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Treatment of Aspirin and CCl_4-Induced Hepatic Damage in Rats by the Aqueous Extracts of some Local Plants Collected from Gombe State in Nigeria

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Abstract

In the present study, the capacity of the aqueous extracts of *Senna singueana* (SS), *Nymphaea lotus* (NL), *Cochlospermum planchoni* (CP) and *Acacia nilotica* (AN) as antitoxicants to protect against aspirin and carbon tetrachloride (CCl_4) induced hepatotoxicity in rats was investigated. Ten groups containing three replicates each were used. Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), direct bilirubin (DB), total bilirubin (TB), total protein (TP) and albumin (ALB) were assayed. SS, NL, CP and AN extracts (250 mg/kg) were given daily by gavage to the animals in the groups V to X for fourteen consecutive days to explore the protective effects against aspirin and CCl_4-induced hepatotoxicity. Animals of negative and standard controls (group I and II) respectively received vehicle and vehicle with 2 mL/kg olive oil by subcutaneous injections twice a week for a period of two weeks. Animals of the CCl_4-treated group (group III) and the aspirin-treated group (group IV) respectively received vehicle with 2 mL/kg CCl_4 in olive oil by subcutaneous injections and vehicle with 1 mL/kg aspirin orally twice a week for a period of two weeks. The results obtained were statistically evaluated using One-Way ANOVA followed by Least Significant Difference (LSD) for the parameters found to be statistically significant at $\alpha = 0.05$. Mean serum AST, ALT and ALP, DB and TB levels/activities of the groups III and IV were statistically ($p < 0.05$) higher than those of the controls. DB and TB levels were slightly ($p > 0.05$) higher when compared with the controls, in contrast with the mean serum TP and ALB levels of the groups III and IV that were found to differ statistically ($p < 0.05$) being lower than those of controls. Conversely, mean serum AST, ALT and ALP, DB and TB levels/activities of the groups V to X differ statistically ($p < 0.05$) being lower than those of the groups III and IV except for DB and TB levels that were slightly lower ($p > 0.05$) when compared with the groups III and IV. Mean serum TP and ALB levels of the groups V to X and the controls were found to differ statistically ($p < 0.05$) being higher than those of the groups III and IV. Taken together, the results of this study showed that the SS or NL extracts were found effective as hepatoprotective agents, and the mixture of SS and CP or the mixture of NL and AN extracts significantly antagonized aspirin and CCl_4-induced liver damage in rats in comparison with control values, as evidenced by the biochemical parameters.

Keywords: *Acacia nilotica*, Aspirin, Carbon tetrachloride, *Cochlospermum planchoni*, Hepatotoxicity, *Nymphaea lotus*, *Senna singueana*.

1. Introduction

According to the World Health Organization (WHO), in 2015, 325 million people worldwide have been estimated to be living with the chronic hepatitis infection (Wikipedia, 2017; CDC, 2017). Globally, 1.34 million people died of hepatitis in 2015. The majority of infants (80 – 90 %) infected during the first year of life developed chronic infections. 30-50 % of the children infected before the age of six years developed chronic hepatitis (Wikipedia, 2017; CDC, 2017). Liver is the first major organ to be exposed to ingested toxins due to its portal blood supply. Toxins may be, at least partially, removed

