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Dose Dependent Hepatotoxicity Study of *Senna tora* Leaves on Wistar Albino Rats

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A B S T R A C T

Medicinal plants and herbs such as *Sennatora* contain substances known to modern and ancient civilizations for their healing properties. Documented evidence shows that the plant has been used to treat various ailments. Still, its toxicity on vital organ such as the liver has not been specifically studied. The study was designed to ascertain the dose dependent hepatotoxicity of *Sennatora* leaves on Wistar albino rats. A total of twenty-five (25) Wistar albino rats were used, divided into 5 groups of 5 rats each (Group 1, 2, 3, 4 and 5). Group 2, 3, 4, and 5 received 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg of the *S. tora* ethanol fraction, respectively while group 1 was the normal control which received only distilled water and feed *ad libitum*. The treatment was carried out for 21 days. At the end of the experiments, the animals were sacrificed. Afterward, liver function tests and lipid peroxidation estimation were carried out. The indicators of liver function which include Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), Total protein (T.P), and Albumin (ALB) were ascertained. Also the parameters for lipid peroxidation which include Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) were determined. The result showed that there was a significant ($p \leq 0.05$) decrease in the level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT) across the groups compared to the normal control. There was a significant ($p \leq 0.05$) increase in the concentration of Total protein (TP) across the group in relation to the normal control while for the concentration of Albumin (ALB), there was a significant ($p \leq 0.05$) increase in group 2 (8.89±1.16), 4 (8.85±0.57), and 5 (7.30±0.84) which received 100 mg/kg, 400 mg/kg, and 800 mg/kg *S. tora* fraction respectively and a significant ($p \leq 0.05$) decrease in group 3 (0.76±0.06) which received 200 mg/kg compared to the normal control (3.45±0.63). The result for lipid peroxidation showed that there was significant ($p \leq 0.05$) increase in the concentration of MDA in group 2 (45.67±0.50) which received 100 mg/kg, however, there was significant ($p \leq 0.05$) decrease in the other groups compare to the normal control. The activity of the antioxidant enzymes: SOD showed significant ($p \leq 0.05$) decrease in group 2 (76.45±3.39) which received 100 mg/kg and a significant ($p \leq 0.05$) increase in group 3 (88.55±2.03), 4 (77.99±4.67) and 5 (83.23±0.89) which received 200 mg/kg, 400 mg/kg and 800 mg/kg of *S. tora* fraction respectively compared to the normal control. While the result for CAT showed a significant ($p \leq 0.05$) increase across the group compared to the normal control. This study showed that at doses of 100- 800 mg/kg body weight, *S. tora* did not cause hepatotoxicity. The use of *S. tora* as a traditional herb for managing liver disease without causing significant organ damage has also been supported by this study.

Introduction

Medicinal plants and herbs contain substances known to modern and ancient civilizations for their healing properties. A number of plants have been used in traditional medicine for many years; some do seem to work, although there may not be sufficient scientific data to confirm their efficacy (Shehu *et al*., 2018). The medicinal value of these plants lies in some chemical substances they contain, that produce definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Arowora *et al*., 2019). Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs (Yakubu *et al*., 2014).

Herbs and spices are generally considered safe and proved to be effective against certain ailments. While literature has documented severe toxic reactions from the use of herbs on many occasions, still the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine (Shehu *et al*., 2018). Patients are often unaware of important similarities and differences between medicinal herbs and approved medications, some mistakenly think of herbs as natural alternative to chemicals, failing to recognize that herbs are composed of bioactive chemicals some of which may be toxic (Arowora *et al*., 2019). Herbal knowledge is gathered through trial and error over a period of time. Traditional herbalists detect effective substances by trying them out on themselves or on patients and therefore, toxicity and side effects may be encountered during treatment (Shehu *et al*., 2018).

Traditional medicines include practices, such as herbal medicine, ayurvedic medicine, unani medicine, acupuncture, as well as other medical knowledge and practices (not orthodox) all over the globe. Inappropriate use of traditional medicines or practices can have negative or dangerous effects and so, further research is needed to ascertain the efficacy and safety of the medicinal plants used in traditional medicine system (WHO, 2003).

