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Hepatoprotective effect of Nescafe in carbon tetrachloride-induced liver injury in Wistar rats

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Abstract

Liver damage is of a growing concern. The study aimed at investigating the effect of Nescafe on carbon tetrachloride (CCL_4)-induced liver injury. Rats were randomly grouped viz; groups: 1 to 5 of 6 rats each. Groups-2 to 5 received intraperitoneal doses of CCl_4, at 2ml/Kg body weight, in 3:1 v/v preparation of CCL_4 and olive oil, weekly for one month. Groups-1 and 2 were normal and positive control respectively and received distilled water (10ml/kg body water). Groups-3 to 5 were fed orally with 41.67, 62.22 and 72.22mg Nescafe suspension respectively, by gavage, weekly for one month. A day after treatment period, rats were anaesthetized and blood withdrawn from heart, using the heart puncture technique. Serum parameters viz: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin, total bilirubin (TB), Total Cholesterol (T-Chol), Triglyceride, High Density Lipoprotein Cholesterol (HDL), Low density lipoprotein cholesterol (LDL) and Very Low-Density Lipoprotein cholesterol (VLDL) were studied. Group-2 activities of AST, ALT, ALP and concentrations of TB, globulin, T-Chol Triglyceride, LDL, VLDL, were increased while HDL, Albumin, TB concentrations were decreased from group-1. Aside from T-Chol and HDL, the changes were significant ($P<0.05$), compared to group-1. Group-3 through 5, the AST, ALT, ALP enzyme activities and globulin, TB, T-Chol, TAG, LDL VLDL concentrations decreased from Group-2. The TP, Albumin and HDL concentrations increased from group-2, ALT activities were significant ($P<0.05$), compared to group-1 while T-Cho and HDL were non-significant, compared to group-1. Results reviewed the protective effect of Nescafe on liver injury by CCl_4.

Key Words: CCL_4 intoxication, liver injury, lipid profile, liver marker enzyme and liver function test.

Introduction

Liver plays crucial role in regulating various physiological and biochemical processes in the body that are central to life sustaining activities.[1] Following its involvements in vital functions, with great capacity in metabolism of macromolecules (protein, lipid, glucose and drug metabolism), storage, secretion, detoxification and excretion of xenobiotics.[2-6] and contributions in regulating the innate immune system, it is open to attack by hepatotoxic agents, with grave consequences.[7] Additionally, the liver is often abused by environmental toxins, which are eating habits, alcohol and overdose of certain drugs which can damage and weaken the liver and eventually lead to many diseases. Navarro and Senior,[8] Russmann et al.,[9] and Ozcelik et al.,[10] have reported at various times, different mechanisms of hepatotoxicity and its importance in medical research. The maintenance of a healthy liver is vital to overall health of the human beings. Since the liver is involved in almost all biochemical processes and there are many different diseases that will affect it.

The main causes of liver injury are viral infection (hepatitis A, B, C, D, et c.), xenobiotics, excessive

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drug therapy, toxic chemicals, environmental pollutants, chronic alcohol ingestion, microbial infections of Entamoeba histolytica, indiscriminate use of herbs/herbal products, anoxic injury, autoimmune hepatitis, drugs and abuse.[11-15]

Liver diseases are fatal and leading cause of illness and deaths worldwide.[16] Various researchers have exerted that liver disorders cause about 18000 to 20000 deaths every year, globally.[17-18] There is alarming increase in liver damage in the world.[2,19] Liver diseases are, one of the world problems. Despite its pandemic, high morbidity, high mortality and enormous advances in modern medicine, there are no completely effective drugs that stimulate hepatic function, offer complete protection to the organ or aid in regenerating hepatic cells.[20] The medical management of liver disease is currently inadequate. There is presently no therapy that can successfully prevent the development of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often secondary medical effects.[21-22] Therefore, research for suitable drugs is essential.[23]

Carbon tetrachloride (CCL_4) is one of the most potent environmental contaminants.[24] and has been widely used for liver studies. Carbon tetrachloride is a routinely used hepatotoxin for experimental study of liver diseases.[25-26] The toxicity profile of CCl_4 is well established worldwide.[27-28] In humans, it simulates acute liver injury and is used as an experimental model to investigate hepatoprotective role of natural products and drugs.[29] It is one of the oldest widely used toxins for experimental induction of liver fibrosis in laboratory animals.[30] In hepatotoxicity studies, CCl_4 is widely used for experimental induction of liver damage.[31-33] CCl_4 induce hepatic damage via lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals.[34-37] Improving antioxidant level is crucial in rectifying and protecting liver health and nescafe is rich in antioxidant.[38]

As one of the most popular and widely consumed drinks, nescafe has long been a staple of breakfast. But, more recently, research has begun to reveal the benefits of nescafe which includes - protection against liver disease,[39-42] by neutralizing free radicals that can cause degradation to cells. Sequel to nescafe's positive health effects on liver, this systematic research was undertaken to evaluate the protective effect of nescafe against CCl_4-induced liver damage in Wistar rats.

