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Hepatoprotective activities of *Autranella congolensis* and *Sapium ellypticum* against carbone tetrachloride induced liver injuries in experimental rats.

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Abstract

Background: Liver injury remains a serious problem worldwide despite advances in drug research. In the perspective of searching for new natural agents that can prevent such abnormalities this study was designed to evaluate the *in vivo* anti-oxidative properties of the methanolic extracts of *Autranella congolensis* and *Sapium ellypticum*.

Methods: The hepatoprotective properties of the extracts were evaluated on 9 groups of 5 *Wistar* rats using the CCl_4 model. Animals were treated orally alone with extracts at doses 100 and 200 mg/kg body weight and 100 mg/kg Silymarin for 10 days except normal and toxic control groups. Animals received an intra-peritoneal injection of CCl_4 except the controls. The efficacy of the extracts was assessed biochemically and histopatologically after 10 days treatment.

Results: Elevated serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, total bilirubin and direct bilirubin observed in toxic groups were restored toward the normal values, when animals received each of both treatments at 100 and 200 mg/kg with each extract. The administration of CCl_4 lowered significantly the level of liver antioxidants markers like superoxide dismutase, catalase, glutathione, and increased the level of lipid peroxidation, which were modulated in the group of animals treated with both extracts at 100 and 200 mg/kg. The histological and biochemical changes induced by both extracts at 200 mg/kg on toxic animals were similar to those obtained with standard silymarin.

Conclusions: These findings indicated that *S. ellypticum* and probably *A. congolensis* could prevent liver damage induced by CCl_4.

Keywords: hepatoprotective; *Sapium ellypticum*; *Autranella congolensis;* liver injury; Carbone tetrachloride

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Background

Liver diseases, including simple lesions, inflammatory and neoplastic changes result from autoimmune disorders, infection, toxicity by poisons from foods and drinks, exposure to chemicals and overdose of drugs, and represent important health threat worldwide [1-3]. The susceptibility of the liver to injuries is attributed to its role as the primary organ which comes in close contact with many harmful substances. Due to its permanent exposure to toxic chemicals and infectious agents, maintaining a healthy liver is a challenge for overall health. Being a vital organ in the body where several biochemical and physiological processes such as major metabolic pathways involved in growth occur, the liver help in fighting against diseases, reproduction, nutrient and energy supply, detoxification and excretion [4, 5]. Liver cells express a battery of microsomal enzymes that catalyse the biotransformation of xenobiotics. Although this biotransformation improves solubility of xenobiotic and consequently their excretion, some derivatives are cytotoxic, and can even induce injuries. Most often these lesions are mediated by oxidative stress, via free radical chain reactions.

The liver pathology is an impaired health condition caused by several agents including host immunity, chemicals and infectious agents. Regardless of the etiology, oxidative stress has been reported as mediator of most of these injuries. Generally, oxidative stress results from over-production of reactive oxygen species (ROS) also known as pro-oxidants or the failure [6] of anti-oxidant defence systems that lead to the destruction of biological macromolecules such as proteins, lipids and nucleic acids [7]. Nevertheless, pro-oxidants are produced by unavoidable side reactions of other important biochemical processes such as oxidative phosphorylation, although aerobic cells have evolved mechanisms of defences since they produce anti-oxidants. These defence molecules either prevent the formation of pro-oxidants, or intercept and destroy them immediately as they are formed. They neutralize the destructive effect of ROS and stop the degenerative chain reaction of free-radical oxidation, thus preventing or repairing oxidative damages of tissues [8]. The production of pro-oxidants is presumed to be in balance with anti-oxidants, even if under certain circumstances the over-production of pro-oxidants associated to the low anti-oxidant protection promotes tissue damage and disease. Components of an anti-oxidant defence system interact with pro-oxidants to protect cells from free radical damages. Some of these components are cellular reducing agents or scavengers such as co-enzyme Q, glutathione, peroxidases, superoxide dismutase and catalase enzymes. This intrinsic defence arm plays key role in emergency protection of the cells and tissues exposed or sensitive to oxidative stress such as hepatocytes, brain, kidney and erythrocytes.