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from the circulation during the first pass, providing protection to other organs while increasing the likelihood of hepatic injury. Liver toxicity is monitored in standard toxicity studies by a range of investigations including clinical biochemical parameters such as enzymes, proteins and lipids (Sharma *et al*., 2012; Jaiswal *et al*., 2015; Gupta *et al*., 2015; Sharma *et al*., 2016). "Hepatitis" refers to the inflammation of the liver which could be a short-term (acute) inflammation or a long-term (chronic) one depending on whether it lasts for less than or more than six months (Wenden & Bernal, 2013). Toxins, chemicals, certain drugs, some diseases, heavy alcohol use, heavy metals and bacterial and viral infections can all cause hepatitis (Sharma *et al*., 2012; Gupta *et al*., 2015; Sharma *et al*., 2016). However, aspirin, carbon tetrachloride (CCl_4) and carbofuran are by far the most essential drugs and chemicals used to induce the oxidative stress not only in the Wistar rats, but in their brain and liver slices as well (Sharma *et al*., 2016;). Hepatitis is also the name of a family of viral infections that affect the liver. Its most common types are Hepatitis A, Hepatitis B, and Hepatitis C (Sharma *et al*., 2012), and to a lesser extent Hepatitis D and Hepatitis E (Sharma *et al*., 2012; CDC, 2017). Perturbations in the activity of the liver function enzymes (LFTs) following the aspirin, carbon tetrachloride (CCl_4) or carbofuran administration in Wistar rats have been extensively studied as previously reported by Sharma *et al*. (2012), Jaiswal *et al*. (2015), Gupta *et al*. (2015), Sharma *et al*. (2016), Gupta *et al*. (2017) and Jaiswal *et al*. (2017). Curcumin and vitamin C have been shown to have protection against carbofuran induced oxidative stress in brain, heart and liver slices in rats (Sharma *et al*., 2016; Gupta *et al*., 2017; Jaiswal *et al*., 2017).

Aspirin is one of the potent non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammatory conditions. A high dose of aspirin can cause damage as it impairs the ability of the gastrointestinal mucosa to respond to the injury (Drugs.com, 2014). Aspirin is a chemical that can induce severe liver damage in experimental animals. In 1971, Vane and his coworkers discovered that aspirin causes mucosal damage by interfering with the prostaglandins synthesis. Aspirin acts by inhibiting cyclooxygenases (COX-1 and COX-2), the enzymes that convert arachidonic acid to prostaglandins, thus reducing the PG levels (Vane, 1971). As prostaglandins play a major role in the maintenance of the gastroduodernal defense, their depletion due to aspirin impairs the cytoprotection which results in mucosal injury, erosions and ulceration (Vane & Botting, 2003). Carbon tetrachloride (CCl_4) has been extensively used in animal models to explore chemical toxin-induced hepatic injuries (Sharma *et al*., 2012). The metabolism of CCl_4 catalyzed by liver microsomal cytochrome P450 rapidly overproduces free radicals that deplete hepatic glutathione, and initiate a chain of lipid peroxidation of the hepatocytes membrane (Recknagel *et al*., 1989). This ultimately results in the overproduction of reactive oxygen species (ROS) and hepatocytes injuries (Mohammed *et al*., 2014). Liver damage induced by CCl_4 involves the biotransformation of the free radical derivatives, increased lipid peroxidation, and excessive cell death in the liver tissues (Mohammed *et al*., 2014).

