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In vivo antioxidant and hepatoprotective activity of ethanol leaf extract of *Senna hirsuta* Mill

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ABSTRACT

Purpose: Liver diseases as a problem all over the world has led to many deaths because the conventional drugs known for the cure are inadequate to effect perfect healing. This study aims to evaluate the *in vivo* antioxidant and hepatoprotective potentials of ethanol leaf extract of *Senna hirsuta* using mice model.

Methods: After the initial effect of hepatic injury in mice with 1 g/kg body weight of paracetamol in a single dose for three days, 200, 400, 800, 1000 and 2000 mg/kg body weight of extract concentrations were used for treating the mice for five days. Blood and liver tissue samples were collected for analyses

Results: The results obtained proved that paracetamol elicited toxicity in the mice. The red blood cells mean count in the negative control (7.67±0.92 million/mm^3) was higher than the values obtained from the positive control (4.36±0.12 million/ mm^3). The WBC mean values of 3.50±0.18 thousand/mm^3 from negative control were lower than the positive control group (9.62±0.39 thousand/ mm^3). Also, a higher value above the permissible level (11-19 %) was obtained in the haemoglobin estimation of the positive control (20.11%) than the negative control (11.50%). Values of the positive control and extract treated were higher than the negative control value while lower values were recorded in GSH and CAT in positive control and extract treated. Higher biochemical values were recorded in the positive control, and the extract administered except for albumin and protein. However, the injuries caused that manifested in the mice haematology, biochemical and liver disorders were significantly improved with the 200 – 2000 mg of extract concentrations.

Conclusion: Leaf extract of *Senna hirsuta* proved the hepatoprotective activity of the employed extracts. The observation did by biochemical, hematological indices, and recovery improvement in liver function by histologic findings. Therefore, established that *S. hirsuta* ethanol leaf extract has hepatoprotective affinity over paracetamol-induced liver toxicity.

Keywords: Hepatoprotective activity; *In vivo* antioxidant; leaf extracts; *Senna hirsuta*; ethanol

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INTRODUCTION

The liver disease ranks as the third leading disease-related cause of death for many people of ages 25 to 59. Particularly common liver disorders include hepatitis and cirrhosis. Some research has studied the cause of liver cell injury. Various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, paracetamol, chronic alcohol consumption, and microbes can cause liver injury.[1]

Because of the non-encouraging results from the use of some modern drugs for liver disease treatment, attention has been shifted to the use of extracts prepared from plants to treat liver diseases effectively. Till date efforts by scientist have revealed many plants with hepatoprotective potentials and the search is continuous; hence there are differences in vegetation.

Quite numbers of herbal formulations or the formulations having extracts from plants for managing liver diseases are sold commercially in some countries. In some other countries, they are used local because there is yet no approval for worldwide acceptance.

Senna (Mill) is a genus in the family Fabaceae. *Senna hirsuta* (L.) common name as Woolly Senna. *S. hirsuta* is widely known for traditional medicine, mostly in African and Asian countries. It has a lot of chemicals properties which have popularized for treatment of some diseases, poison and as a laxative. Despite these functions, from our best knowledge, no attempt has been considered to study the hepatoprotective activity of the plant.

MATERIAL AND METHODS

Plant material

The leaves of *Senna hirsuta* were collected from Aule forest in Akure, Ondo State; authenticated in Forestry and Wood Technology Department of Federal University of Technology, Akure, Nigeria

and the voucher specimen was deposited in the University herbarium.

Plant leave extraction

The leaves were air-dried for three weeks at between 25 to 27 °C before ground with a grinder (Thomas Wiley machine, model 5 USA). 500 g of leave powder was soaked with 1 litre of ethanol for 24 h and filtered. The filtrate was vaporized in vacuo and refrigerated in a glass vial before use.