The use of *Sennatora* as a healing herb stems from Ayurveda. Rural as well as tribal people of Madhya Pradesh, India have been using the leaves and the seeds of this plant for different kinds of aliments. Further, Traditional Chinese Medicine (TCM), has plenty of proof and experience regarding the application of *Senna* seeds (also known as Jue Ming Zi) and leaves in drug preparation as well as using them to cure various diseases. The weed form of the crop has been analyzed since ancient times and as per Ayurvedic records, it was known to contain acrid, laxative, anthelmintic, ophthalmic, cardiotonic and expectorant properties. Since ancient times in China, the seeds of *Senna* have been used as aperients, antiasthenic and diuretic agents (Parth *et al*., 2021). It is a semi-wild annual herb grown widely in different places of south-east Asia including India, Northern Australia and Americas (Sarwa *et al*., 2014).

Many xenobiotics are capable of causing some degree of liver injury (Singh *et al*., 2011). The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure (Singh *et al*., 2011).

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Singh *et al*., 2011). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, and natural chemicals like microcystins, herbal remedies and dietary supplements (Tostmann *et al*., 2008). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Singh *et al*., 2011). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Singh *et al*., 2011). Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or

legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool (Tostmann *et al*., 2008).

Materials and Methods

Sample Collection and Preparation

The stem leaves of the *Sennatora* plant were collected within Wukari metropolis in Wukari Local Government Area of Taraba State, Nigeria. The leaves were critically examined to be free from disease and contamination of any sort. Only healthy plants were selected for the analysis. The plant material was dried in the laboratory at room temperature for one week and pulverized using traditional Mortar and pestle.

Ethanol Extraction

The method of Yakubu *et al*., (2014) was adopted for this protocol. Four hundred grams (400 g) of pulverized sample each of leaf was weighed into a plastic container and filled with 1200 mL ethanol (1:4 w/v) for 72 hours at room temperature with occasional shaking. After finishing maceration, the sample was sieved with clean white mesh before filtering using Whitman No 1 filter paper. Next, the filtrate was poured into a beaker.

Fractionation of crude ethanol extract of *S. tora* leaves

50 ml of crude ethanol extract was measured using a measuring cylinder into a separating funnel, 100 ml of n-Hexane was also measured into the separating funnel and the funnel was shaken several times, it was kept undisturbed to separate into two layers. The ethanol fraction settled at the bottom of the separating funnel and n-hexane settled at the top of the separating funnel. The n-hexane fraction was collected into a beaker. The ethanol fraction was then collected into a beaker and was concentrated using rotary evaporator under reduced pressure and concentrates transferred into air-tight container and preserved in the refrigerator at 4°C prior to administration.

Experimental Animals

Healthy male Wistar albino rats of about 120-150 g in weight were used for this study. They were purchased from animal house of College of Health Science, Benue State University, Nigeria and transported to the Animal house of Department of Biochemistry, Federal University Wukari, Nigeria.

They were acclimatized for 2 weeks and fed with finisher mash and water *ad libitum*. They were weighed prior to the commencement of the experiment.

Experimental Design

The Wistar albino rats of the average weight of 135 g were randomly assigned into five (5) groups: 1, 2, 3, 4, and 5 of five (5) animals in each group. Group 2, 3, 4, 5 served as treatment groups, while group 1 served as the control group.

The rats in the treatment groups (2, 3, 4 and 5) received 100 mg/kg, 200 mg/kg, and 400 mg/kg and 800 mg/kg body weight of ethanol fraction of *S. tora* orally through an or gastric tube daily for 21 days, respectively. The control group received an equal volume of distilled water without the *S. tora* ethanol fraction.

Animal sacrifice and Serum preparation

After the 21 days, the animals were fasted overnight and sacrificed under Chloroform anaesthesia. Blood samples were collected from each of the animals through cardiac puncture into a plain tubes and serum was separated after centrifugation.

