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Phytochemistry Investigation of the Hepatoprotective and Liver Regeneration Potential of the Leaf Extract of *Struchium sparganophora*

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Abstract
Phytochemistry and *in-vivo* potential leaf extract of S*truchium sparganophora* (SS) to treat liver damage was examined in this study using liver function biochemical assays. A total of sixty-seven (67) chemical components were identified in the extract using gas chromatography-mas spectrometry (GC-MS) 3α,4α-epoxymurolan-9(11)-en-10-ol identified as the main component (11.5%). The biochemical results showed that increased in GSH concentrations indicates its protective potency to cells from toxins and diseases. Significant superoxide dismutase (SOD) concentrations showed greater potential to remove reactive oxygen species (ROS). An increased concentration of catalyse (CAT) activities regulates cellular level of hydrogen peroxide, and hydrogen catabolism protection of the cells from oxidative assault. Increased levels of RBC attributed to the presence of phytochemicals responsible in reversing diclofenac induced anaemia. Lipid profile resulted in the raising of the HDL levels which enhances a normal and good heart functioning of the system. Reduction of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzymes in the extract is an indication of the hepato-protective potency of extract. This study showed that *S. sparganophora* (SS) have good prospects of being hepatoprotective drug candidate.

Key words: *Struchium sparganophora*, hepatoprotective, oxidative stress, antioxidant, liver regeneration.

Introduction

Vegetables are group of edible medicinal plants that are consumed by humans or other animals as food because they play important roles in human's health. Vegetables as natural products have been important part of the human diets for thousands of years (Samtiya *et al*., 2021; Adesina *et al*., 2023a; Fayehun *et al.*, 2024; Ololade *et al.,* 2024a). Vegetables offer many health benefits as source of antioxidant, anti-aging, anti-inflammatory, roughages that improve digestive health, normalize blood pressure, and prevent heart diseases (Zhou et al., 2021; Gbenga-Fabusiwa et al., 2024; Ololade et al., 2024b; Salemcity et al., 2024; Anuoluwa et al., 2025; Ololade et al., 2025). *S. sparganophora* (SS) known as "ewuro odo" in Yoruba; it is a medicinal herb used in Nigerian and other African countries' traditional medical systems to treat pain, fever, arthritis, rheumatism, neurological, diarrhea, antidote to poisons and mental diseases (Jose, 2014; Ganiyu *et al.*, 2012; Jeyadevi *et al*., 2019). SS has been demonstrated to possess high nutrient such as protein, crude fibre and minerals (Aderibige *et al*., 2015; Alemayehu *et al*., 2021). The liver is the body's largest visceral organ in animal, lies in the upper right part of the abdomen (Pomohaci et al., 2025). The body's capacity to digest medications and other foreign compounds is dependent on it. The liver is where medicines and other foreign substances are primarily metabolized (Marcia, and Ariane, 2013; Omoboyowa, 2021). To the best of our knowledge, there is paucity on the phytochemical, hepatoprotective and liver regeneration potential of the extract of SS. Therefore, this study was undertaken to investigate the hepatoprotective and liver fuctions potential of phytochemicals in SS.

Materials and Methods

Plant Collection and Preparation of extract

Fresh leaves of the sample were plucked from their stalk and air-dried at room temperature. The dried leaves were pulverized. The sample was soaked in methanol and ethyl acetate for 3 days (2:1) and then shaken intermittently. The slurry formed were filtered. The filtrates were transfered into beakers and evaporated in a waterbath at a temperature of 50°C. The concentrated extract was collected and kept in a refrigerator until use (Ololade *et al*., 2024a).

Analysis of Leaf Extract using GC-MS

The leave methanol-ethyl acetate extract of SS was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The specification and conditions for analysis were done according to Ololade *et al*., 2021. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature (Ololade *et al*., 2021; Adesina *et al*., 2022a; Ololade et al., 2024d).

Biochemical Analysis

Assessment of liver function: Serum was used for the assay of liver function: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) were done using spectrophotometer and total bilirubin

concentrations using Rx Monza Analyzer, and standard laboratory kits from Randox Laboratories Ltd. Other tests carried out include: Lipid Profile-High Density Lipoprotein, Low Density Lipoprotein, Total Cholesterol and glyceridehydes. Antioxidants effects on the organs (liver), Malondialdehyde (MDA), Superoxide Dismutase (SOD), Gluthathione (GSH) and Catalyse (CAT) and histopathological analysis (Kuyoro *et al*., 2017; Abdulrazzaq *et al*., 2019; Onifade *et al*., 2020; Yahaya *et al*., 2021; Kathak *et al*., 2022; Ololade et al., 2024e).

Statistical Analysis

Graph pad prism 6.0 was used for statistical analysis. The obtained data were expressed as mean ± Standard error of mean (SEM). Statistical significance, $p < 0.05$ was evaluated with one-way Analysis of Variance (ANOVA).

Results and Discussion

Chemical constituents of the leaf extract of SS analyzed by GC-MS. A total of sixty-five (65) chemical components were identified in the methanolic/ethyl acetate extract of SS, accounting for 97% of the total scomponents in the extract, the constituents with higher percentage composition were 3α,4α-epoxymurolan-9(11)-en-10-ol (11.5%), 3-methoxypyrocatechol (4.1%), glycerol (4.0%), and catechol (3.8%) as presented in Table 1.