Acute toxicity

This was conducted according to World Health Organization[2] guidelines for evaluating the safety and efficiency of herbal medicines, and the Organization of Economic Co-operation and Development[3] guidelines for testing of chemicals. Both male and female mice with the weight of between 30 – 35 g were used. Seven groups of five mice was made. Group 1 mice served as control and were dosed with normal saline. Groups II – VI were dosed with extract concentrations of 200, 500, 1000, 2000, 3000 and 4000 mg/kg body weight of rat respectively. The mice were observed for toxic symptoms and mortality in each group within 14 days (Lorke.[4] The median lethal dose of the extract (LD50) was estimated using probit analysis.[5]

Experimental animal

Sixty Swiss albino mice (*Mus mausculus*) were used. They were feed for two weeks with regular rat feed and water to acclimatize. Before experimentation, they were denied of feed for 18 h and conducted in compliance with NIH guide.

Experimental design

Seven groups of five mice per group were conducted for the experiment. Group I was allowed access to feed and water as negative control. Group II mice were dosed with 1 g/kg body weight of paracetamol (positive control) three times daily for three days. Groups III-VII were orally dosed with 1 g/kg body weight of paracetamol in a single dose for three days and treated with 200, 400, 800, 1000 and 2000 mg/kg body weight of extract concentrations respectively for five days.

Mice haematology determination

Red blood cells and white blood cells, leucocytes differential counts, neutrophil, monocyte, eosinophil and lymphocyte differential counts were estimated by the criteria of Dacie and Lewis[6] with the automated haematologic analyzer SYSMEX KX21 (SYSMEX Corporation, Japan), haemoglobin was estimated with the use of Sahli's Hemoglobinometer by standard procedures according to the criteria of D'Armour et al.[7]

In vivo antioxidant activity assays

Liver tissue was washed repeatedly with cold 10% saline (w/v) and homogenized with mixture of cold solutions of 1.15% (w/v) KCL and 0.1 M potassium phosphate buffer (pH = 7.4). The homogenate was spurned at 10000 g for 1 h and obtained supernatant was used for the study.

Determination of the antioxidant status was carried out by measuring the level of its lipid peroxidation,[8] the amount of non-enzymatic antioxidant system (reduced glutathione) by the method of Ellman[9] while the enzymatic lipid peroxidation (Catalase) was with the criteria described by Cohen *et al.*[10]

Biochemical assay

The methods of Bergmeyer *et al.*[11,12] was adopted for the aspartate aminotransferase and alanine aminotransferase activities assay. Alkaline phosphate activity based on the principle that serum alkaline phosphate hydrolyzes a colourless substrate of phenolphthalein monophosphate to give phosphoric acid and phenolphthalein, which at alkaline pH value turns to a pink colour which can be determined by photometric method. Assessment of bilirubin used the method of Watson and Rogers,[13] total albumin used the method of Doumas et al.,[14] total protein concentration was estimated using Biuret method Donninger et al,[15] urea assessed with the method described by Fenech and Tommasini,[16] uric acids determined by the method of Carroll et al,[17] creatinine by the method of Lustgarten and Wenk,[18] and total cholesterol by the method of Abel et al.[19]

Liver histopathology

Small pieces of liver were cut from each treatment, washed with normal sali0ne and dehydrated in grades of ethanol. Xylene was used to clear traces of ethanol and water from the tissues after dehydration before impregnating in paraffin wax for one h at controlled temperature of 60 °C. The tissues were after that embedded in molten paraffin wax and sectioned with microtome (Bright, England) at 4 - 6 µm. The sectioned tissues were floated in water bath regulated at 35 °C and picked with slides previously robbed with egg albumin. The tissues were then dewaxed with xylene, hydrated, cleared with xylene, stained with haematoxylin and eosin; and mounted with DPX. The prepared slides were then allowed to dry, photographed and then observed with binocular microscope for the level of damages or safety.

Statistical analysis

Obtained results expressed as Mean±SD.

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Differences and compared by One-way Analysis of Variance (ANOVA) and was followed by Dunnet Multiple Comparison Test using SPSS version 16.