Tissue Homogenization

The liver of each Wistar albino rat in each group was harvested carefully and placed in normal saline so as to clean from unnecessary tissue remnant. After weighing, liver tissues were homogenized in phosphate buffer (1:10 w/v) at 7.4 pH. The supernatants were used for the determination of Thiobarbituric acid reactive substances (TBARS), Superoxide dismutase (SOD) and Catalase (CAT).

Determination of Biochemical Parameters

Assessment of Aspartate Aminotransferase (AST) Activity

AST activity was determined by the method described by Reitman and Frankel (1957) assay kits (Randox Laboratories Ltd, UK).

Assessment of Alanine Aminotransferase (ALT) activity

ALT activity was determined by the method described by Reitman and Frankel (1957) using assay kits (Randox Laboratories Ltd, UK)

Assessment of Alkaline Phosphatase (ALP) activity

The serum activity of Alkaline Phosphatase (ALP) was determined by the method (Schlebusch *et al*., 1946) described using Agappe reagent kit.

Assessment of Gamma- glutamyl transferase (GGT)

The serum activity determination of GGT was carried out by the method described by Szasz (1976) using Agappe reagent kit.

Determination of Total Protein (T.P)

The total protein concentration was determined using the method described by Weichselbaum (1942) using assay kits (Randox Laboratories Ltd, UK).

Determination of Albumin (ABL)

The serum albumin concentration was determined by the method described by Doumas *et al*., (1971) using Agappe kit.

Estimation of Thiobarbituric Acid Reactive Substance (TBARS)

Thiobarbituric Acid Reactive Substances (TBARS) are products of the oxidative degradation of polyunsaturated fatty acids, in particular, malondialdehyde (MDA). Lipid peroxidation was assayed by the measurement of MDA levels on the basis of MDA reacted with thiobarbituric acid at 532 nm, according to Torres *et al*., (2004).

Estimation of Superoxide Dismutase (SOD) activity

Superoxide dismutase activity was measured using the method described by Martin *et al*., (1987).

Estimation of Catalase (CAT) activity

Catalase activity was determined using the method described by Aebi (1983).

Statistical Analysis

Statistical analysis was carried out with the use of one-way analysis of variance (ANOVA) and furthers with Duncan multiple comparisons using Statistical Package for Social Sciences (SPSS), version 23. The result means were compared for significance at $p<0.05$ and the group results presented as mean±standard deviation (n = 5).

Results and Discussion

Result Data Showing Effect of *S. tora* Ethanol Fraction on Liver Function Tests

Effect of *S. tora* Ethanol Fraction on Aspartate Aminotransferase (AST)

From the result obtained on the activity of AST, it showed that there was significant ($p\leq0.05$) difference in both group 2 (25.75 ± 1.35^{b}) and 3 (25.70 ± 1.92^{b}) which received 100 mg/kg and 200 mg/kg compare the normal control (26.25 ± 0.54^{c}). In the same vein, there was a significant ($p\leq0.05$) difference in the activity of AST in group 4 (13.50 ± 3.45^{a}) and 5 (12.45 ± 3.34) which received 400 mg/kg and 800 mg/kg of ethanol fraction of *S. tora*. There was a significant ($p\leq0.05$) decrease in the activity of AST across the group in relation to the normal control (26.25 ± 0.54^{c}).

Effect of *S. tora* Ethanol Fraction on Alanine Aminotransferase (ALT)

From table 1, the result on the activity of ALT showed that, there was no significant ($p\leq0.05$) difference in group 2 (37.00 ± 3.43^{b}) that received 100 mg/kg and the normal control (38.80 ± 4.16^{b}). However, there existed a level of significant ($p\leq0.05$) in group 3 (33.7 ± 1.14^{a}), 4 (31.5 ± 4.52^{a}), and 5 (30.60 ± 8.61^{a}) that received 200 mg/kg, 400 mg/kg and 800 mg/kg compare to the normal group. There was a significant ($p\leq0.05$) decrease in the level of ALT across the treated groups compared to normal group (38.80 ± 4.16^{b}).