The extrinsic anti-oxidant defence system is mediated by dietary anti-oxidants and drugs. Such anti-oxidants include vitamins E and C, alpha-lipoic acid, carotenoids, flavonoids, tannins and other dietary phenol derivatives [9]. Due to high level of free radicals released during the oxidative stress, the need for anti-oxidants is increasing. Although the body responds by a burst in the production of intrinsic anti-oxidants, the extrinsic defence is required because of inadequate recycling of intrinsic anti-oxidants in addition to their low concentrations. Therefore, an administration of supplements may improve the outcome of disease mediated by oxidative stress.

Herbal medicine constitutes the main source of extrinsic anti-oxidants as well as some effective drugs. Due to the availability of plants and their accessibility in remote localities mainly in areas where synthetic drugs are unaffordable; the investigation of anti-oxidant profiles of natural species used in the treatment of some diseases that involve oxidative stress in their pathogenesis is of great interest. The use of plants as drugs is a tradition in developing countries despite the advent of modern medicine. Most of the plant ingredients are recognized and metabolized by human enzymes as well as other endogenous compounds and xenobiotics. Silymarin, a standardized milk thistle extract, of which silybin is the main component, known for its hepato-protective, anti-inflammatory, anti-oxidant activities, and hepatocyte regeneration. This mixture of polyphenolic compounds (lignanes) is an example of herbal product used to attenuate oxidative liver damage.

Anti-oxidants are widely distributed in plants, and they belong to several classes of secondary metabolites such as phenols, flavonoids, terpenoids, steroids and glycosides [9-11]. There is therefore a greater need of dietary polyphenols, and the search for natural sources of medicinal products that have anti-oxidant and radical scavenging activity [12, 13]. The anti-oxidant properties of Cameroonian medicinal plants and their ingredients have been proven in numerous studies. Here, we explore the hepatoprotective properties of two medicinal plants, *Autranella congolensis* and *Sapium ellipticum* used in traditional medicine on the liver induced injury by carbon tetrachloride (CCl_4) using both biochemical and histological markers.

Methods

Plant material and extract preparation

Two (2) medicinal plants used for the treatment of diseases whose pathogenesis involves oxidative stress were selected. Stem bark of these plants were harvested in different localities and identified at the Cameroon National Herbarium (CNH), where voucher specimens were conserved under their reference numbers. They were extracted by maceration in methanol as previously described [14]. Their characteristics are summarised in Table 1.

Table 1

Experimental animals and ethical consideration

Adult *Wistar* albino rats (four weeks) of either sex weighing between 150-200g were used for the study. They were housed in a cleaned animal room, in temperature control environment (25±1°C), relative humidity, with regular 12 h light/12 h dark cycle. All animals were fed with commercialised standard rat diet, and water *ad libitum*. Rats were used in chemically induced liver injury studies (CCl_4) while Balb/C mice were used both for acute toxicity study. The protocol of experimentation was approved by the Institutional Animal Ethical Committee (Ref:SSCPT/IAEC.Clear/141/2012-13) of Sree Siddganga College of Pharmacy, Tumkur, Karnataka, India.

Chemical hepatotoxicants

Carbon tetrachloride (CCl_4) was chemical hepatotoxicant used to induce liver injury in experimental rodents. It was provided by Dr. Typpeswamy BD, pharmacologist at Sree Siddaganga College of Pharmacy (SSCP), in India.

Study design

Acute toxicity study was previously performed as described in our published work [19]. These extracts were considered safe at 2000 mg.kg body weight (bw). Then, one tenth (1/10) and one twenty (1/20) of this maximum experimental dose (i.e. 200 and 100 mg/kg bw respectively) were selected for the evaluation of their hepato-protective activity.

Fourty-five (45) albinos' rats were divided in 9 groups of 5 animals each. On the first day, Groups 1, 2 and 3 were treated alone with a single dose of paraffin oil (0.7 mL, i.p.), 4 hours before treatment with extract or vehicle, whereas groups 4, 5, 6, 7, 8 and 9 were treated with CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p).

Animals from group 1 (G1) served as a normal control, and were treated with 1 mL of 0.5% gum acacia (p.o.) alone daily for 10 days. Group 2 and 3 (G2 & G3) served as extract controls and were treated with *S. ellypticum* and *A. congolensis* extract alone respectively (200 mg/kg, p.o.), daily for 10 days. Group 4 (G4) served as toxic control, and were treated alone with 1 mL of 0.5% gum acacia (p.o.) daily for 10 days. Group 5 and 6 (G5 & G6) served as *S. ellypticum* treated groups. They received *Sapium ellipticum* extract at low (100 mg/kg, p.o.) and high (200 mg/kg, p.o.) dose respectively daily for 10 days. Group 7 and 8 (G7 & G8) served as *A. congolensis* treated groups. They received *Autranella congolensis* extract at low (100 mg/kg, p.o.) and high (200 mg/kg, p.o.) dose respectively daily for 10 days. Animals from group 9 were treated daily with the standard drug Silymarin (100 mg/kg, p.o.) for 10 days.