Table 1: Chemical Composition of the Leaf Extract of *S. sparganophora* (SS)

Compound	Retention Index	Percentage Composition
tetrahydro-2H-pyran	708	0.20
1,4:3,6-Dianhydro-α-d-glucopyranose	916	2.20
2-methyl-2-pentenoic acid	959	1.00
glycerol	967	4.00
corylone	972	1.90
3-methylcyclopentane-1,2-dione	1003	1.90
3,4-anhydro-d-galactosan	1039	0.90
1-methyl-2(1H)-pyrimidinone	1042	1.60
2,4-dimethyl-1,3-cyclopentanedione	1064	1.00
p-guaiacol	1090	2.80
catechol	1122	3.80
trimethyl[2-(trimethylsilyl)phenyl]silane	1124	1.00
β-cyclocitral	1204	0.60
erythritol	1229	2.20
l-threitol	1229	2.20
2-methyl-6-oxoheptyl acetate	1254	1.00
α-hydroxy-*p*-cresol	1257	1.80
8-nonenoic acid	1262	1.00
6-amino-5-methyl-1,3-diazaadamantane	1263	0.60
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	2.50
3,4-dimethoxyphenol	1279	2.40
syringol	1279	2.70
9,10-dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	1303	1.80
3-methoxypyrocatechol	1311	4.10

1-methoxy-2-methyl-4-(methylthio)benzene	1347	1.90
neopentyl phenyl sulfide	1358	3.40
E-3-decenoic acid	1380	2.00
2,5-dimethoxyphenylmethanol	1415	1.80
4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene	1443	1.60
dl-arabinitol	1491	3.00
2-hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	1507	1.50
dihydro-β-agarofuran	1514	0.40
acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester	1562	1.80
3α,4α-epoxymurolan-9(11)-en-10-ol	1624	11.50
2,3-dehydro-4-oxo-*β*-ionol	1637	1.40
5,5,8a-trimethylhexahydro-2H-chromen-4a(5H)-yl acetate	1639	1.10
eudesm-7(11)-en-4-ol	1647	0.30
4-(1*E*)-3-hydroxy-1-propenyl-2-methoxyphenol	1653	1.40
2,3,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol	1658	0.50
arteannuin b	1690	1.20
myristic acid	1769	0.30
E-11-tetradecenoic acid	1777	0.90
(8S,14)-cedran-diol	1786	0.30
glutaric acid, ethyl octyl ester	1847	0.80
6-(isopropylidenehydrazino)pyridazine-3(2H)-thione	1939	1.80
acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene)oxepan-2-yl ester	1950	0.10
palmitic acid	1968	2.00
3,3a-epoxydicyclopenta[a,d]cyclooctan-4β-ol, 9,10a-dimethyl-6-methylene-3β-isopropyl-	1981	0.60
3-oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	2031	0.40
trans-phytol	2045	1.00
(1R,2S,8R,8Ar)-8-acetoxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-*trans*-decalin	2101	0.90
azuleno[4,5-b]furan-2(3H)-one, decahydro-8,9-dihydroxy-6,9a-dimethyl-3-methylene-, [3aS-(3aα,6β,6aα,8α,9α,9aβ,9bα)]-	2166	0.30
10αH-ambros-11(13)-en-12-oic acid, 3α,4α,6β-trihydroxy-γ-lactone	2166	0.30
ambrosiol	2166	0.30
2,5-anhydro-1-O-octyl-d-glucitol	2201	0.30
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	2203	0.60
2H-cyclohepta[b]furan-2-one, 6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-	2262	0.40

dodecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	2348	0.50
2-myristynoyl-glycinamide	2439	0.50
dihydropseduosarsasapogenin	2906	0.30
cholestan-3,22,26-triol/cholest-5-ene-3,16,22,26-tetrol	2991	0.30
lupan-3-one, cyclic 1,2-ethanediyl acetal	3000	0.30
2H-cyclohepta[b]furan-2-one, 6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-	3189	2.60
α-D-glucopyranoside, O-α-D-glucopyranosyl-β-D-fructofuranosyl	4506	0.90
stevioside	6530	0.30
Pecentage Total		97.00

The Effect of Diclofenac, leaf extract of SS and Silbon 140 mg standard drug on Antioxidant of Oxidative Stress Markers (GSH)

Figure 1 present treatment of induced rats liver tissues antioxidant enzymes with SS 200 mg (9.09±0.64), 400 mg (8.57±1.21) and silbon 140 (10.43±0.31) which gave rise to significant ($p < 0.05$) increase in the antioxidant enzyme (GSH) when compared with the Control group (8.36±0.47). There was no significant ($p > 0.05$) decrease in SS 200 mg (9.09±0.64) and 400 mg (8.57±1.21) in antioxidant enzyme (GSH) when compared with the group treated with Silbon 140 (10.43 ± 0.31). However, there was no significant ($p > 0.05$) difference in the activities of antioxidant enzyme (GSH) between the groups treated with SS 200 mg (9.09±0.64) and 400 mg (8.57±1.21). Significant ($p < 0.05$) increase inreduced glutathione concentrations on treating with SS 200 mg and 400 mg in comparison with Diclofenac only (7.99±1.19) was strong indication of its protective potency to cells from toxins and diseases. Reduction of antioxidant enzyme (GSH) level in the serum of induced rats' liver tissue in Diclofenac only (7.99±1.19) group in comparison with the Control group (8.36±0.47) was due to generation of reactive O_2^- species as GSH consumption increased which resulted to deadness of the liver/hepatocytes (Pomohaci et al., 2025). Figure 2 displayed the activities of SOD antioxidants in all experimental groups. The result showed that there was significant ($p < 0.05$) increase in SOD concentrations in the liver tissues of rats when treated with SS 400 mg (4.15±0.48) in comparison to what was obtainable in control group (2.53±0.27). There was no significant ($p > 0.05$) decrease between Diclofenac only (3.77±0.56) and Silbon 140 (3.76±0.62). There was significant ($p < 0.05$) decrease in concentration of SOD in the liver tissues of treated induced rats with SS 200 mg (3.66±0.39) when compared with 400 mg (4.15±0.48). There was significant ($p < 0.05$) increase in Diclofenac Only in comparison with control group.