Effect of *S. tora* Ethanol Fraction on Alkaline Phosphatase (ALP)

The activity of ALP from the table below showed that there was a significant ($p\leq0.05$) difference in group 2 (2.73 ± 8.33^{c}) and 3 (2.60 ± 0.26^{c}) that received 100 mg/kg and 200 mg/kg compare to the normal control group. The

was significant ($p \leq 0.05$) difference in group 4 (1.46 ± 0.26^{d}) which received 400 mg/kg in relation to normal control. In the same vein, there was a significant ($p \leq 0.05$) in group 5 (0.79 ± 1.54^{b}) which received 800 mg/kg compare to normal control (2.76 ± 0.93^{a})

The overall result indicated a significantly ($p \leq 0.05$) decrease in the level of ALP activity across the treatment groups.

Effect of *S. tora* Ethanol Fraction on Gamma-glutamyl transferase (GGT)

Result from table 1, showed that there was a significant ($p \leq 0.05$) difference in level of GGT activity in group 2 (1.46 ± 0.16^{a}) and 3 (0.81 ± 0.03^{a}) which received 100 mg/kg and 200 mg/kg in relation to the normal control group (2.28 ± 0.28^{c}).

In the same vein, there was a significant ($p \leq 0.05$) difference in the activity of GGT in group 4 (0.69 ± 0.22^{b}) that received 400 mg/kg compare to the control group. Lastly, group 5 (0.94 ± 0.22^{ab}) that received 800 mg/kg showed a level of significance ($p \leq 0.05$) in the activity of GGT. There was a significantly ($p \leq 0.05$) decrease in the activity of GGT across the treated groups in comparison with the normal control group (2.28 ± 0.28^{c}).

Effect of *S. tora* Ethanol Fraction on Total Protein (T.P)

From the result shown on table 2, there was a significant ($p \leq 0.05$) difference on the of Total protein level in group 2 (19.24 ± 1.64^{a}), 3 (19.70 ± 0.07^{a}), 4 (21.95 ± 1.89^{a}), and 5 (22.00 ± 1.28^{a}) which received 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg of *S. tora* fraction respectively in relation to the normal control group (13.50 ± 5.82^{b}). There was significantly ($p \leq 0.05$) increase in of Total protein level across the treated groups compare to the normal control group.

Effect of *S. tora* Ethanol Fraction on Albumin (ALB)

The result of serum albumin in table 2, showed that, there was a significant ($p \leq 0.05$) difference in group 2 (8.89 ± 1.16^{c}), 4 (8.95 ± 0.57^{c}) and 5 (7.30 ± 0.84^{c}) which received 100 mg/kg, 400 mg/kg and 800 mg/kg respectively, still there was a significant ($p \leq 0.05$) difference in group 3 (0.76 ± 0.057^{a}) which received 200 mg/kg of *S. tora* ethanol fraction compare to the normal control group (3.45 ± 0.63^{b}). There was a significantly ($p \leq 0.05$) increase in the concentration of albumin in group 2 (8.89 ± 1.16^{c}), 4 (8.95 ± 0.57^{c}) and 5 (7.30 ± 0.84^{c}), and the albumin level decrease significantly ($p \leq 0.05$) in group 3 (0.76 ± 0.057^{a}) all compare to normal control group (3.45 ± 0.63^{b}).

Result Data Showing Effect of *S. tora* Ethanol Fraction on Lipid Peroxidation Parameters

Effect of *S. tora* Ethanol Fraction on Malondialdehyde (MDA)

Based on the result shown in table 3, there was no significant ($p \leq 0.05$) difference on the level of MDA in group 2 (45.67 ± 0.50^{c}) which received 100 mg/kg compare with the normal control (43.33 ± 0.60^{c}). However, there was a significant ($p \leq 0.05$) difference on the level of MDA in both group 3(38.56 ± 2.02^{b}) and 4 (38.56 ± 3.08^{b}) which received 200 mg/kg and 400 mg/kg in relation to the normal group (43.33 ± 0.60^{c}).

Lastly, there was a significant ($p \leq 0.05$) difference in the concentration of MDA in group 5 (29.68 ± 2.14^{a}) which received 800 mg/kg of *S. tora* ethanol fraction compared to the normal group.