At the end of treatment, animals were sacrificed and biochemical serum and liver markers of hepatotoxicity and oxidative stress, as well as histopathological changes of the livers were evaluated. The total serum protein (TP), total Bilirubin (TB), direct bilirubin (DB), serum albumin (ALB), Serum Glutamate Oxaloacetate Transaminase (GOT) and serum Glutamate Pyruvate Transaminase (GPT) were quantified using the sero-diagnostic kits. Assays were carried out according to the protocol indicated by the manufacturer, and analysed using a semi-automatic analyser.

Preparation of samples for biochemical and histological analysis

On the day 11th the overnight fasted animals were anesthetized with ether and the blood was collected by puncturing the orbital plexus and was allowed to coagulate on ice for 30 min. Serum was separated by centrifugation at 3500 rpm for 10 min and analysed immediately for various biochemical markers. After 24 hours, rats were scarified by decapitation; the livers of all animals were perfused with ice cold phosphate buffer saline (PBS), removed and processed for other investigations. The hepato-protective activity was evaluated biochemically and morphologically.

Preparation of liver homogenates for estimation of antioxidant markers

Liver homogenates were prepared as follow. An incisional biopsy of 500 mg of the fresh liver was made and homogenized with ice cold PBS using a glass homogenizer. The homogenates were stored at -20 ºC for not more than 4 days, and they were used for assessment of the anti-oxidant contents.

Table 1. Characteristics of plants collected

Plant name (Abbreviation)	Family	Voucher number	Medicinal properties
Autranella congolensis (AC)	Sapotaceae	39458/HNC	Diarrhea and chronic dysentery, malaria [15, 16].
Sapium ellipticum (SE)	Euphorbiaceae	47266/HNC	Wounds healing, pain in chest, headache, anaemia, guinea worms, and rheumatic problems [17, 18].

Estimation of liver proteins

Liver proteins were estimated by the method of Lowry [20]. The working reagent copra-alkaline solution was prepared by mixing 48 mL of 2% Na_2CO_3, 1 mL of 1% potassium sodium tartrate and 1 mL of 0.5% copper sulphate. This solution was freshly prepared and kept for 2 hours before use. The reagent (2 mL) was mixed with 100 µl of homogenate and the tubes were incubated for 10 minutes at room temperature. Then, 200 µl of 1N Folin reagent was added, and the tubes were vortexed immediately and incubated at room temperature for 30 minutes. The absorbance was read at 600 nm using a UV / visible double beam spectrophotometer (LABINDIA). The standard protein Bovine Serum Albumin was used to prepare the calibration curve.

Estimation of reduced glutathione (GSH)

The reduced glutathione was estimated using previously described method [21]. GSH reacts with DTNB to give a yellow coloured complex with maximum absorption at 412nm. To 0.5 mL of (10%) homogenate, 125 µl of 25% TCA was added to precipitate proteins. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 0.6 mL of TCA, centrifuged for 10 minutes at 1500 rpm and 0.3ml of resulting supernatant was taken for GSH estimation. The volume of the aliquote was made up to l mL with 0.2 M phosphate buffer. Then 2 mL of freshly prepared 0.6mM DTNB (HIMEDIA) was added to the tubes and intensity of yellow colour formed was read at 412 nm. Standard curve of GSH (Himedia) was prepared using concentrations varying from 5 to 100 nM in 5% TCA for assay. Values were expressed as nmol/mg protein.

Estimation of lipid peroxidation (LPO)

The LPO was assessed by estimating the thio-barbitturic acid reactive substances (TBARS), equivalent to the end product of lipid peroxidation Malone dialdehyde (MDA). An aliquot of 1.0 mL of the sample homogenate was combined with 2.0 mL of the reagent (equivalent volumes of 15% w/v trichloroacetic acid, 0.375% w/v thio-barbituric acid and 0.25N Hydrochloric acid) and mixed thoroughly. The solution was then heated on a boiling water bath for 15 min. The resultant flocculent precipitate was removed by centrifugation. The absorbance of the supernatant was read at 532 nm against a blank. The concentration of the TBARS was determined using a molar extinction coefficient of MDA ($1.56\times10^5.M^{-1}.cm^{-1}$) and the results were expressed as nmoles MDA/mg protein.