RESULTS

Median Lethal dose (LD_{50}) of *S. hirsuta* extract on the analysis found to be safe at 2700 mg/kg. At 4000 to 5000 mg/ml of the plant extract, 50% death in mice recorded while at 200 to 3900 mg/ml death rate of 0%. Thus, the choice of between 200 – 2000 mg/ml of extract concentration employed in this study.

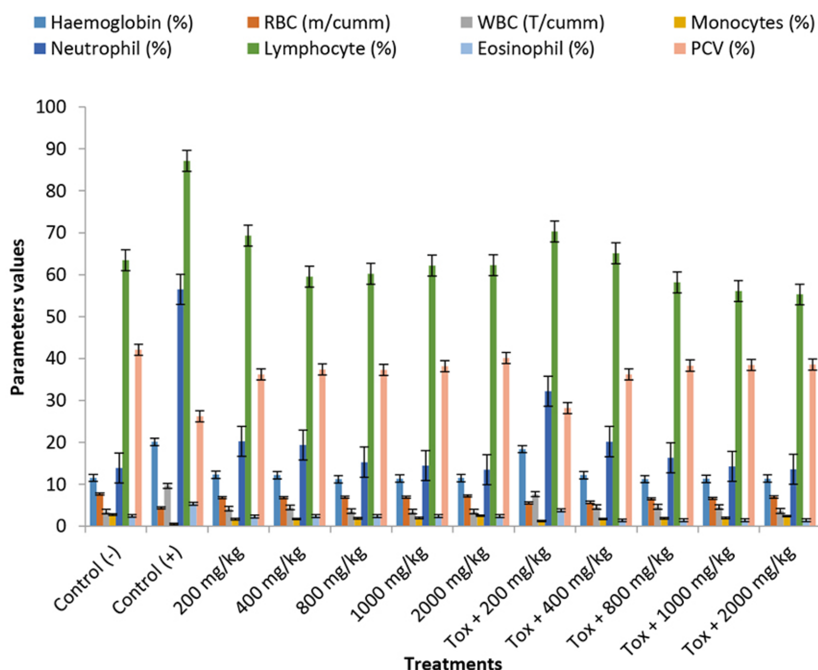

Figure 1. Effect of ethanol leaf extract of *S. hirsuta* on haematological parameters of mice
Legend: Tox = Toxicant

Table 1. Effect of *Senna hirsuta* on the antioxidant system of treated and paracetamol induced mice

	LPO(μM/g)	GSH(μM/g)	CAT(μM/g)
Control (-)	98.94±6.66^{a}	34.76±1.40^{a}	70.58±6.66^{a}
Control(+)	138.40±2.10^{c}	22.34±2.00^{c}	47.36±3.76^{e}
200mg/kg^{bw}	127.63±1.14^{b}	24.18±1.16^{bc}	48.19±2.14^{e}
400mg/kg^{bw}	125.31±2.31^{b}	24.34±1.73^{bc}	50.38±1.13^{c}
800mg/kg^{bw}	122.74±3.17^{b}	26.12±2.11^{b}	58.13±1.27^{b}
1000mg/kg^{bw}	120.53±2.60^{b}	28.63±1.31^{b}	65.43±2.44^{ab}
2000mg/kg^{bw}	120.12±2.75^{b}	28.78±1.43^{b}	66.18±1.01^{ab}
TOX.+ 200mg/kg^{bw}	134.16±2.08^{c}	22.26±2.14^{c}	49.14±2.18^{bc}
TOX.+ 400mg/kg^{bw}	132.21±1.17^{c}	22.34±1.33^{c}	52.31±2.05^{bc}
TOX.+ 800mg/kg^{bw}	127.43±1.25^{b}	23.74±3.16^{bc}	54.18±1.76^{c}
TOX.+ 1000mg/kg^{bw}	120.11±2.20^{b}	24.17±2.45^{bc}	56.27±1.34^{b}
TOX.+ 2000mg/kg^{bw}	119.55±1.70^{b}	25.36±1.18^{b}	60.28±1.13^{b}