There was an increase in the level of MDA in group 2 (45.67 ± 0.50^{c}) which received 100 mg/kg, while a decrease in the level in group 3 (38.56 ± 2.02^{b}), 4(38.56 ± 3.08^{b}) and 5 (29.68 ± 2.14^{a}) compared with the normal control (43.33 ± 0.60^{c}).

Effect of *S. tora* Ethanol Fraction on Superoxide Dismutase (SOD) activity

The result of the activity of SOD showed that, there was no significant ($p \leq 0.05$) difference in group 2 (76.45 ± 3.35^{a}) and 4 (77.99 ± 4.67^{a}) which received 100 mg/kg and 400 mg/kg compared with the normal control (77.45 ± 3.39^{a}).

However, there was a significant ($p \leq 0.05$) difference in group 3 (88.55 ± 2.03^{b}) and 5(83.23 ± 0.89^{b}) which received 200 mg/kg and 800 mg/kg. There was a significantly ($p \leq 0.05$) increase in the activity of SOD across the treated group with the highest in group 3(88.55 ± 2.03^{b}) compared to the normal control group (77.45 ± 3.39^{a}).

Effect of *S. tora* Ethanol Fraction on Catalase (CAT) activity

As shown in table 3 on the activity of Catalase enzyme, there was a significant ($p \leq 0.05$) difference in group 2 (9.45 ± 0.60^{c}) and 3 (28.45 ± 4.00^{c}) which received 100 mg/kg and 200 mg/kg when compared with the normal control group (6.93 ± 1.22^{a}). However, there was no significant ($p \leq 0.05$) difference in group 4 (7.25 ± 0.50^{a}) which received 400 mg/kg.

Lastly, there was significant difference ($p \leq 0.05$) in group 5 (9.22 ± 3.26^{b}) which received 800 mg/kg of *S. tora* ethanol fraction. Across the treated groups, there was a significantly ($p \leq 0.05$) increase in the activity of catalase enzyme with the highest in group 3 (28.45 ± 4.00^{c}) with dose of 200 mg/kg compare to normal control group (6.93 ± 1.22^{a}).

Liver Function Parameters

The liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. Any alteration or abnormality associated with the liver could lead to non-performance or inefficiency in carrying out its functions.

The abnormalities associated with liver function could be ascertained by evaluating the levels of some liver function parameters such as liver enzymes (AST, ALT, ALP and GGT). Liver synthetic molecule assay such as: Total protein, Albumin etc.

Based on the liver function parameters tested, there was a significant ($p \leq 0.05$) decrease in the level of Aspartate aminotransferase (AST) in both group 2 (25.75 ± 1.35^{b}), 3 (25.70 ± 1.92^{b}), 4 (13.50 ± 3.45^{a}) and 5 (12.45 ± 3.34^{a}) which received 100 mg/kg, 200 mg/kg 400 mg/kg and 800 mg/kg ethanol fraction of *S. tora* respectively compared to the normal control (26.25 ± 0.54^{c}).

There was a significant ($p \leq 0.05$) decrease in the level of Alanine amino transferase (ALT) in both group 2 (37.00 ± 3.43^{b}), 3 (33.70 ± 1.14^{a}), 4 (31.50 ± 4.52^{a}) and 5 (30.60 ± 8.61^{a}) which received 100 mg/kg, 200 mg/kg 400 mg/kg and 800 mg/kg ethanol fraction of *S. tora* respectively compared to the normal control (38.80 ± 4.16^{b}). Based on the results, it showed that there was significant ($p \leq 0.05$) decrease in the level of Alkaline phosphatase (ALP) in both group 2 (2.73 ± 8.33^{c}), 3 (2.60 ± 0.26^{c}), 4 (1.46 ± 0.26^{d}) and 5 (0.79 ± 1.54^{b}) which received 100 mg/kg, 200 mg/kg 400 mg/kg and 800 mg/kg ethanol fraction of *S. tora* respectively compared to the normal control across the group compared with normal control (2.76 ± 0.93^{a}).