Materials and Method

Nescafe collection and Identification: A tin of nescafe was bought from The Commandant market in Nigeria Police Academy, Wudil-Kano State on August, 2017 and were authenticated at the Department of Biochemistry and Forensic Science, Nigeria Police Academy.

Chemicals: All chemicals and reagents used were of analytical grade while the water was glass distilled.

Animal Maintenance: Thirty rats of wistar strain weighing between 60 -90 g of either sex, purchased from the central animal house of Bayero University Kano were used for the study. The rats were kept in polypropylene cages at 25 ± 3 °C, with relative humidity 45-55% under 12-hour light and dark cycles, in the Forensic Laboratory unit of Biochemistry and Forensic Science Department. All the rats were allowed for two weeks to acclimatize to the laboratory conditions before commencement of the research. They were provided with standard pelleted feed and water ad libitum. After acclimatization period, the rats were divided into groups.

Ethical statement: There are no potential sources of conflict of interest. This study was conducted in accordance with the Declaration of Helsinki and all animal protocols were in accordance with the guideline in Nigeria Police Academy, ethical committee. The experiments with animals were performed in accordance with the legislation for the protection of animals used for scientific purposes.

Animals Grouping: The thirty albino rats were randomly allocated to five groups of six rats each as follows:

Group-I: (Normal control) received only normal food and water.

Group-2: (CCl_4 treated, Positive control) received only normal food and water.

Group-3: (CCl_4 treated) received normal food, water and 30g/72kg body weight of Nescafe.

Group-4: (CCl_4 treated) received normal food, water and 40g/72kg body weight of Nescafe.

Group-5: (CCl_4 treated) received normal food, water

and 50g/72kg body weight of Nescafe.

Administration of Ccl_4

Rats of groups-2, 3, 4 and 5 were administered 2ml/Kg of body weight, in 3:1 v/v preparation of CCL_4 and olive oil. (15ml of CCL_4 was made to 20ml with 5ml of olive oil). The injection of CCL_4 and Olive mixture were by intraperitoneal route, once per week for one month (four-times in a month) to induce liver damage. Simultaneously once weekly for one month, rats of groups-3, 4 and 5 were fed with nescafe suspension, orally by gavage according to the required doses for one month. Rats of group-1 and 2 were given distilled water in a volume of 10 ml/kg body weight.

Estimation of serum Biochemical Markers

On the day, after the treatment period, all the rats were anaesthetized and blood was withdrawn from heart, using the heart puncture technique. The serum was separated by centrifugation at 3000 rpm for 15 min. This was subsequently analysed for various biochemical parameters viz: serum aspartate aminotransferase (AST),[43] serum alanine aminotransferase (ALT),[43] albumin,[44] alkaline phosphatase ALP,[45] total Protein,[46] total bilirubin,[47] total cholesterol,[48] high density lipoprotein cholesterol,[49] and triacylglycerol[50,51] with their respective methods. Low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol[52,53] were calculated using the formula. The globulin concentration, by the difference between total protein and albumin

Statistical Analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) using SPSS 16 for windows software package. Post hoc testing was performed for inter-group comparisons using the Least Significant Difference (LSD) test according to the method described by Zar.[54] In all instances p values < 0.05 were considered statistically significant.

Results

The result of effects of nescafe on selected liver function parameters, liver function enzymes and lipid profile are presented in table 1, 2 and 3.

In table 1, the positive control rats of group-2 (induced untreated) have the highest increase in serum activities of aspartate aminotransferase (462.75U/L), alanine aminotransferase (145.45U/L) and alkaline phosphatase (141.60U/L) as compared with corresponding serum enzyme activities of the same group. These increased in aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in group 2 were significantly different compared to group-1 of respective enzyme at $p<0.05$. Administration of Nescafe solution to the CCl_4-induced rats in group-3 (41.67 mg of Nescafe), group-4 (62.22 mg Nescafe) and group-5 (72.22 mg Nescafe) caused decrease in serum enzyme activities in these groups, as compared with group-2 (Table 1) of the corresponding enzyme. The decreased in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) from their corresponding group-2, follows a reverse order of dose dependent fashion.