Values are mean ± standard deviations of triplicate determination.
Values with different superscript (a-c) per column are significantly different.
Legend: LPO = Lipid peroxidation, GSH = Glutathione, CAT = Catalase, TOX = Toxican

Figures 1 shows the haematological profile of positive control, negative control and toxicant co-administered with the various extract concentrations (satellite or treatment) groups of mice. The red blood cells mean count in the negative control was valued at 7.67±0.92 million/ mm^3 and higher than the values obtained from the positive control (4.36±0.12 million/ mm^3). The WBC mean values of 3.50±0.18 thousand/mm^3 from negative control were lower than the positive control group (9.62±0.39 thousand/ mm^3). Also, a higher value above the permissible level (11-19 %) was obtained in the haemoglobin estimation of the positive control (20.11%) than the negative control (11.50%)

Table 1 illustrates the *in vivo* efficacy of the extracts on the enzymatic antioxidant in mice. A significant LPO level increase ($P<0.05$) in the positive control was observed when compared with the negative control. However, the decrease in LPO level was recorded in both the extract-treated and the satellite groups alongside extract concentration compared with the values obtained in the negative control group. But GSH and CAT values increased significantly alongside extract concentrations in both the extract-treated and satellite groups of mice.

Table 2 presented the effects of extracts on liver function. The AST values from low extract dose (200 mg/kg BW) to high dose (2000 mg/kg BW) ranged from 35.53±1.53 U/L to 35.45±1.60 U/L. The ALT and ALP values also decreased in that order from 53.22±1.19 U/L to 45.85±1.54 U/L and 112.44±1.21 U/L to 94.28±1.16 U/L respectively. Observed in the liver function of the extracts treated mice was in decrease values on dose-dependent as it affects bilirubin, total albumin urea, uric creatinine and cholesterol. Significant differences ($P<0.05$) occurred when compared with the values obtained from the positive control group. While these observations, the total protein contents of the mice were on the increase in dose-dependent towards the values of the negative control (7.15±0.45 mg/dL). Values from 5.28±0.44 mg/dL to 6.72±0.03 mg/dL were recorded in the extract-treated mice and 5.27±0.34 - 6.60±0.17mg/dL in the satellite groups. Urea content in the negative control was 18.15±1.50 mg/dL and in the positive control was 23.40±0.16 mg/dL. From the positive control perspective, increase ($p>0.05$) in values observed in both the extract-treated and the satellite group. Also, values from 22.63±1.43 mg/dL to 21.18±1.07 mg/dL were recorded in the extract-treated mice groups and values of 28.14±0.63 mg/dL to 22.03±0.34 mg/

Table 2. Effect of ethanol leaf extract of *S. hirsuta* on biochemical parameters in liver functions of mice