Senna singuena (Delile) Lock, commonly known as "Winter cassia" (English) and "Rumfu" (Hausa) is a widespread plant in the semi-arid parts of tropical Africa. It is usually found in the savanna and is abundantly available where shrubs used to grow, either on the lowlands or the hills. *S. senguena* has an anti-oxidant, anti-inflammatory, anti-dysentric, anti-cancer, anti-fever antipyretic, anti-worms, anti-syphilis, anti-ulcer (leaf/root), and antibacterial activities against both gram positive and negative bacteria (Sepasal, 2006). According to Kawanga and Bosch (2007), and Mebrahton *et al.* (2016), the phytochemical screening results of *S. Singuena* confirmed the presence of Alkaloids, carbohydrate, glycosides, phenols, steroids, tannins and triterpenes. *Nymphaea lotus* L, popularly known as "Water lily" (English) and "Bado" (Hausa) grows in various parts of East Africa and Southeast Asia. It consists of various phenols, tannins, saponins, steroids, proanthocyanidins, flavanols, alkanoids and flavonoids (Madhusudhanan *et al.*, 2011; Afolayan *et al.*, 2013). *N. lotus* is very rich in phytochemicals, and is a good source of natural antioxidants. This may justify its use in the treatment of several diseases affecting humans (Afolayan *et al.*, 2013). *N. nymphaea* has antioxidant, antitumor, sedative, anti-inflammatory, anti-cancer, aphrodisiac, antiviral, antibacterial, and demulcent activities (Madhusudhanan *et al.*, 2011; Afolayan *et al.*, 2013). It is used in traditional medicine systems as an aphrodisiac, astringent, cardiotonic, sedative, demulcent, analgesic, and as anti-inflammatory agent (Madhusudhanan *et al.*, 2011). *Cochlospermum planchoni* Hook.f., variously called "False cotton" (English) and "Rawaya" (Hausa) is a perennial plant with a woody subterranean found in the savannas and the savanna forests. It is found in dried rocky areas (Anaga and Oparah, 2009). *C. planchoni* has alkaloid, phenolics, carbohydrates, glycosides, anthraquinones, saponins, steroidal triterpenes, flavonoids, tannins, cardenolides and dienoloides (Nafiu *et al.*, 2011; Isah *et al.*, 2013). *C. planchoni* possesses antibacterial, antimalarial, antityphoid, anti-hepatobililary infections (black toilets fever) antidiabetic, anti-inflammatory and analgesic activities (Anaga and Oparah, 2009). *Acacia nilotica* (L.) Delile, popularly known as "Tomentosa Babul" (English) and "Bagaruwa" (Hausa) is usually found in the regularly flooded areas. The chemical constituents of *A. nilotica* include alkaloids, flavonoids, glycosides, saponins, tannins, stearic acid, vitamin C, polysaccharides and terpenoids (Amos *et al.*, 1999; Deshpande, 2011). Several bioactive agents have been identified from *A. nilotica* which include: androstene steroid, gallic acid, ellagic acid, kaempferol, naringenin, rutin, lupine, niloticane, umbelliferone catechin, and sitosterol (Lee *et al.*, 2011; Kannan *et al.*, 2013). Previous scientific studies on different parts of *A. nilotica* revealed that the plant has anti-inflammatory, hypoglycemic, antitumor, anti-fungal, antiplatelets aggregation, spasmogenic and vasoconstrictor, antihypertentive, antihepatitis (Lee *et al.*, 2011). Many researches have been previously conducted on the hepatoprotective effects of many medicinal plants, such as the aqueous extracts of *Pterocarpus erinaceus* and *Bauhinia rufescens* by Usman *et al.* (2017); nutraceuticals by Mohammed *et al.* (2014); *Xylopia aethiopica* by Adekeye *et al.* (2014); dandelion by Al-Malki *et al.*

(2013); *Cnicloseous aconititolius* by Saba *et al.* (2010); honey and aloe vera by Adewoga and Sebiomo (2014); *Bauhinia racemosa* by Gupta *et al*. (2004); *Vernonia amygdalina* by Adesanoye *et al.* (2010); among others. However, little or no work has been carried out on the assessment of the hepatoprotective effects of the aqueous extracts of *S. singuena*, *N. lotus*, *C. planchoni* or *A. nilotica* singly or in combination with one another in both aspirin and CCl_4-induced liver damaged rats. This research is designed to investigate these gaps. It is aimed at assessing the hepatoprotective effects of the aqueous extracts of *S. singuenas*, *N. lotus*, *C. planchoni* and *A. nilotica* in albino rats for a period of two weeks. The specific objectives of this research are as follows: 1) Induction of liver damage using aspirin and CCl_4. 2) Assaying the activities/levels of AST, ALT, ALP, TB, DB, TP and Albumin in the experimental groups. 3) Assessing the hepatoprotective effects of SS, NL, CP and AN, by the context of liver function tests (LFTs), following the induction of liver damage using aspirin and CCl_4.

2. Materials and Methods

2.1. Study Area

This research was carried out at the Department of Biological Sciences' Laboratory, Federal University of Kashere in Gombe State, Nigeria. The study was approved by the ethical committee of the University prior to the experimentation, and all the experiments were performed according to the guidelines of the Institutional Animal Ethical Principle.

2.2. Chemicals

All chemicals were of the highest commercially analytical grade, and were obtained from Sigma-Aldrich Co., USA.