Estimation of catalase (CAT)

Catalase activity was estimated according to the method of Beers and Sizer [22] with slight modifications. The working reagent was 10.3 mM hydrogen peroxide solution in phosphate buffer (pH 7). In a cuvete, were immediately mixed 950 µl of reagent and 50 µl of diluted homogenate. The absorbance was recorded at 240 nm during the first 30 seconds with 10 second interval. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2. A Unit of catalase is defined as the quantity which decomposes 1.0 µmol of H_2O_2 per min at pH = 7.0 at 25°C.

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was estimated using the enzyme inhibition of the oxidation of epinephrine [23]. The superoxide anion (O_2^-) substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O_2^- builds in the solution, the formation of adrenochrome accelerates because O_2^- also reacts with epinephrine to form adrenochrome. To 925 µl of 50 mM sodium carbonate buffer (pH 10.25, with 0.1 mM EDTA) were added 50 µl of homogenate and 25 µl of 9.74 mM adrenaline bitartrate (freshly prepared in 10 mM HCl). The absorbance was immediately recorded at 480 nm within 5 min with 30 seconds interval. The initial rates were computed from the graph Abs vs time and the percentage of inhibition was calculated. A unit of SOD activity is defined as that amount of SOD required to cause 50% inhibition of the oxidation of the epinephrine (SOD_{50}).

Histopathology studies

Fixation

A biopsy of the liver was immediately fixed with 10% buffered formalin for more than 24 hrs. Specimens were further introduced in labelled cassettes and then incubated in 60% ethanol for 8 hrs, before they were transferred into the tissue processing device (Tissue Tek).

Processing

The tissues were dehydrated in a series of graduated changes with different strength of alcohol (75%, 80%, 96% and 100%) for approximately two hours each. Xylene was used as clearing agent. From xylene, melted paraffin was infiltrated into the tissue.

Embedding

Following complete processing, the tissues were oriented in embedding mould. Casting is the enclosing of the tissue in a solid mass of the embedding medium (melted paraffin from the dispensing console machine or paraffin dispenser).

Sectioning

The tissue embedded blocks were allowed to solidify on the cool surface of the thermal console. The blocks were faced by cutting it down to the desired tissue plane and the paraffin ribbon discarded. After facing the tissue embedded blocks were put in soapy water for 5 minutes.

Acu-edge blades were mounted into the microtome for sectioning. Sections (5 microns each) were picked with forceps and kept floated on the surface of a 37ºC water bath, then collected with the surface of clean glass slides and later kept in an oven at 56ºC for 40 minutes.

Staining

The slides were deparaffinised by heating in an incubator set at 56ºC for 45 minutes, followed by immersion in xylene for 10 min. Rehydration was performed successively in 100% ethanol, 95% ethanol and distilled water. After the slides were rinsed, they were stained with Lerner 2 hematoxylin for 1 minute, 30 seconds, and then immersed one time in 1% glacial acetic acid. Slides were rinsed in running tap water for 2-3 minutes and then dipped in 95% ethanol for 2 minutes. Thereafter the slides were counter-stained with eosin-phloxine for 2 minutes and then rinsed in 95% ethanol. Slides were further dehydrated successively in 95% and 100% ethanol. Specimens were cleared thrice in xylene for 15 minutes and mounted with 1-2 drops of xylene based mounting medium.

Results

Extracts

The crude methanolic extracts of *Sapium ellipticum* and *Autranella congolensis* (AC) were prepared and their extraction yields were 16.2 % and 11.4 % respectively.

Effect of Sapium ellypticum and Autranella congolensis extracts on serum biochemical parameters

Animals treated with CCl_4 (Toxic control group) developed a significant level of liver injury proven by a significant elevation of serum biochemical markers like SGPT, SGOT, SALP, TP, total bilirubin and direct bilirubin when compared with the normal control group (Table 2). Treatment of animals with *S. ellypticum* and *A. congolensis* extracts at the dose of 200 mg/kg, p.o significantly prevented the CCl_4 induced elevation of serum SGPT, SGOT, SALP, bilirubin (total and direct) and increased the level of total protein. Hepato-protective potency of the test extract at the dose 200 mg/kg was found closer to that of the standard.