For the activity of Gamma-glutamyl transferase (GGT), there was a significantly ($p \leq 0.05$) decrease in the level of GGT in both group 2 (1.46 ± 0.16^{a}), 3 (0.81 ± 0.03^{a}), 4 (0.69 ± 0.22^{b}) and 5 (0.24 ± 0.22^{ab}) which received 100 mg/kg, 200 mg/kg 400 mg/kg and 800 mg/kg ethanol fraction of *S. tora* respectively compared to the normal control across the group compared with normal control across the group compared to the normal control (2.28 ± 0.28^{c}).

The lack of significant increase in the levels of AST, ALT, ALP, and GGT good indicators of liver functions, which suggests that the repeated administration of ethanol fraction of *S. tora* do not have toxic effects on liver.

By defining the liver's functionality (albumin and lipid profile), cellular integrity (transaminases), and connection to the biliary system (ALP), liver function tests provide information about the condition of the liver (Ezejiofor *et al*., 2013).

The normal range of acceptable values for liver function tests, above which liver damage may be suspected, according to Thapa and Anuj (2007), is ALT (10-55 /L), AST (10-40 /L), and ALP (45-115 /L).

This was supported by Amin and AlMuzafar (2015), who showed that an increase in AST, ALT, and ALP values over these thresholds indicates an early diagnosis of hepatotoxicity and tissue damage. According to reports, hepatocyte destruction leads to an increase in a number of serum liver enzymes, which is correlated with liver toxicity.

It has been discovered that the *Sennatora* chemicals chrysophanol, emodin, and rhein work as antioxidants necessary for minimizing oxidative damage to the liver cells by scavenging free radicals, protecting cells. It might have also been the main element responsible for stabilizing every liver parameter (Yakubu *et al*., 2021b). However, improper antioxidant consumption can lead to oxidative stress (Galati and O'Brien, 2004) as a result, it is important to maintain the use of *S. tora's* ethanol fraction at a healthy level.

Table.1 Treatment details

Groups	Treatment
Control 1	Water and feed
Group 2	100 mg/kg ethanol fraction
Group 3	200 mg/kg ethanol fraction
Group 4	400 mg/kg ethanol fraction
Group 5	800 mg/kg ethanol fraction

Table.2 Effect of *S. tora* fraction on Liver function Parameters

Parameters	AST(U/I)	ALT(U/I)	ALP(U/I)	GGT(U/I)
GROUP 1	26.25±0.54^{c}	38.80±4.16^{b}	2.76±0.93^{a}	2.28±0.28^{c}
GROUP 2	25.75±1.35^{b}	37.00±3.43^{b}	2.73±8.33^{c}	1.46±0.16^{a}
GROUP 3	25.70±1.92^{b}	33.70±1.14^{a}	2.60±0.26^{c}	0.81±0.03^{a}
GROUP 4	13.50±3.45^{a}	31.50±4.52^{a}	1.46±0.26^{d}	0.69±0.22^{b}
GROUP 5	12.45±3.34^{a}	30.60±8.61^{a}	0.79±1.54^{b}	0.24±0.22^{ab}

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05)

Table.3 Effect of *S. tora* fraction on Liver function Parameters

Parameters	T.P(g/dL)	ALB(g/dL)
GROUP 1	13.50±5.82^{b}	3.45±0.63^{b}
GROUP 2	19.24±1.64^{a}	8.89±1.16^{c}
GROUP 3	19.70±0.07^{a}	0.76±0.06^{a}
GROUP 4	21.95±1.89^{a}	8.95±0.57^{c}
GROUP 5	22.00±1.28^{a}	7.30±0.84^{c}

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05)

Table.4 Effect of *Senna tora* Ethanol Fraction on Lipid Peroxidation Parameters

Parameters	MDA(nmol/mg protein)	SOD(U/mg protein	CAT(U/mg protein)
GROUP 1	43.33±0.60^{c}	77.45±3.39^{a}	6.93±1.22^{a}
GROUP 2	45.67±0.50^{c}	76.45±3.35^{a}	9.45±0.60^{c}
GROUP 3	38.56±2.02^{b}	88.55±2.03^{b}	28.45±4.00^{c}
GROUP 4	38.56±3.08^{b}	77.99±4.67^{a}	7.25±0.50^{a}
GROUP 5	29.68±2.14^{a}	83.23±0.89^{b}	9.22±3.26^{b}