The AST activities of group-4 and 5 were lower than that of group-1. The decreased in serum AST activities in Group-3, 4 and 5 were not significant from their corresponding group-1 ($P>0.05$). The changes in reverse dose dependent pattern in serum ALT activities in group-3 (90.33U/L), group-4 (73.00U/L) and group-5 (70.70U/L) were significantly different at $p <0.05$ from the group-1 (22.30U/L). The ALT activities in CCl_4-induced treated groups (Group-3, 4 and 5) decreased from CCl_4-induced untreated (Group-2). These decreased, are in dose dependent pattern with the group-3 (90.33U/L) and group-5 (70.70U/L) as highest and lowest activity respectively. The ALT activities in CCL_4 induced treated of group-3, 4 and 5 did not show any significant difference with the group-1 (22.30U/L) at $P>0.05$. The ALP activities in CCl_4-induced treated groups (Group-3, 4 and 5) decreased from CCl_4-induced untreated (Group-2). These decreased, is in dose dependent pattern with the group-3 (129.50U/L) and group-5 (100.50U/L) as highest and lowest activity respectively. The ALP activities in CCL_4 induced treated of group-3, 4 and 5 did show significant difference with the group-1 (91.70U/L) at $P<0.05$.

Table 2 presents the changes in concentrations of total protein, albumin, globulin, albumin -globulin ratio and total bilirubin. The results showed that total protein, albumin, globulin, albumin -globulin ratio and total bilirubin concentrations in normal

Table 1: Effect of nescafe on serum maker enzymes (U/I) in CCl4-induced liver damage rats

Group	Treatments	AST	ALT	ALP
Group-1	10ml (water)	60.60±10.10	22.30±6.89	91.70±8.25
Group-2	10ml (water)	462.75±5.25*	145.45±12.89*	141.60±14.72*
Group-3	41.67mg (NF)	70.70±8.90	90.33±9.02*	129.50±.9.0
Group-4	62.22mg (NF)	52.70±15.80	73.00±12.87*	124.83±4.81
Group-5	72.22mg (NF)	50.50±0.90	70.70±5.05*	100.50±6.90

Values bearing * indicate significant difference at $p<0.05$ when compared to normal untreated group
AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase
All values are shown as mean ± Std and n=6

Table 2: Effect of nescafe on serum protein and total bilirubin in CCl4-induced liver damage rats.

GPS	Treatments	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin -globulin ratio	Total Bilirubin (mg/dL)
GP1	10ml (water)	6.37±1.10	4.43±0.47	1.94±0.53	2.28	0.43±0.58
GP 2	10ml (water)	3.88±3.10*	1.76±0.87*	2.12±2.23*	0.83	0.73±0.10*
GP 3	41.67mg (NF)	4.94±0.96	2.93±0.8	2.01±0.08	1.46	0.69.±0.05*
GP 4	62.22mg (NF)	5.20±0.72	3.57±0.74	1.63±0.20	2.19	0.51±0.10
GP 5	72.22mg (NF)	6.19±2.40	4.40±0.50	1.79±1.21	2.46	0.40. ±0.12

Values bearing * indicate significant difference at $p<0.05$ when compared to normal untreated group
All values are shown as mean ± Std and n=6

Table 3: Effect of nescafe on serum lipid profile (mg/dl) in CCl4 -induced liver damage rats

Lipid profile	Group-1	Group-2	Group-3	Group-4	Group-5
T- Chol	120.00±20.00	165.80±4.89	160.80±7.59	150.50±9.95	120.40±7.59
TAG	130.00±6.08	187.55±2.04*	180.03±1.64*	166.13±2.64	152.13±2.64
HDL	24.67 ±0.58	19.25±1.07	22.94±1.37	24.59±2.33	25.68±1.37
LDL	83.73±20.46	141.79±5.38*	129.01±5.89*	118.48±8.00	86.69±5.69
VLD L	26.00±1.22	37.51±0.41*	36.00±0.33*	33.23±0.53	30.42±0.53

Values bearing * indicate significant difference at $p<0.05$ when compared to normal untreated group
T-Chol: Total cholesterol, TAG: Triacylglycerol, HDL: High Density lipoprotein Cholesterol, LDL: Low Density Lipoprotein Cholesterol and VLDL: Very Low-Density Lipoprotein Cholesterol
All values are shown as mean ± Std and n=6

control (group-1) rats were 6.37, 4.43, 1.94, 2.28 and 0.43 respectively.

