflammatory drugs: A systematic review of randomized controlled trials. International Journal of Hepatology. https://doi.org/10.1155/2018/5253623
Walter M, Gerard H (1970). Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchemistry Journal15:231–236.
Wright PJ. Walker S, Plummer DT (1972). Enzymes in rat urine alkaline phosphatase. Enzymologia 42:317-327.
Yakubu MT, Akanji MA, Oladiji AT (2007). Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem in on some testicular function indices in rats. Journal of Ethnopharmacology 111(27):288-292.