According to Mukesh and Che, (2018) and Younus, (2018), superoxide dismutases (SODs) are group of metalloenzymes found in all kingdoms of life. SODs form the front line of defense against reactive oxygen species (ROS)-

mediated injury and oxidative stresses in plants and play most vital role in scavenging the reactive oxygen species produced during metabolic processes as well as under abiotic stress conditions. These proteins catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) and decrease O_2^- level which damages the cells at excessive concentration. It constitutes one of the major enzymatic components of detoxification of superoxide radicals (O_2^-) generated in biological system by catalyzing its dismutation to H_2O_2 and finally to H_2O and O_2 by catalase and peroxidase. Superoxide dismutase has therapeutic effects in various physiological and pathological conditions such as cancer, inflammatory diseases, cystic fibrosis, ischemia, aging, rheumatoid arthritis, neurodegenerative diseases, and diabetes. Numerous studies revealed that the higher the SOD activity the greater the potential to remove oxygen reactive species (ROS). ROS are the species generated through the reduction of molecular oxygen (O_2) that includes some free radicals such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), alkoxyl ($RO^{\bullet}$), and peroxyl ($ROO^{\bullet}$), and non-radical products like hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). SOD concentration was increased as a result of treatment with high dose of the extract SS 400 mg and therefore has potential to remove oxygen reactive species and converts superoxide anions into H_2O_2 and O_2 in the liver. Increased SOD concentrations in Diclofenac only were however in contrast with other works while treatment with the extract was in line with report of Oyinloye *et al*., 2016.

In Figure 3 concentrations of catalase (CAT) activities withtreated induced rats' liver tissues with SS 400 mg (19.50±2.59) showed significant ($p < 0.05$) increase when compared with control group (15.03±2.00). There was significant ($p < 0.05$) decrease between SS 200 mg (12.90±3.21) and 400 mg (19.50±2.59). There was significant ($p < 0.05$) increase between SS 400 mg (19.50±2.59) and Silbon 140 (16.67±1.37). Diclofenac only group (15.88±3.60) had no significant ($p > 0.05$) increase in comparison with Control group (15.03±2.00).

Ankita-Nandi *et al*., (2019) stated that a catalase (E.C. 1.11.1.6) is one of the most important antioxidant enzymes present in almost all aerobic organisms which mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen. Catalase is a key enzyme which uses hydrogen peroxide, a nonradical ROS, as its substrate. Catalase deficiency or malfunctioning is associated with many diseases such as diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, some dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia. Catalase has a prime role in regulating the cellular level of hydrogen peroxide, and its hydrogen peroxide catabolism protects the cells from oxidative assault, for example, by securing the pancreatic β cells from hydrogen peroxide injury. It has been postulated that catalase in the liver may confer cellular protection by degrading the hydrogen peroxide to water and oxygen. It can be deduced that SS 200 mg has reduced catalase enzymes reasons being unknown while 400 mg had remarkedly CAT concentrations whose level evaded oxidative stressin the liver. This finding is in line with Ijaz *et al*., (2023).

Figure 4 showed the MDA concentrations in different groups. The administration of Diclofenac only (6.57±1.34) increased the concentration of MDA levels significantly ($p < 0.05$) when compared with the Control group (3.22±0.85). There was significant ($p < 0.05$) decrease in MDA concentrations in SS 200 mg (4.92±0.78), 400 mg (4.49±0.95) and silbon 140 (3.88±0.44) in comparison to diclofenac only (6.57±1.34). Biomonitoring of MDA has been used in both *in-vivo* and *in-vitro* studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer. Higher levels of MDA are reported in patients of various categories including lung cancer patients, complex regional pain syndrome patients and glaucoma patients. Findings by Khoubnasabjafari *et al.*, (2015) suggestted the validity of the MDA assay as a reliable tool in finding out the oxidative stress in different disease pathologies. Administration of diclofenac only resulted into increased activity of MDA antioxidant enzyme which was due to increase in oxidative stress. This can be harmful to the circulatory system which can result into various health disorders. The reduction in MDA concentrations in the liver of groups treated with the extract in comparson with diclofenac only group suggests the ability of the extract to ameliorate lipid peroxidation. The finding in this study is in line with the report of Famurewa *et al.*, (2020).