2.3. Laboratory Animals

Apparently thirty healthy male and female Wistar rats (*Rattus norvegicus*) with body weights ranging from 152 to 309 g were obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were acclimatized for a period of one week in a well-ventilated room, and were housed in a well-ventilated plastic cage maintained under standard laboratory conditions (twelve hours light/dark cycle; 25 – 32 ^oC) prior to experimentation. They were fed with commercial rat chow (Vital Feeds LMT, Plateau State, Nigeria) and sachet water *ad libitium*, and were handled according to the standard protocols.

2.4. Collection of Plant Materials

Fresh leaves of *S. singuena* and roots of *C. planchoni* were collected from Kashere town, Akko Local Government, Gombe State, Nigeria, in December, 2017. Fresh leaves of *N. lotus* were collected from Wuro ibba Dam, Dukul town, Kwami Local Government, Gombe State, Nigeria, in January, 2017. Pod of *A. nilotica* was collected from the Bajoga town, Funakaye Local Government, Gombe State, Nigeria, in January, 2017. The plants were identified and authenticated by the Department of Biological Sciences, Federal University, Kashere, Gombe State – Nigeria. The voucher specimen No. was prepared and deposited at the Herbarium of Federal University of Kashere (FUKH) for reference. A Batch/code number for all the plant samples were issued by Mr Umar Galadima, (Head of Biological laboratory, FUK) as follows: *Senna singueana* leaf; FUKH077, *Nymphaea lotus* leaf; FUKH078, *Cochlospernum planchonmi* root; FUKH076 and *Acacia nilotica* seed; FUKH079.

2.5. Induction of Liver Damage by Aspirin and CCl4

The concentration of aspirin was determined by using the following formula: Wt/ Vol (mg/mL). Each tablet of aspirin was dissolved in three mL of distilled water. A total of thirty-three tablets of aspirin (each 300 mg, 33 tab X 300 mg = 9900 mg) were dissolved in 99 mL of distilled water, making 100 mg/mL. 1 mL/kg was administered orally to the experimental groups. 16 mL of CCl_4 from the stock was dissolved up to 100 mL of olive oil. 2 mL/kg was administered subcutaneously to the experimental groups.

2.6. Experimental Design

A total of thirty Wistar rats were randomly divided into ten groups consisting of three rats each as follows:

Group I: served as negative/normal control that is allowed free access to vital food and water only.

Group II: neutral/standard control: olive oil (2 mL/kg) twice a week (day 0, day 4, day 8, day, 12) for a period of two weeks.

Group III: positive control: CCl_4 (2 mL/kg) dissolved in olive oil, twice a week for a period of two weeks.

Group IV: positive control: Aspirin (1 mL/kg) twice a week for a period of two weeks.

Group V: CCl_4 (2 mL/kg) twice a week for a period of two weeks plus *Senna singueana* (250 mg/kg) daily for two weeks;

Group VI: CCl_4 (2 mL/kg) twice a week for a period of two weeks plus *Nymphaea lotus* (250 mg/kg) daily for two weeks;

Group VII: Aspirin (1 mL/kg) twice a week for a period of two weeks followed by *Senna singueana* (250 mg/kg) daily for two weeks;

Group VIII: Aspirin (1 mL/kg) twice a week for a period of two weeks followed by *Nymphaea lotus* (250 mg/kg) daily for two weeks;

Group IX: CCl_4 (2 mL/kg) twice a week for a period of two weeks followed by *Senna singueana* (250 mg/kg) plus *Cochlospernum planchoni* daily for two weeks;

Group X: Aspirin (1 mL/kg) twice a week for a period of two weeks followed by *Nymphaea lotus* (250 mg/kg) plus *Acacia nilotica* daily for two weeks.