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Total albumin (mg/dL)	Total protein (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)
Control (-)	34.50±1.60	43.20±4.01	87.25±2.54	1.08±0.10	4.90±0.11	7.15±0.45	18.15±1.50	6.05±0.60	1.38±0.30	113.70±0.50
Control (+)	67.47±4.02	64.92±3.69	321.12±2.11	2.11±0.63	2.21±0.60	5.18±0.60	23.40±0.16	8.02±1.10	1.96±0.09	218.14±0.64
200mg/kg^{bw}	55.53±1.53	53.22±1.19	112.44±1.21	1.57±0.25	3.71±0.34	5.28±0.37	22.63±1.43	8.24±1.13	1.74±1.08	122.61±1.26
400mg/kg^{bw}	37.76±2.71	52.30±2.44	112.07±1.58	1.50±0.10	3.74±0.10	5.41±0.14	22.34±0.54	8.15±0.44	1.70±0.17	120.13±0.47
800mg/kg^{bw}	36.70±1.28	50.14±2.10	95.48±2.64	1.27±0.02	3.75±0.09	6.55±0.08	21.51±0.18	7.12±1.14	1.57±0.13	118.24±0.13
1000mg/kg^{bw}	36.20±1.08	48.18±1.68	95.24±3.48	1.18±0.34	3.84±0.26	6.64±0.21	21.26±0.03	7.10±0.26	1.48±0.42	116.30±0.48
2000mg/kg^{bw}	35.45±1.60	45.85±1.54	94.28±1.16	1.13±0.18	4.41±0.25	6.72±0.03	21.18±1.17	6.14±0.43	1.45±0.35	115.21±0.16
TOX+ 200mg/kg^{bw}	40.32±1.60	58.24±3.45	198.18±3.74	2.18±0.14	2.48±0.31	5.27±0.34	28.14±0.63	8.33±0.41	2.35±0.41	138.18±1.24
TOX+ 400mg/kg^{bw}	53.61±1.53	56.38±1.12	140.02±3.44	2.06±0.32	2.54±0.12	5.38±0.28	28.06±0.18	8.20±1.06	2.21±0.36	133.64±1.18
TOX+ 800mg/kg^{bw}	51.71±2.26	54.42±1.6	126.25±1.73	1.53±0.06	3.66±0.28	6.45±0.16	24.19±0.11	7.45±1.18	1.63±0.24	127.22±0.68
TOX+ 1000mg/kg^{bw}	52.20±1.08	48.37±1.52	100.30±2.43	1.48±0.21	3.60±0-.16	6.53±0.28	22.18±0.02	6.36±2.14	1.50±0.17	126.41±1.12
TOX.+ 2000g/kg^{bw}	50.74±2.17	48.45±2.04	98.20±3.61	1.44±0.16	3.51±0.11	6.60±0.17	22.03±0.34	6.28±0.18	1.47±0.16	130.12±0.64

Legend: Tox = Toxicant

dL from the satellite groups. Uric acid content decreased significantly to normal from low to high dose of extract (8.24±1.13 - 6.14±0.43 mg/dL). In the satellite groups, values of between 8.33±0.41 - 6.28±0.18 mg/dL were recorded, which ascertained uric acid elimination from circulation with the leaf extracts. Creatinine content in the negative control was 1.38±0.30 mg/dL and 1.96±0.09 mg/dL in the positive control. The values obtained from the extract-treated and satellite groups suggested significant decrease and was on dose-dependent. The cholesterol values in the extracts treated and the satellite was close to the values of the negative control. While it was 113.70±0.50 mg/dL in the negative control, the 200-2000 mg/kg BW of extracts treated mice were 122.61±1.26, 120.13±0.47, 118.24±0.13, 116.30±0.48 and 115.21±0.16 mg/dL respectively. The satellite groups in that order were 138.18±1.24, 133.64±1.18, 127.22±0.68, 126.41±1.12 and 124.12±0.64 mg/mL respectively. On the bases of the value from positive control (218.14±0.64 mg/dL) significant decrease in values on extract dose-dependent was observed on the various extracts of *S. hirsuta* able to reduced high cholesterol level manifested by paracetamol.

Various liver damages caused by paracetamol were observed. Such of the damages are severe hepatic cellular degenerations, hepatic drainage, dilated sinusoid, focal necrosis, among others. There was a recovery in the liver of mice induced with paracetamol and co-administered with extract concentrations (Figure 2).

DISCUSSION

The plant ethanol leaf extracts on the haematological parameters were of varied reactions on extract concentrations. The low hemoglobin recorded which fell within the standard value of 11-19% in the negative control remains a point of no consultation that blood cells were intact. In contrast, the high hemoglobin value in the positive control denotes alteration in the red blood cells. Confirmation of these was manifested in the red blood cells counts and PCV values. The reduced level of hemoglobin could be associated with hemolysis which was regulated by the plant extracts for necessary recovery of the mice physiological status. The significance of such change suggests the alteration of erythrocytes in the positive control group. That may be related to oxidative stress and lipid peroxidation in the circulating erythrocytes. The significant elevation in WBC of the positive control mice is a sign of toxicity initiated by paracetamol whereby the WBC on account of regulating the changes is multiplied into circulation by lymph glands. Supporting this