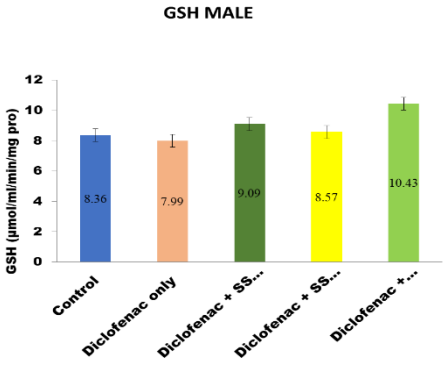


Figure 1: Reduced Glutathione (GSH)

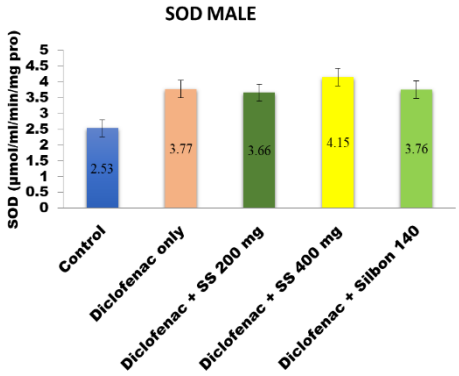


Figure 2: Superoxide Dismutase (SOD)

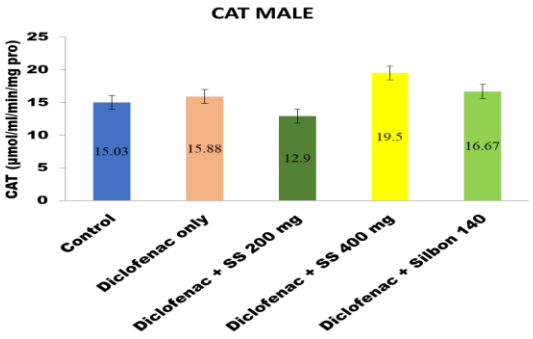


Figure 3: Catalase (CAT)

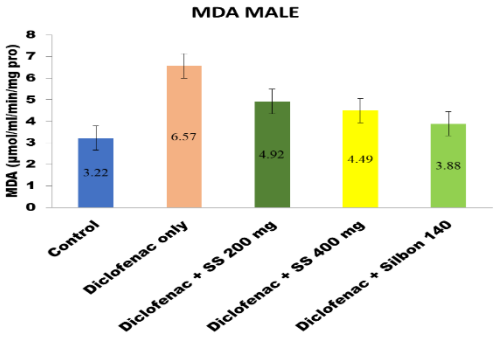


Figure 4: Malondialdehyde (MDA)

The Effect of Diclofenac (Toxicant), Leaf Extract of SS and Silbon140 mg (Sylimarin) Standard Drug on Haematology Parameters

In Figure 5, high significant ($p < 0.05$) increase in WBC concentration was generated in the serum of diclofenac only group (11.67±4.49) induced rats when compared with the control group (6.23±2.19). Other experimental groups SS 200 mg (4.80±0.23), 400 mg (8.20±1.55) and Silbon 140 (5.43±0.95) decreased significantly ($p < 0.05$) when compared with serum of induced rats with diclofenac only. A significant ($p < 0.05$) increase in WBC concentration was observed in the group treated with SS 400 mg (8.20±1.55) when compared with the groups treated with SS 200 mg (4.80± 0.23) and Silbon 140 (5.43±0.95) while there was no significant ($p > 0.05$) decrease between the groups treated with SS 200 mg (4.80±0.23) and Silbon 140 (5.43±0.95). Induction of Diclofenac only gave rise to increased WBC concentration due to inflammation of the liver, stress, trauma and infection in the body, this study is similar to Umaru *et al*., (2018). In Figure 6, there was no significant ($p > 0.05$) increase between control (6.81±0.56) and SS 200 mg (6.39±0.52) groups and these had the highest concentrations of RBC. There was no significant ($p > 0.05$) decrease between treated induced rats with SS 400 mg (5.89±0.35) and Silbon 140 (5.97±0.18). Diclofenac only (4.35±1.42) had high significant ($p < 0.05$) decrease in RBC levels. It has been known that some herbal formulation could restore the hepatic architecture and protect the liver tissue from fatty and degenerative changes, by preventing the toxic chemical reaction, oxidative stress, lipid peroxidation, molecular changes in the liver tissues, micro and macro vesicular fatty changes that lead to necrosis (Tan *et al*., 2016). The induction of hepato-toxicity with diclofenac only resulted in reduction of red blood cells (RBCs) leading to depletion of iron and RBCs destruction resulting into anaemic state. The significant increase of RBC when the extract was administered in varying doses could be attributed to the presence of phytochemicals responsible in reversing diclofenac induced anaemia. This study is similar to the result obtained by Yamoah *et al.,* 2020.

In Figure 7, Diclofenac only (11.43±0.30) serum of induced rats had significant ($p < 0.05$) increase in HGB concentrations when compared with the control group (10.10±0.26). Treated groups with SS 200 mg (10.33±0.73), 400 mg (9.97±0.47) and Silbon 140 (10.40±0.80) had significant ($p < 0.05$) decrease when compared with diclofenac only group. High HGB in the diclofenac only group could be health implicating which include, blood clots, bleeding, weight loss, dizziness and fatigue while low level indicates anaemia due to insufficient oxygen-carrying capacity in meeting the body's physiological demands Yamoah *et al*., 2020. This result is in contrast with Yamoah *et al*., 2020. The reason may be due to the toxicant used for hepato-toxicity.