2.7. Preparation of the Plants Aqueous Extracts and Biochemical Analysis

The fresh leaves of *Senna singuena* and *Nymphaea lotus*, roots of *Cochlospermum planchoni*, and the pods of *Acacia nilotica* were washed, air-dried until constant weight was obtained, and were grinded into fine powder using a mortar and pestle. The powder was poured into a Bama bottle for each sample and was labeled. 350 g of each of the powder of *Senna singuena*, *Nymphaea lotus*, *Cochlospermum planchoni* and *Acacia nilotica* was soaked in 1 L (1000 mL) of distilled water, shaken for three minutes and was then allowed to stay for three days (seventy-two hours). The mixtures were filtered with Whatman No. 1 filter paper (25 cm) on the third day. The filtrates were evaporated to dryness using a water bath evaporator at 40 – 50 ^oC which took two weeks in order to obtain the crude extract that was reconstituted up to 200

mL distilled water (Won *et al.*, 2005). At the end of the experiment, the rats were sacrificed twenty-four hours after the last administration of aqueous extracts, and the blood samples were collected into lithium heparin tubes for Liver Function Tests (LFTs). The samples for LFTs were transported to the Biochemistry Laboratory of Gombe State University (GSU) and were centrifuged for ten minutes at 3000 rpm to separate the serum. Sera were carefully separated into clean dry Wassermann tubes by using a Pasteur pipette, and were tested for the activity of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) (Reitman and Frankel, 1957), and Alkaline Phosphatase (ALP) (Rec, 1972), as well as the level of Direct Bilirubin (DB) and Total Bilirubin (TB) (Jendrassik and Grop, 1938), Total Protein (TP) (Keller, 1984) and Albumin concentration using Randox Kit.

2.8. Statistical Analysis

The results obtained were statistically evaluated using One-Way Analysis of Variance (ANOVA). Differences were considered statistically significant at $p < 0.05$ followed by Least Significant difference (LSD) to determine where the difference among the ten groups containing three rats each actually lies.

3. Results

Table 1 shows the results of mean serum levels/activities of AST, ALT, ALP, TP and ALB of group I to IV. When mean serum levels of AST, ALT, ALP, TP and ALB of group III and IV are compared with controls (group I and group II), the difference is statistically significant ($p< 0.05$).

Mean serum levels of AST, ALT and ALP of group III and IV were found to be statistically ($p < 0.05$) higher when compared with controls (group I and II). Conversely, mean serum levels of TP and ALB of group III and IV were found to differ statistically ($p < 0.05$) being lower than those of controls. Thus, the subcutaneous injection of CCl_4 (group III) or the oral administration of Aspirin (group IV) induced significant elevation ($p < 0.05$) of the ALT, AST and ALP levels, and somehow caused significant reduction ($p < 0.05$) in the TP and ALB levels when compared with controls (group I and II).

The results of Table 2 reveal mean serum levels/activities of AST, ALT, ALP, TP and ALB of the groups I, IV, VII, VIII and X. When mean serum levels of AST, ALT, ALP, TP and ALB of group IV are compared with control, the difference is statistically significant ($p < 0.05$). Mean serum levels of AST and ALP of the groups VII, VIII and X were found to differ statistically ($p < 0.05$) being lower when compared with group IV in contrast to the ALT levels of these groups that were found to differ slightly ($p > 0.05$) being lower when compared with group IV. Conversely, the mean serum levels of TP and ALB of the groups VII, VIII and X were found to differ statistically ($p < 0.05$) being higher than those of group IV. Therefore, the pre-treatment with the SS, NL or a mixture of NL and AN extracts caused significant reduction ($p < 0.05$) in the AST, ALT and ALP activities, and in the same vein induced significant elevation in the TP and ALB levels when compared with the Aspirin-induced group (IV).