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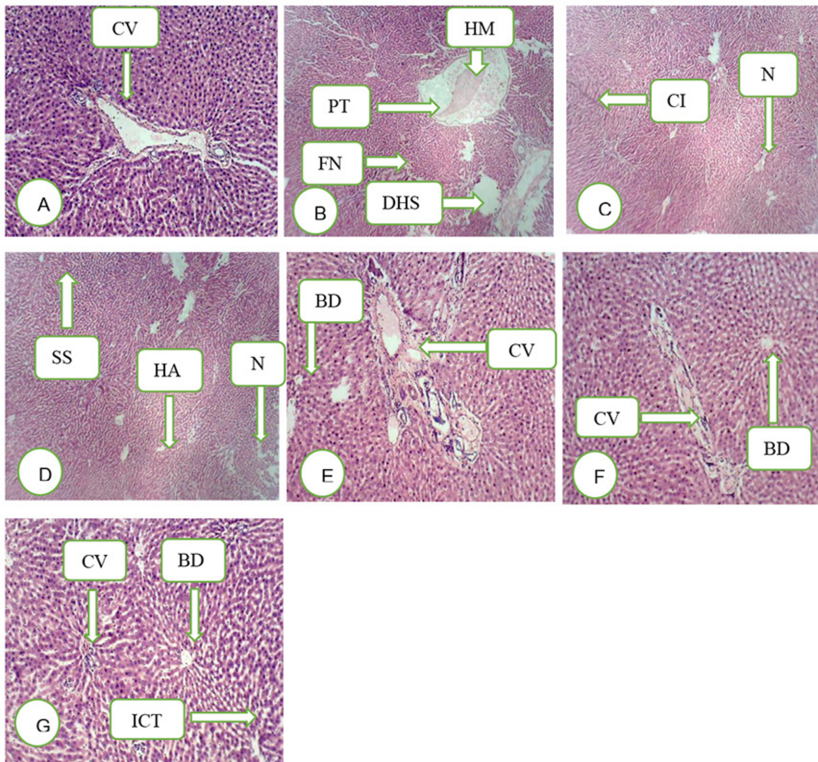

Figure 2. Photomicrographs of liver (H & E stain, ×100). A= Negative control with normal architecture showing central vein (CA), bile duct (BD) and distinct cellular boundaries. B= Positive control showing focal necrosis (FN), distorted hepatic structure (DHS), portal triad (PT) and hemorrhage HM). C= Paracetamol-induced and pretreated with 200 mg/kg extract showing Cellular infiltration (CI), Necrosis, D= Paracetamol-induced and pretreated with 400 mg/kg extract showing sinusoid (SS), cellular infiltration (CI), hepatic artery (HP), Necrosis (N), E= Paracetamol-induced and pretreated with 800 mg/kg extract showing bile duct (BD), central vein (CV), F= Paracetamol-induced and pretreated with 1000 mg/kg extract showing central vein (CV), bile duct (BD), G= Paracetamol-induced and pretreated with 2000 mg/kg extract showing interlobular connective tissue (ICT), central vein (CV), bile duct (BD).

observation as confirmatory of toxic substances in the circulation of the positive control mice are the decreased values in RBC and PCV, which resulted in the high hemoglobin counts encountered. The values in the haematology parameters obtained from the extracts treated groups did not suggest toxicity; hence values were not significantly different when compared with the negative control mice values. The tendency of the extracts non-toxicity elicited the improvements in changes observed in the hematology parameters towards normalcy in the satellite group of mice.

The excessive increase above the standard of neutrophil count in the positive control mice as well denotes severe hematology defect in the WBC of the mice. Leukocyte count was found to significantly increase in the paracetamol-induced mice in comparison with that of normal mice. The observed decrease is in agreement with the results obtained by Patcharee *et al.*[20] However, the improvement of leukocytes in circulation after the treatment of the extract in the satellite group of mice indicates that oxidative stress was manifested with the induced paracetamol.