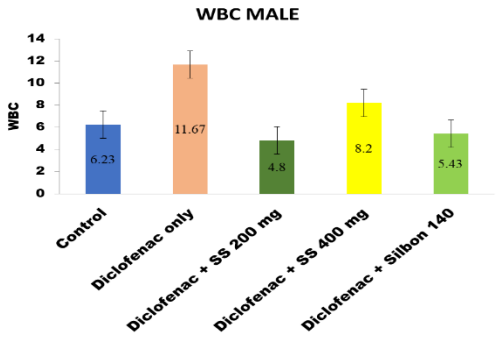


Figure 5: White blood cells (WBC)

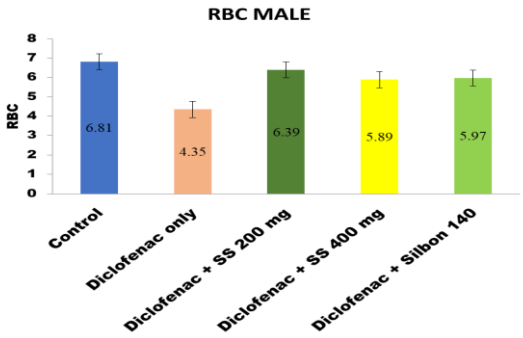


Figure 6: Red blood cells (RBC)

The Effect of Diclofenac, leaf extract of SS and Silbon 140 mg standard drug on Lipid Profile

In Figure 8, serum of treated induced rats with SS 200 mg (1.44±0.19) has significant ($p < 0.05$) increase when compared with control group (0.83±0.10). Serum of treated induced rats with SS 400 mg (0.80±0.32) had significant ($p < 0.05$) decrease when compared with serum of the treated rats with SS 200 mg (1.44±0.19) and Silbon 140 (1.23±0.11). Serum of rats induced with Diclofenac only (0.70±0.17) had high significant ($p < 0.05$) decrease when compared with treated rats serum with SS 200 mg (1.44±0.19) and Silbon 140 (1.23±0.11).

High Density Lipoprotein (HDL) or good cholesterol should be high. Treatment of induced rats with SS 200 mg and Silbon 140 resulted in the raising of HDL levels while the induction of rats with Diclofenac only reduced the level of HDL (Figure 8). HDL helps to remove accumulation of LDL- which is a bad cholesterol from the blood thereby promoting good heart functioning and good health conditions. Reduction of HDL levels in the blood was a sign of lipid disorder which might lead to life threatening conditions like heart attacks, strokes or coronary artery diseases. This finding is in similarity to the report of Oyinloye *et al*., (2016).

In Figure 9, all experimental rats'groups have significant ($p < 0.05$) increase in comparison with control group (1.49±0.05). Diclofenac only group (2.25±0.21) and serum of treated induced rats with SS 200 mg (2.24±0.48) have no significant ($p > 0.05$) decrease. Silbon 140 (2.05±0.19) and SS 400 mg (2.09±0.41) have no significant ($p > 0.05$) decrease in cholesterol level. Cholesterol (CHOL) is a waxy substance needed to produce certain hormones and build the outer membrane of every cell. Induction of rats with diclofenac only increased the level of CHOL. A certain amount of cholesterol is essential, too much of it can build up the blood vessels and raise one's risk of heart disease, clogging or hardening ofarteries and stroke. Total cholesterol increased in diclofenac only group could be attributed to the harmful effects of diclofenac on cell membrane (Ghosh *et al.*, 2016). SS 400 mg remarkably attenuated the alterations in the levels of total cholesterol when administered. This work is similar to Bagheri *et al*., (2020).

In Figure 10, serum of treated induced rats with Silbon 140 (0.52±0.32) had no significant ($p > 0.05$) decrease when compared with control group (0.58±0.11). Diclofenac only (0.92±0.09) had significant ($p < 0.05$) increase when compared with serum of treated induced rats with SS 200 mg

(0.75±0.17), SS 400 mg (0.68±0.22) and Silbon 140 (0.52±0.32). Serum of treated rats with SS 400 mg (0.68±0.22) had no significant ($p > 0.05$) decrease when compared with SS 200 mg (0.75±0.17). Low Density Lipoprotein (LDL) or bad cholesterol should be low. Induction of rats with diclofenac only increased the level of LDL which could cause cholesterol to build up on the walls of the arteries and thereby resulting into risk of having heart attack, stroke and atherosclerosis while administration of the extract attenuated the increased alteration levels. Lipoproteins are involved in different processes such as immune reactions, coagulation, and tissue repair. They include macromolecules of lipid and protein which is responsible in the transportation of lipids through the vascular and extravascular fluids in the body. Oxidative alteration of lipoproteins, particularly the LDL-C, which commonly follow the reduction of lipoprotein antioxidants, especially Vitamin E, is identified to cause accumulation of cholesterol and enhance vulnerability of atherosclerosis (Olayinka *et al*., 2011; Ololade et al., 2025).

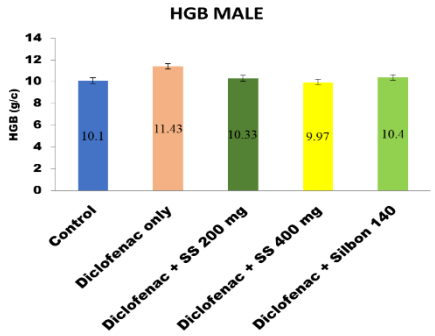


Figure 7: Haemoglobin (HGB)

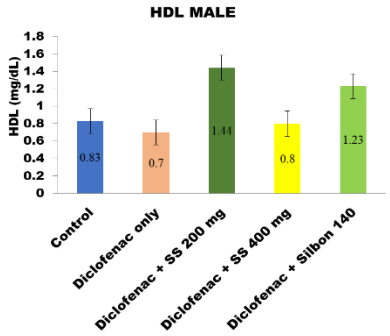


Figure 8: High Density Lipoprotein (HDL)