Table 1. Serum activities of biochemical parameters in rats after 14 days of subcutaneous injection of CCl_4 and oral administration of Aspirin

Group/ Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dl)	ALB (g/dl)
Group I negative control	70.67 ± 1.5	22.33 ± 2.31	82.00 ± 24.25	4.80 ± 0.71	3.37 ± 0.29
Group II(2 mL/kg olive oil)	74.00 ± 00	22.00 ± 1.73	116.00 ± 1.73	6.23 ± 0.12	3.77 ± 0.12
Group III (2 mL/kg CCl_4)	234.33 ± 7.02[a]	69.33 ± 2.52[a]	330.67 ± 17.04[a]	4.17 ± 0.12[a]	2.83 ± 0.25[a]
Group IV(1 mL/kg Aspirin)	189.33 ± 15.31[a]	52.33 ± 4.16[a]	204.33 ± 7.37[a]	4.37 ± 0.35[a]	3.00 ± 0.10[a]
$LSD_{0.05}$	11.62	18.00	26.33	0.34	0.09

Values are expressed as mean ± standard deviation of 3 replicates; a= significant difference at $p < 0.05$ when CCl_4-induced group or Aspirin-induced group is compared with Groups I and II; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin.

Table 2. Serum levels of biochemical parameters in aspirin-treated rats after 14 days of oral administration of SS, NL, mixture of NL and AN extracts.

Group/ Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dl)	ALB (g/dl)
Group I negative control	70.67 ± 1.5	22.33 ± 2.31	82.00 ± 24.25	4.80 ± 0.71	3.37 ± 0.29
Group IV(1 mL/kg Aspirin)	189.33 ± 15.31[a]	52.33 ± 4.16[a]	204.33 ± 7.37[a]	4.37 ± 0.35[a]	3.00 ± 0.10[a]
Group VII (1 mL/kg Aspirin +1 mL/kg SS)	154.00 ± 11.36[a,b]	39.67 ± 2.52	149.33 ± 7.51[a,b]	5.20 ± 0.10[a,b]	3.33 ± 0.21[b]
Group VIII (1 mL/kg aspirin+2 mL/kg NL)	114.33 ± 6.66[a,b]	37.00 ± 2.00	124.67 ± 7.02[b]	5.47 ± 0.06[a,b]	3.20 ± 0.10[a,b]
Group X (1 mL/kg aspirin+ 2 mL/kg NL +1 mL/kg AN)	112.00 ± 0.00[a,b]	37.00 ± 0.00	120.00 ± 0.00[b]	5.80 ± 0.00[a,b]	3.30 ± 0.00[b]
$LSD_{0.05}$	11.62	18.00	26.33	0.34	0.09

Values are expressed as mean ± standard deviation of 3 replicates; a = significant difference at $p < 0.05$ when Aspirin-induced group or pretreated groups are compared with Group I; b = significant difference at $p < 0.05$ when pretreated groups are compared with Aspirin-induced group; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin.

Table 3 reveals the results of mean serum levels/activities of AST, ALT, ALP, TP and ALB of the groups I, III, V, VI and IX. When the mean serum levels of AST, ALT, ALP, TP and ALB of group III are compared with controls (group I and II), the difference is statistically significant ($p < 0.05$). Mean serum levels of AST, ALT and ALP of the groups V, VI and IX were found to differ statistically ($p < 0.05$) being lower when compared with group III. In contrast, mean serum levels of TP and ALB of the groups V, VI and IX were found to differ statistically ($p < 0.05$) being higher than those of group III (CCl_4-induced group). Hence, the pre-treatment with SS, NL or a mixture of SS and CP induced significant reduction ($p < 0.05$) in the AST, ALT and ALP activities and at the same time brought significant elevation in the TP and ALB levels when compared with CCl_4-induced group (group III).

Table 3. Serum levels of biochemical parameters in CCl_4-treated rats after 14 days of oral administration of SS, NL, mixture of SS and CP extracts**.**