Effect of Sapium ellypticum and Autranella congolensis extracts on endogenous anti-oxidant enzymes and relative liver weight

It was observed that animals treated with CCl_4 developed hepatic damages, shown by an increase in LPO and decrease in GSH, CAT and SOD when compared to normal control (3). Administration of extracts only did not alter these parameters. Animals treated with standard (Silymarin) showed extremely significant ($P<0.001$) increase in GSH, CAT, SOD and decrease in LPO. Treatment with *A. congolensis* and *S. ellypticum* extract (200 mg/kg, p.o) significantly prevented the increase in LPO levels and brought them near to normal level, whereas GSH, SOD and CAT levels were significantly raised, thus providing protection against CCl_4 toxicities.

The relative liver weight was increased in CCl_4 treated animals when compared to normal control. The animals which were treated with *A. congolensis*and *S. ellypticum* 200 mg/kg showed significant decrease in liver weight (Table 3) as compared to the toxic group.

Table 2. Serum parameters of animals after 10 days of treatment

Experimental groups	Serum parameters						
	ALP (U/L)	SGOT (U/L)	SGPT (U/L)	TB (mg/dl)	DB (mg/dl)	TP (mg/dl)	SALB (mg/dl)
G1	136.7± 7.8	87.1± 5.5	60.1± 5.1	0.95± 0.02	0.24± 0.01	8.11± 0.06	3.69± 0.10
G2	144.1± 8.5	74.7± 4.7	66.1± 5.3	1.01± 0.03	0.21± 0.01	7.99± 0.06	3.51± 0.07
G3	148.9± 7.7	73.9± 4.9	68.6± 5.1	1.11± 0.03	0.22± 0.01	8.01± 0.06	3.24± 0.07
G4	361.3± 17.1[a]	478.8± 23.7[a]	336.4± 18.5[a]	2.88± 0.02 [a]	0.52± 0.01[a]	4.74± 0.12[a]	2.73± 0.06[a]
G5	303.9± 15.0 [*]	153.6± 18.3[*]	107.8± 15.5 [*]	2.59± 0.02 [*]	0.51± 0.01	6.69± 0.06 [*]	3.51± 0.06 [*]
G6	253.3± 19.5 [*]	108.0± 15.7 [*]	98.0± 16.2 [*]	2.81± 0.02	0.48± 0.00 [*]	7.29± 0.07 [*]	3.34± 0.06 [*]
G7	266.0± 12.9 [*]	242.3± 18.7 [*]	224.2± 17.3 [*]	2.15± 0.04 [*]	0.45± 0.02 [*]	6.55± 0.06 [*]	3.30± 0.05 [*]
G8	226.1± 17.7 [*]	119.3± 17.0 [*]	101.4± 6.2 [*]	1.95± 0.02 [*]	0.42± 0.02 [*]	6.99± 0.06 [*]	3.41± 0.07 [*]
G9	179.3± 10.5 [*]	98.9± 5.6 [*]	80.3± 5.9 [*]	1.13± 0.02 [*]	0.27± 0.01 [*]	7.71± 0.06 [*]	3.30± 0.06 [*]

*: significantly different (P<0.05) to group 4. Groups 5- 9 were compared to group 4.
[a]: significantly different (P<0.05) to group 1. Groups 2- 4 were compared to group 1.
G1: Normal control; G2: *S. ellypticum* control (200 mg/kg b.w.); G3: *A. congolensis* control (200 mg/kg b.w.); G4: Toxic control, CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G5: SE (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G6: *S. ellypticum* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G7: *A. congolensis*(100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G8: *A. congolensis* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G9: Silymarin (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); ALP: Alkaline phosphatase; SGOT: serum glutamate oxaloacetate transaminase; SGPT: serum glutamate pyruvate transaminase; TB: total bilirubin ; DB: direct bilirubin; TP: total protein; SALB: serum albumin.