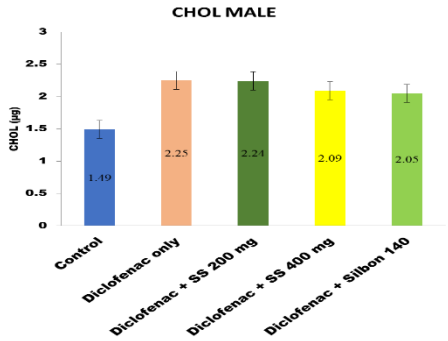


Figure 9: Cholesterol (CHOL)

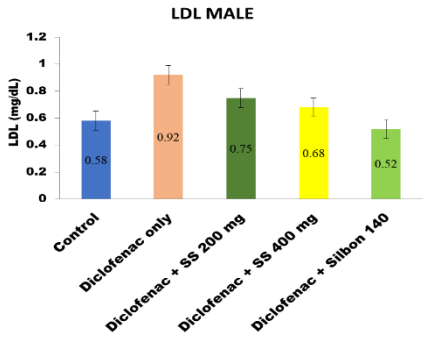


Figure 10: Low Density Lipoprotein (LDL)

Effect of Triglyceride concentrations level in experimental animals

Serum of induced rats with diclofenac only (1.68±0.34) gave rise to significant ($p < 0.05$) increase in TG concentrations in comparison with control group (0.72±0.80) Figure 11. Serum levels were reduced significantly ($p < 0.05$) on treatment with SS 200 mg (0.49±0.04), 400 mg (0.93±0.15) and Silbon 140 (0.57±0.19) while it was significantly (p

< 0.05) attenuated by the administration of SS 200 mg dose of the leaf extract. This finding is in line with Nwozo *et al*., (2012) and Oyinloye *et al.*, (2016). Elevated level in diclofenac only could be attributed to harmful effect of diclofenac on cell membrane resulting in the elevation of hepatic synthesis of triglyceride and reduced clearance rate of triglyceride-rich lipoproteins while the significant reduction of TG may be attributed to the presence of secondary metabolites in plants. This showed that extract of SS has a great potential for protection against atherosclerosis and its accompanying risk of cardiovascular diseases Oyinloye *et al*., (2016).

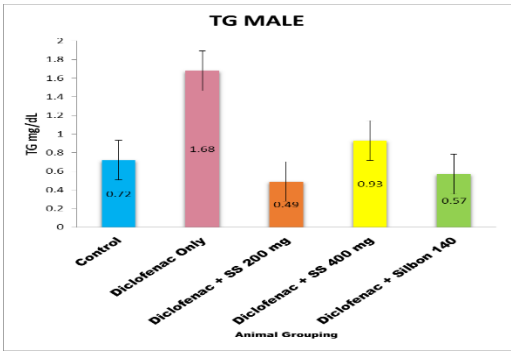


Figure 11: Triglyceride (TG)

The Effect of Diclofenac, leaf extract of SS and Silbon140 mg (Sylimarin) Standard Drug on Serum biochemical parameters

Liver function marker

Figure 12 demonstrated that diclofenac only (95.83±11.14) group has high significant ($p < 0.05$) increase in SGPT (ALT) enzymes when compared with the Control group (55.20±4.28). Treatment of induced rats with SS 200 mg (61.90±8.83), 400 mg (61.10±13.91) and Silbon 140 (43.47±6.27) decreases SGPT enzymes significantly ($p < 0.05$) when compared with the group treated with Diclofenac only. The high rise of SGPT-ALT in diclofenac only group showed that the liver was highly inflamed and this established the fact that the intake of high dose or prolong use of diclofenac can result in hepato-toxicity of the liver. Significant ($p < 0.05$) decrease of SGPT-ALT levels in the treated groups with SS 200 mg and 400 mg proved the potencyof the leaf extract of SS as agent of liver regeneration and hepato-protectiveness.

Figure 13 demonstrated that rats induced with Diclofenac only (176.47±8.55) has high significant ($p < 0.05$) increase in SGOT (AST) enzymes when compared with the Control group (132.10±7.99) and other experimental groups. Treatment of induced rats with SS 200 mg (158.17 ± 18.35), 400 mg (94.30±41.52) and Silbon 140 (100.50±19.63) decreases SGOT (AST) enzymes significantly ($p < 0.05$) when compared with the group treated with diclofenac only. Rats induced treated with SS 200 mg (158.17±18.35) has significant ($p < 0.05$) increase when compared with 400 mg (94.30±41.52) and Silbon 140 (100.50±19.63) in SGOT (AST) enzymes. SS 200 mg has significant ($p < 0.05$) increase when compared with the control group. SS 400 mg has significant ($p < 0.05$) decrease when compared with the control group. The detection of high level of SGOT (AST) enzymes in

diclofenac only induced rats' serum is in line with Salim, (2016) which showed that the liver was injured and there could be skeletal muscle damage. Administration of the extract in varying doses SS 400 mg and 200 mg ameliorated hepatic damage of the liver. However, SS 400 mg proved to be more potent as agent of liver regeneration and hepatoprotectiveness.