Administration toxic dose of paracetamol showed adverse reactions in lipid peroxidation (LPO), Glutathione (GSH) and Catalase (CAT). Ascertaining these results are the higher value of the positive control than the negative control in LPO, lesser values in GSH and CAT than the negative control. In this process, where the higher value in LPO was observed in positive control than the negative control value. Non-enzymatic lipid peroxidation or enzymatic peroxidation would have occurred.

Lipid peroxidation increased in the paracetamol and extract-treated mice than the negative control. It could likely be the increase in free radicals developed during abnormal metabolic activities and the variances in antioxidant defence causing oxidative stress which decreased glutathione level leading to oxidative damage and resulting to an increase in lipid peroxidation level. Palanisamy *et al.* showed similar results.[21] Meanwhile, reduction in lipid peroxidation was observed in the satellite groups and was on extracts dose dependant. This decrease might be due to the available phenol contents in the leaf extracts that motivated the recovery of liver cells from the sustained injuries even as supported by the report by Khan and Sultana.[22] Though the reduction in values for the period of preliminary treatment with the extracts were not precisely the same with the negative control value. There exist the correlations which signified that if preliminary treatment extended, there will be the tendency of values coming inline or equal to the negative control values.

The observed elevation of GSH in the liver of mice after preliminary treatment with the leaf extract may caused by the automatic response brought about by oxidative-reduction of the cell as result of excessive hydrogen peroxide (H_2O_2) production and progressive decrease of glutathione peroxide. The enhancement efficacy of the extract exhibited such inhibitory effects. This study supported the observations recorded by Sanapala and Kumar.[23] The Reduced GSH level in paracetamol dosed mice may be due to conjugation of GSH with NAPOI to form mercapturic acid as reported by Parmar et al.[24]

The CAT increase and LPO decrease in the satellite group is an indication of the effectiveness of the plant antioxidant. Literature confirmed that

catalase converts harmful hydrogen peroxide into water and oxygen. Catalase can protect the liver from paracetamol toxicity-induced highly reactive hydroxyl radicals. Reduction in the activities of this enzyme in the positive control mice may result in the number of harmful effects due to the accumulation of highly toxic metabolites and hydrogen peroxide on paracetamol administration. The co-administration of the extracts increased the activities of catalase. It prevented the accumulated excess free radicals, thereby protecting the liver from intoxication realized from paracetamol.

Microcytic hypochromic anemia confirmed by the reduced red blood cells, high hemoglobin counts, and the low pack cell volume (PCV) encountered.

High values in biochemical enzyme markers of AST, ALT and ALP activities observed in the overdose of paracetamol administered mice. On the other hand, decreased protein and total albumin levels, AST, ALT and ALP are known marker enzymes for liver health. In acute liver toxicity, these enzymes are generally high but become lowered with extension in the intoxication process because of liver damage.[25] The ability of overdose of paracetamol to effect damages of the liver is in connection with varieties of alteration in biochemical indices, and this is capable of manifesting intracellular constituents such as AST, ALT, ALP, and cholesterol among others into circulation. The amount of these biochemical markers in circulation accounted for the lever cells damage. Stabilization activities of the biochemical parameters investigated with the treatment of the plant extracts manifested distinct improvement in the health condition of liver cell, which may be due to free radical scavenging action of the extracts. In previous study, *Senna hirsuta* leaf extract contained the phytochemicals such as saponnins, steroids, flavonoids, tannins, glycosides, alkaloids and phenol.[26]

CONCLUSION

The overdose of paracetamol caused the hepatic injuries in mice. The pretreatment of the ethanol leaf extract with the 800 to 2000 mg/ml showed hepatoprotective activity which observed by hematology and biochemical assessment. It has established that *S. hirsuta* ethanol leaf extract can have hepatoprotective affinity over paracetamol-induced liver toxicity.

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There is no conflict of interest among us the authors of this manuscript.

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FCA conceived the research idea, designed and wrote the first draft of the manuscript. LEI assisted in literature search and tests analyses. All authors read and approved the final manuscript before sending out.

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