Table 3. Relative liver weight (RLW) and anti-oxidant parameters in liver homogenates after 30 days of treatment

Experimental groups	RLW and anti-oxidant parameters				
	LPO (nmol MDA/mg protein)	SOD (U/mg protein)	GSH (nmol/mg protein)	CAT (U/mg protein)	RLW (g/100g b.w.)
G1	0.79± 0.07	20.83± 0.78	0.32± 0.06	20.97± 1.80	3.41± 0.06
G2	0.92± 0.05	22.16± 1.65	0.34± 0.06	22.16± 1.66	3.61± 0.06
G3	0.79± 0.06	20.63± 1.30	0.33± 0.06	22.15± 1.53	3.51± 0.05
G4	4.56± 0.06[a]	5.56± 0.61[a]	0.13± 0.06[a]	10.17± 1.80[a]	5.01± 0.08[a]
G5	3.26± 0.06[*]	8.06± 0.61[*]	0.16± 0.08	12.49± 0.97[*]	4.26± 0.66
G6	3.00± 0.05[*]	6.56± 0.61[*]	0.24± 0.07[*]	12.96± 1.66[*]	3.91± 0.08[*]
G7	3.13± 0.10[*]	9.57± 0.69[*]	0.20± 0.04[*]	13.77± 1.80[*]	4.06± 0.62
G8	2.61± 0.08[*]	7.90± 1.56[*]	0.21± 0.08[*]	14.60± 2.08[*]	3.81± 0.09[*]
G9	1.96± 0.08[*]	16.42± 1.73[*]	0.28± 0.08[*]	16.99± 1.94[*]	3.81± 0.07[*]

*: significantly different (P<0.05) to group 4. Groups 5- 9 were compared to group 4.
[a]: significantly different (P<0.05) to group 1. Groups 2- 4 were compared to group 1.
G1: Normal control; G2: *S. ellypticum* control (200 mg/kg b.w.); G3: *A. congolensis* control (200 mg/kg b.w.); G4: Toxic control, CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G5: SE (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G6: *S. ellypticum* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G7: *A. congolensis*(100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G8: *A. congolensis* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); G9: Silymarin (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p); LPO: Lipid peroxidation; MDA: Malone dialdehyde; SOD: superoxide dismutase; GSH: Glutathione; CAT: catalase; RLW: relative liver weight.

Histopathology

Histopathological studies also provided a supportive infirmation to biochemical analyses (Figures 1-2). The CCl_4 intoxicated group of animals showed inflammation, centrilobular degeneration and necrosis, sinusoidal dilatation associated to Kupffer cell hyperplasia (Figure 1G4). Treatment with *S. ellypticum* and *A. congolensis* extract (100 and 200 mg/kg) showed reduced inflammation, centrilobular and bridging necrosis (Figures 2G5-G8). The same finding was observed with section from animals treated with the standard Silymarin (Figure 2G9). The liver sections of these groups revealed normal hepatocytes with significant reduction in areas of necrosis when compared to the toxic group. These changes showed protective effect of the drug against hepatic damage induced by CCl_4.

Discussion

This work was designed to investigate the potential of anti-oxidant plant extracts in protecting the liver using rodent models. The methanolic extract of a total of 2 medicinal plants from Cameroon were subjected to hepato-protective activities using chemical induced models. Carbon tetrachloride induced hepatic injuries has been a widely used model for the screening of hepato-protective drugs and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and transaminases in circulation, as well as histopathological changes. It is well documented that CCl_4 is bio-transformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites [24]. These metabolites are attributed to damage of the structural integrity of liver and induced an increase in the levels of SGPT, SGOT, ALP and bilirubin. Further, depletion of GSH, decreased protein synthesis, triglycerides accumulation and increased lipid peroxidation results in hepatocyte damage [25-27]. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical ($^oCCl_3$) in presence of cytochrome P450 which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles. Due to liver injury, the transportation function of hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum. The wet liver weight and liver volume was found to be increased, probably due to enlargement of liver cells, accumulation of fluids and fatty changes in the liver. CCl_4 has an important role in reduction of intracellular anti-oxidant reduced glutathione (GSH), increased LPO, membrane damage, decreased protein synthesis and alteration of hepatic enzymes level.

The present study revealed a significant alteration in physical and biochemical parameter after exposure of rats to CCl_4. The plant extracts *S. ellypticum* 100 and 200 mg/kg showed dose dependent decrease in the elevated serum biomarkers (SGPT, SGOT, SALP and bilirubin) and endogenous enzymes (GSH, SOD, CAT), and increase in total protein levels which were comparable to the standard. A significant reduction in the level of lipid peroxidation was also noted. Lipid peroxidation has been suggested to be the destructive process in liver injury due toCCl_4 administration [28-30]. The present study revealed a significant alteration in physical and biochemical parameters after exposure to the CCl_4. The plant extract *A. congolensis* at 200 and 100 mg/kg BW showed dose dependent decrease in the elevated serum biomarkers (SGPT, SGOT, ALP and bilirubin) and endogenous enzymes (GSH, SOD, CAT). Increase in total proteins levels which were comparable to the standard and significant reduction in the levels of lipid peroxidation were also observed, therefore suggesting the neutalization of oxidative stress as the mechanism of hepato-protection by methanolic extracts. The hepato-protective activity of plants ingredients such as flavonoids, saponins and other phenolic compounds is documented in animal models. It is therefore predicted that these phyto-constituents found in plant extracts are responsible for the observed hepato-protective activity [14].