Figure 14 demonstrated that the serum of rats induced with Diclofenac only (1086.43±30.21) has high significant ($p < 0.05$) increase in ALP enzymes when compared with the Control group (575.50±116.74) and other experimental groups. Treatment of inducedrats with SS 200 mg (630.50 ± 95.73), 400 mg (698.20±108.66) and Silbon 140 (415.70±151.58) decreased the activity of ALP enzymes significantly ($p < 0.05$) when compared with the group treated with diclofenac only. Treated induced rats's serum with SS 200 mg and 400 mg increased significantly ($p < 0.05$) when compared with Silbon 140 mg. The detection of high level of ALP enzymes in the serum of Diclofenac only induced rats which indicates the level of liver injury and bone diseases. Raised level of ALP could also mean that bile flow out of the liver is blocked. Administration of the extract however ameliorated the hepatic damage and healed bone diseases.

Figure 15 showed that the serum of induced rats with Diclofenac only (3.10±0.17) has significant ($p < 0.05$) increase when compared with control group (1.60±0.15). SS 400 mg (2.43±0.35) has no significant ($p > 0.05$) increase when compared with Silbon 140 (2.30±0.32) and SS 200 mg (2.13±0.09). Bilirubin is the catabolic product of haemoglobin produced within the reticuloendothelial system, released in unconjugated form which enters into the liver, converted to conjugated forms bilirubin mono and diglucuronides by the enzyme UDP-glucuronyltransferase (Kumbhar et al., 2024). In viral hepatitis, hepato-cellular damage, toxic or ischemic liver injury higher levels of serum conjugated bilirubin is seen (Ramírez-Mejía *et al*., 2024). A rise in the concentrations of TBILI in Diclofenac only group showed hepato-cellular injury while its attenuation was indicated in the decreased values of plant extract (Figure 15).

Decrease in the level of serum liver enzyme markers (AST, ALT, ALP and TBILI) as a result of treatment with Silbon 140, SS 200 mg and 400 mg is in conformation with the study carried out by Chantarojanasiri et al., 2023.

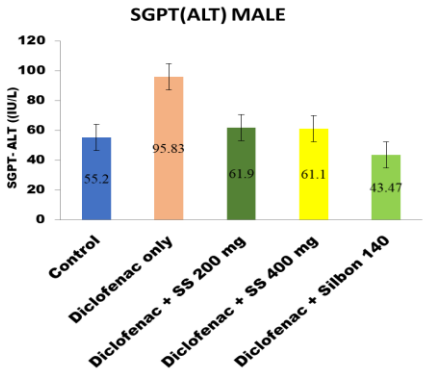


Figure 12: SGPT (ALT)

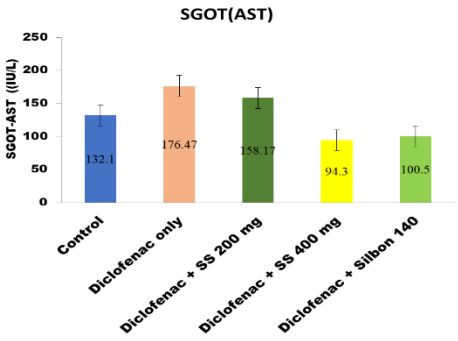


Figure 13: SGOT (AST)

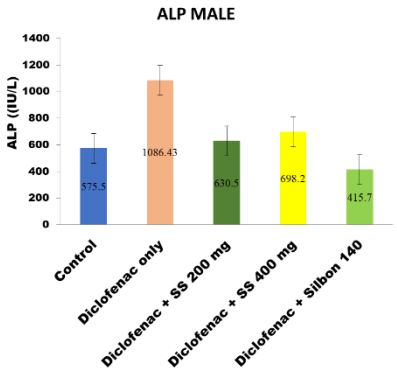


Figure 14: ALP

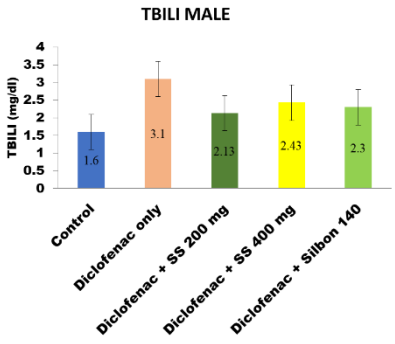
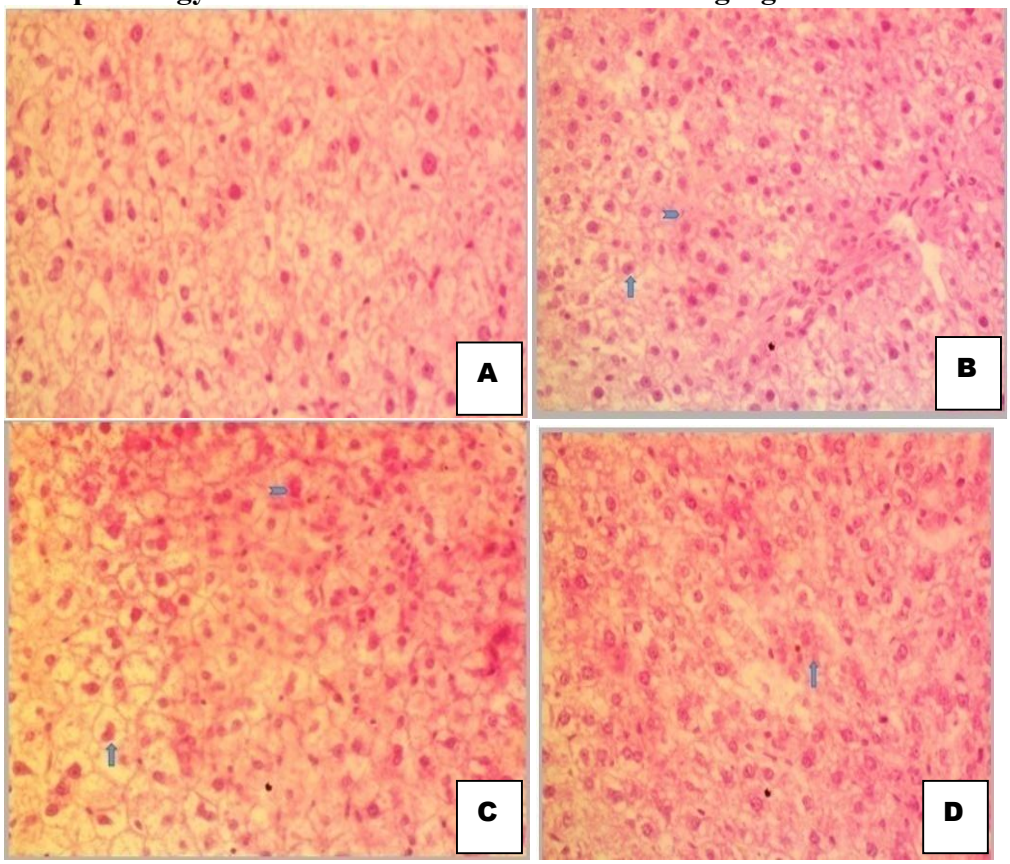