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05)

The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins like albumins and globulins are produced in the liver (Thapa and Walia, 2007). Normal range of total protein is 6.0 to 8.3 g/dl. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury. From table 3, it showed that there were significant (p≤0.05) increase in the concentration of total protein on the both the treatments that received *Senna tora* ethanol fraction compared to the control group in the same vein, there was a significant (p≤0.05) increase in group 2 (8.89±1.16^{c}), 4(8.95±0.57^{c}) and 5 (7.30±0.84^{c}) which received 100 mg/kg, 400 mg/kg and

800 mg/kg respectively compared to the control group (3.45±0.63[b]). However, there was a significantly ($p \leq 0.05$) decrease in level of ALB in group 3 (0.76±0.06[a]) which received 200 mg/kg compared to the control group.

Lipid Peroxidation

Oxidative stress, which is an imbalance between the production of deleterious reactive oxygen species (ROS) and existing antioxidant defence system, plays a pivotal pathophysiological role in the development of liver disease, cancer, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases (Auten and Davis, 2009). Overproduction of ROS such as hydroxyl radical (OH·), superoxide radical (O_2^{-}), hydrogen peroxide (H_2O_2), and nitric monoxide (NO·) readily attacks the polyunsaturated fatty acids in the plasma membrane, resulting in the oxidative degradation of lipids (Ott *et al*., 2007). Consequently, lipid oxidation induces cellular and tissue damages through covalent binds, resulting in lipid peroxidation, DNA injury, inflammation, and subsequent cell death (Birben *et al*., 2012).

MDA is the most widely used indicator of lipid peroxidation, quantified as TBARS (Ikuo *et al*., 1991). Despite years of widespread criticism of the TBA test's use to assess the lipid peroxidation product, MDA in vivo (Baumgartner *et al*., 1975), some investigations have found that the TBA assay's results correspond well with other measurements of lipid peroxidation (Pitkanen *et al*., 1991). The concentration of TBARS in the control and experimental groups is shown in table 3. The concentration of TBARS was significantly reduced in group 3 (38.56±2.02[b]), 4 (38.56±3.08[b]) and 5 (29.68±2.14[a]) which received 200 mg/kg, 400 mg/kg and 800 mg/kg of *S. tora* ethanol fraction ($p \leq 0.05$) compared to the normal control group (43.33±0.60[c]).

SOD converts superoxide to hydrogen peroxide and is a major defence for aerobic cells in combating the toxic effects of superoxide radicals (McCrod *et al*., 1971). As presented in table 3, administration of *S. tora* ethanol fraction at 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg significantly increased the activity of SOD ($p \leq 0.05$), when compared with the control group.

Catalase decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance *et al*., 1952). Table 3 illustrates the activity of catalase in control and experimental groups. There was a significant increase in the catalase activity of across the treated groups in relation to the normal control group ($p \leq 0.05$).

Based on the results obtained on antioxidant parameters, it showed that *S. tora* has a significant antioxidant action, which could exert a beneficial action against pathological alterations, especially in inflammatory diseases.

The increase in the antioxidant enzyme activity and the reduction in the lipid peroxidation by *S. tora* may result in reducing a number of deleterious effects due to the accumulation of oxygen radicals. A medicinal plant removing ROS is the most effective defence of a living body against disease (Lin *et al*., 1995). Therefore, the natural antioxidants contained in this medicinal plant might contribute towards the total or partial alleviation of some clinical disorders.

It can be concluded that *S. tora* extract administration up to 800 mg/kg body weight did not result in hepatotoxicity. Additionally, without causing serious organ damage, this discovery supports the use of as a traditional herb for managing a number of illnesses, including liver ailments.

Author Contributions

Ojochenemi Ejeh Yakubu: Investigation, formal analysis, writing—original draft. Genesis Ayuka: Validation, methodology, writing—reviewing. Jankada Patience Audu:—Formal analysis, writing—review and editing. Emmanuel Gabriel Onuh: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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