The protective effect in CCl_4 induced liver toxicity was also shown by decrease in relative organ weights in animals treated with both SE-200 and AC-200 mg/kg bw. Histopathological studies of liver, treated with CCl_4 revealed the architecture of liver parenchyma with damaged hepatocytes. Treatment with *S. ellypticum* (200 and 100 mg/kg) revealed the significant improvement in architecture of liver parenchyma and regenerating hepatocytes indicating hepato-protection.

It can be concluded from the above data that the hepato-protective activity was found to be more significant in high dose (SE-200 mg/kg) compared to low dose (SE-100 mg/kg) in CCl_4model. The treatment with methanolic extract of *S. ellypticum* could restore the organ (liver) weight at considerable range, which were elevated in hepatotoxic animals. The hepato-protective potential of this extract in this experimental model might be due to the presence of flavonoids, saponins and other polyphenolic compounds previously reported in these plants [14].

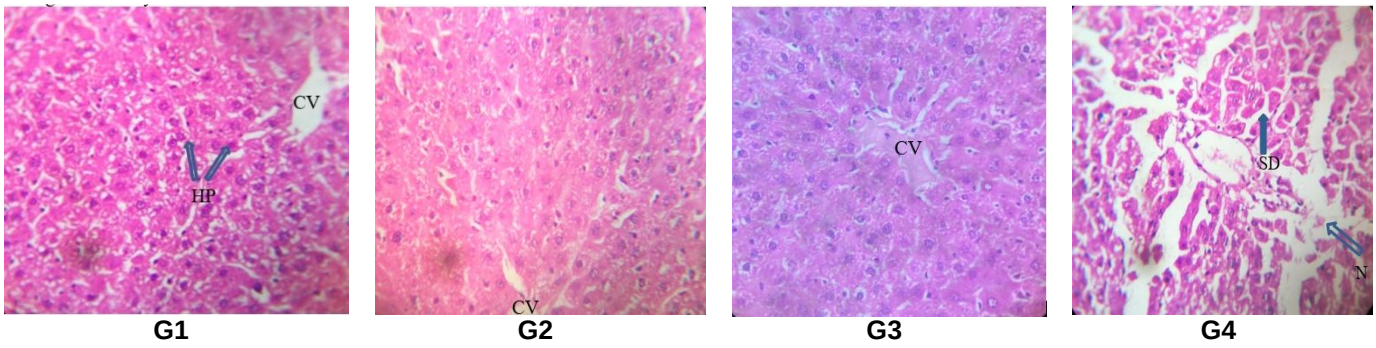

Figure 1. Histopathological slice of the liver stain section of Normal control, AC control, SE control and toxic control.
G1: Normal control. This micrograph shows well organized hepatocyte plates (HP) around the central vein (CV), and well defined sinusoidal spaces; G2: *S. ellypticum* control (200 mg/kg b.w.). No major changes were found in this micrograph as compared to the normal control; G3: *A. congolensis* control (200 mg/kg b.w.). No major changes were found in this micrograph as compared to the normal control; G4: Toxic control, CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). This micrograph from toxic group of animal shows centrilobular necrosis (N) associated with sinusoidal dilatation (SD) in the parenchyma.