Group/ Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dl)	ALB (g/dl)
Group I negative control	70.67 ± 1.5	22.33 ± 2.31	82.00 ± 24.25	4.80 ± 0.71	3.37 ± 0.29
Group II (2 mL/kg olive oil)	74.00 ± 00	22.00 ± 1.73	116.00 ± 1.73	6.23 ± 0.12	3.77 ± 0.12
Group III (2ml/kg CCl_4)	234.33 ± 7.02[a]	69.33 ± 2.52[a]	330.67 ± 17.04[a]	4.17 ± 0.12[a]	2.83 ± 0.25[a]
Group V (2 mL/kg CCl_4 + 1 mL/kg SS)	155.67 ± 14.19[a,c]	50.00 ± 1.00[a,c]	242.33 ± 30.[a,c]	4.70 ± 0.10[c]	3.00 ± 0.10[a,c]
Group VI (2ml/kg CCl_4 + 2 mL/kg NL)	123.33 ± 5.03 [a,c]	42.00 ± 2.65[a,c]	180.00 ± 5.57[a,c]	5.07 ± 0.38[a,c]	3.17 ± 0.21[a,c]
Group IX (2ml/kg CCl_4+ 1 mL /kg SS+ 2 mL/kg CP)	76.33 ± 1.15 [c]	30.00 ± 1.73[c]	103.67 ±19.63[c]	6.03 ± 0.06[c]	3.50 ± 0.00[a,c]
$LSD_{0.05}$	11.62	18.00	26.33	0.34	0.09

Values are expressed as mean ± standard deviation of 3 replicates; a = significant difference at $p < 0.05$ when CCl_4-induced group or pretreated groups are compared with Groups I and II; c = significant difference at $p < 0.05$ when the pretreated groups are compared with CCl_4-induced group; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin.

Table 4. Serum levels of biochemical parameters in CCl_4 aspirin-treated rats after 14 days of oral administration of SS, NL, mixture of SS and CP, and mixture of NL and AN **extracts.**

Group	Treatment	TB (mg/dl)	DB (mg/dl)
Group I	negative control	0.07 ± 0.02	0.04 ± 0.00
Group II	2 mL/kg olive oil	0.06 ± 0.00	0.05 ± 0.01
Group III	2 mL/kg CCl_4	0.11 ± 0.01	0.08 ± 0.01
Group IV	1 mL/kg aspirin	0.07 ± 0.02	0.07 ± 0.01
Group V	2 mL/kg CCl_4 + 1 mL/kg SS	0.06 ± 0.01	0.05 ± 0.01
Group VI	2 mL/kg CCl_4 + 2 mL/kg NL	0.06 ± 0.01	0.05 ± 0.01
Group VII	1 mL/kg aspirin + 1 mL/kg SS	0.06 ± 0.01	0.05 ± 0.01
Group VIII	1 mL/kg aspirin + 2 mL/kg NL	0.07 ± 0.00	0.05 ± 0.01
Group IX	2 mL/kg CCl_4 + 1 mL/kg SS + 2mL/kg CP	0.08 ± 0.01	0.06 ± 0.01
Group X	1 mL/kg aspirin + 2 mL/kg NL + 1 mL/kg AN	0.07 ± 0.00	0.06 ± 0.00

Values are expressed as mean ± standard deviation of 3 replicates; TB = Total Bilirubin; DB = Direct Bilirubin.

Table 4 reveals the results of mean serum levels/activities of TB and DB of the groups I to X. When mean serum levels of DB and TB of the groups III and IV are compared with controls (group I and group II), the difference is statistically insignificant ($p > 0.05$). Mean serum levels of DB and TB of the groups III and IV were found to be slightly ($p > 0.05$) being higher when compared with controls (group I and II) and the groups V, VI, VII, VIII, IX and X. Thus, the oral administration of SS, NL, a mixture of SS and CP, and a mixture of NL and

AN extracts caused slight reduction in the TB and DB activities when compared with the groups III and IV.