Figure 15: TBILI

Histopathological studies of male albino rats' liver

Histopathology Examination Result and Photomicrograghs of rats' liver



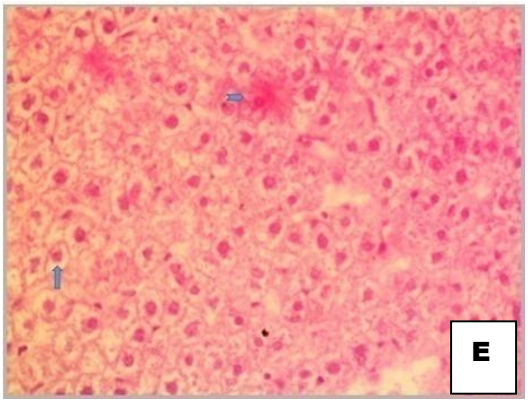


Plate 1: Photomicrograghs of liver**:** (A) Control (Normal) group (B) Diclofenac Only group (C) Diclofenac + SS 200 mg/kg (D) Diclofenac + SS 400 mg/kg, (E) Diclofenac + Silbon 140 mg/kg (Stain: H & E; x400)

Plate 1 present histopathology examination of male albino rats liver.

Control group

Examination of isolated liver organs from male albino rats microscopically revealed that there were no alterations in the rats liver from control group Plate 1A.

Diclofenac Only group

Histology of diclofenac only treated group showed that there were severe vacuolar degenerations (arrow) and severe necrosis (arrow head) of hepatocytes (x400; H & E) Plate 1B.

Diclofenac + SS 200 and 400 mg/kg

No significant ($p > 0.05$) vacuolar degenerations and necrosis of hepatocytes (x400; H & E) were observed in rats liver administered with SS 200 mg/kg and 400 mg/kg doses of leaf extract of SS separately Plate 1 C & D respectively.

Diclofenac + Silbon 140 mg/kg

Microscopic examination of liver section using (x400; H & E) revealed scanty vacuolar degeneration and mild necrosis changes Plate 1 E.

Severe vacuolar degenerations and necrosis observed in liver photogragh of Diclofenac Only in Plate 1 B was a strong indication of hepatocellular damage in comparison with Control group. However, hepato-protective effect of the leaf extract (Plate 1 C & D) and Silbon 140 (Plate 1 E) was observed as the minor vacuolar degenerations and necrosis seen in them.

Conclusion

This study presents novel natural application of *S. sparganophora* (SS) as the hepatoprotective agent responsible for liver treatment. Secondary metabolites in the sample with medicinal property is responsible for significant reduction of ALT, AST, BILI and MDA levels. The results further showed that increased in GSH concentrations in the sample at 200 mg indicates its protective potency to cells from toxins and diseases. Raised levels of SOD concentrations in the sample at 400 mg showed greater potential to remove oxygen reactive species (ROS). Increased concentrations of CAT activities with the extract at 400 mg regulate cellular level of hydrogen peroxide, and hydrogen catabolism protection of the cells from oxidative assault. Increased levels of RBC on treatment with the extract at varying doses of 200 mg and 400 mg could be attributed to the presence of phytochemicals responsible in reversing diclofenac induced anaemia. In lipid

profile, treatment with the sample at 200 mg resulted in the raising of the HDL levels which enhances a normal and good heart functioning of the system. Increase in ALT, AST, ALP enzymes and TBIL in diclofenac only group indicates liver injury while rapid reduction of ALT, AST and ALP enzymes in the extract 200 mg and 400 mg is an indication of the hepatoprotective potency of extract. It has therefore proven the efficiency and efficacy of the extract of SS as source of natural hepatoprotective drugs. The histopathology result was a proof of the damaging effect of diclofenac and the hepatoprotective potential of the leaf extract of SS on the liver of male albino rats.

Conflict of Interest Statement: We have no conflit of interest.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

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