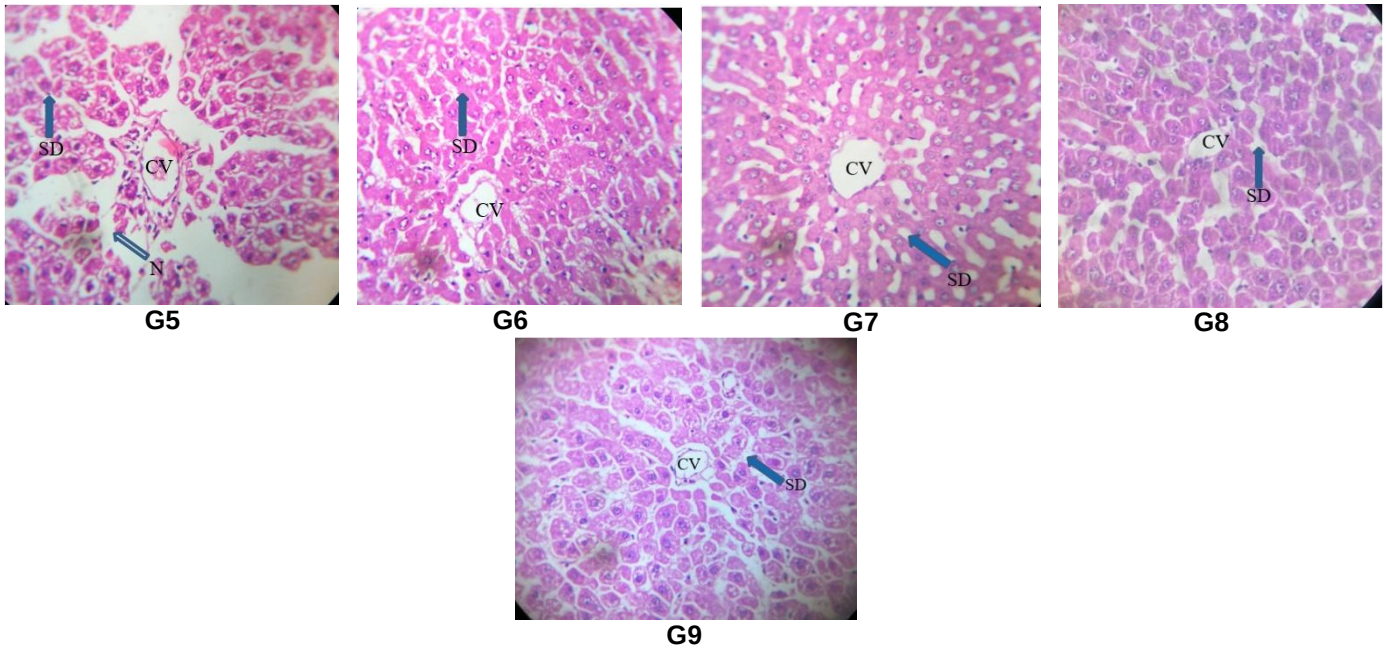

Figure 2. Histopathological slice of the liver stain section animals treated with extracts and CCl_4
G5: SE (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). Centrilobular necrosis was still severe here as compared to the toxic group of animals; G6: *S. ellypticum* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). The micrograph shows slight improvement of tissue repair. The extern of damage is less than that of the toxic group. Prominent sinusoidal dilatations were still present; G7: *A. congolensis* (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). The micrograph indicates evidence of tissue repair. The extern of damage is low as compared to the toxic group. However, sinusoidal dilatations remain prominent changes; G8: *A. congolensis* (200 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). The micrograph shows evidence of tissue repair. The extern of damage is low as compared to the toxic group.
G9: Silymarin (100 mg/kg b.w.) & CCl_4/paraffin oil (1:1 v/v, 0.7 mL/kg, i.p). The micrograph indicates evidence of tissue repair. The extern of damage is low as compared to the toxic group.

Conclusions

In this study, it was found that *A. congolensis* and *S. ellypticum* methanolic extracts administered to rats at 200 mg/kg bw can reverse changes biochemical and histological features in the liver induced by CCl_4. The observed protective effects of *A. congolensis* and *S. ellypticum* methanol crude extracts justify scientifically

the local use of these two plants for the treatment of various ailments involving oxidative stress.

Additional file

Supplement: **Figure Suppl1**. Illustration of the mean serum parameters of animals after 10 days of treatment; **Figure Suppl2:** Illustration of the mean relative liver weight (RLW) and anti-oxidant; **Figure Suppl3:** Calibration curve for liver protein titration. Doc [230 kb]

Authors' Contribution

AJN designed the protocol, carried out the experiments and drafted the manuscript; JCNA PNF, VPB and SW contributed to design the protocol, read and substantially revised the manuscript; APNE collected the plant and prepared the extracts; MOT and KMB contributed to the laboratory work, SB supervised the research activities; all authors read and approved the manuscript.

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Conflict of interest

The author(s) declare that they have no competing interests.

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