4. Discussions

Liver function tests are of immense importance in the diagnosis and monitoring of liver diseases. Serum levels of AST, ALT and ALP were assayed with a view to testing the liver injury and/or cholestasis (Burtis *et al.*, 2008; Vasudevan *et al.*, 2013). In the same vein, serum levels of TB and DB, and TP and ALB were investigated with the aim of testing hepatic excretory and synthetic functions respectively (Burtis *et al.*, 2008; Vasudevan *et al.*, 2013). The result of this study indicated that the subcutaneous injection of CCl_4 or the oral administration of aspirin induced a marked and significant elevation ($p < 0.05$) in ALT, AST and ALP activities, and conversely caused a significant reduction ($p < 0.05$) in TP and ALB levels (Table 1). These findings are corroborated with the findings of previous studies on CCl_4 and aspirin-induced hepatic damage by Kannan *et al.* (2013), Gupta *et al.* (2004), Adekeye *et al.* (2014), Mohammed *et al.* (2014), Adewoga and Sebiomo (2014) and Lee *et al.* (2011). The results in Table 2 revealed that the pre-treatment with SS, NL or a mixture of NL and AN extracts caused significant reduction ($p < 0.05$) in AST, ALT and ALP activities and conversely, induced significant elevation in TP and ALB levels. However, the co-administration with the NL and AN extract is by far efficacious in antagonizing the liver damage than do the SS or NL extracts alone. These findings were tallied with the findings of previous studies on aspirin-induced hepatic damage by Kannan *et al.* (2013), Gupta *et al.* (2004), Adewoga and Sebiomo (2014) and Lee *et al.* (2011). The significant reduction in the AST, ALT and ALP activities, and the induced elevation in the TP and ALB levels following the administration of the SS, NL or a mixture of NL and AN extracts may be attributed to the presence of phytochemicals found in SS, NL and AN as reported formerly by Kawanga and Bosch (2007), and Mebrahton *et al.* (2016), Madhusudhanan *et*

al. (2011), Afolayan *et al.* (2013), Amos *et al.* (1999) and Deshpande (2011). The results in Table 3 show that the pre-treatment with SS, NL or a mixture of the SS and CP extracts induced a marked and significant reduction ($p < 0.05$) in the AST, ALT and ALP activities and conversely caused a significant elevation in the TP and ALB levels; the co-administration of SS and CP extracts antagonized the liver damage in comparison with the control groups. The findings of the current study conformed to the findings of Usman *et al.* (2017), Adekeye *et al.* (2014), Adesanoye *et al.* (2010), Nafiu *et al.* (2011), Mohammed *et al*. (2014), Al-malki *et al*. (2013). The marked decrease in the AST, ALT and ALP activities, and the significant increase in the TP and ALB levels following the administration of SS, NL or a mixture of the SS and CP extracts may be attributed to the presence of phytochemicals found in SS, NL and CP as confirmed earlier by Kawanga and Bosch (2007), and Mebrahton *et al.* (2016), Madhusudhanan *et al.* (2011), Afolayan *et al.* (2013), Nafiu *et al.* (2011) and Isah *et al.* (2013). The results in Table 4 entailed that the oral administration of SS, NL, a mixture of SS and CP, and a mixture of NL and AN extracts caused slight reduction in the TB and DB activities when compared with both controls (group I and II) and positive controls (group III and IV). These findings are also corroborated with the findings of Adekeye *et al.* (2014) and Mohammed *et al*. (2014). Although the excretory function of the liver from this study was not significantly perturbed, the oral administration of SS, NL, a mixture of SS and CP, or a mixture of the NL and AN extracts was capable of antagonizing the liver damage caused by CCl_4 and aspirin in comparison with the control groups. This may be attributed to the presence of phytochemicals found in SS, NL , CP and AN as reported formerly by Kawanga and Bosch (2007), and Mebrahton *et al.* (2016), Madhusudhanan *et al.* (2011), Afolayan *et al.* (2013), Nafiu *et al.* (2011), Isah *et al*. (2013), Amos *et al.* (1999) and Deshpande (2011).

5. Conclusion

According to this study, liver damage at 16 mL CCl_4 dissolved in up to 100 mL olive oil and 9900 mg aspirin dissolved in up to 100 mL distilled water was apparent. Pretreatment of CCl_4-treated and aspirin-treated groups with SS, NL, a mixture of SS and CP or a mixture of NL and AN extracts orally administered at a dose of 250 mg/kg were efficacious in terms of antagonizing the liver damage caused by CCl_4 and aspirin.

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Competing Interests

The authors have declared that no competing interests exist.

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