In the CCL_4-induced untreated (group-2), there are decreased in total protein (3.88), albumin (1.76), globulin (2.12), albumin-globulin ratio (0.83) and total bilirubin concentrations (0.73) as compared with group-1 in the respective parameters. The group-2 was significantly different compared with group-1, in total protein, albumin, globulin and total bilirubin concentrations of the respective parameter at $p<0.05$. The changes in concentration of total protein after the CCl_4-induced treatment were 4.94 in group-3 (treated with 41.67 mg of nescafe suspension), 5.20 in group-4 (treated with 62.22 mg of nescafe suspension), in group-5 (treated with 72.22mg of nescafe suspension). The concentrations of total protein in CCl_4-induced treated (group-3, 4 and 5), increased from group-2 (3.88) in a dose dependent fashion, with group-5 (6.19) and group-3 (4.94), as the highest and lowest respectively. The group-5 protein concentration is in a close concentration level of group-1. The group-3 and 4 were of lower total protein concentration levels than group-1. The changes in total protein concentrations were not significantly different from the group-1at $p>0.05$ (Table 2). The concentrations of total albumin in CCl_4-induced treated (Table 2), increased from group-2 (1.76) in a dose dependent fashion, with group-5 (4.40) and group-3 (2.93), as the highest and lowest respectively. The group-5 albumin concentration is a close concentration level of group-1. The group-3 and 4 were of lower albumin concentration levels than group-1. The changes in albumin concentrations were not significantly different from the group-1at $p>0.05$ (Table 2). Still in table 2, the concentrations of globulin in CCl_4-induced treated, decreased from group-2 (2.12) in a dose dependent fashion, with group-5 (1.79) and group-3 (2.01), as the lowest and highest respectively. The changes in globulin concentrations of CCl_4-induced treated (group-3, 4 and 5) were of a lower concentration levels to group-1 globulin concentration. The changes in globulin concentrations were not significantly different from the group-1at $p>0.05$ (Table 2).

The Albumin -globulin ratio in table 2 in CCl_4-induced treated, increased from group-2 (0.83) in a dose dependent fashion, with group-5 (2.46) and group-3 (2.19), as the highest and lowest respectively. The changes in globulin concentrations of CCl_4-induced treated (group-3, 4 and 5) were of lower concentration levels to group-1 globulin concentration. The group-4 and 5 albumin-globulin ratio are a close level of group-1. From the total bilirubin concentration in table 2, the concentrations of total bilirubin in CCl_4-induced treated, decreased from group-2 (0.73) in a reverse dose dependent fashion, with group-5 (0.40) and group-3 (0.69), as the lowest and highest respectively. The changes in total bilirubin concentrations of CCl_4-induced treated of group-3 and 4 (0.51) were of higher concentration levels to group-1 total bilirubin concentration (0.43). The group-5 total bilirubin concentration is a close level of group-1.

The lipid profile as presented in table 3 shows the concentration of the total cholesterol (T-Chol), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low-density lipoprotein (VLDL). The T-Chol concentration increased in the CCL_4-induced untreated (165) in group-2 (positive control) as compared with normal untreated (120) in group-1 (normal control). The concentrations of total cholesterol of CCl_4-induced treated of group-3 (160.80), group-4 (150.50) and group-5 (120.40) decreased from the CCl_4-induced untreated in group-2 (positive control). The group-3 (treated with 41.67mg of Nescafe) and group-5 (treated with 72.22mg of Nescafe) has the lowest and highest concentration of T-Chol among the CCl_4-induced treated groups. These decreased in T-Chol concentration follow a reverse in dose dependent fashion. There was no significant difference in T-Chol concentration of all the groups as compared with the group-1 at $p>0.05$. The concentration of total cholesterol in group-5 was almost on the concentration level with the group-1.

The TAG concentration in the CCl_4-induced untreated in group 2 (187.55) increased from the group-1 (130.00). In the CCl_4-induced treated, the concentrations of TAG in group-3 (180.03), group-4 (166.13) and group-5 (152.13) decreased from the group-2. The changes in concentrations of TAG in the CCl_4- induced treated were in reverse dose dependent manner, with the group-5 (treated with 72.22mg of nescafe) and group-3 (treated with

41.67mg of nescafe) as lowest and highest respectively. Group-2, 4 and 5 were significantly different with the group-1 at $p< 0.05$ (Table 3).

The high-density lipoprotein cholesterol (HDL) concentration of CCl4-induced untreated in Group-2 (19.25) increased from the normal untreated in group-1 (24.67). In the CCl_4-induced treated, the HDL concentration in Group-3 (22.94), group-4 (24.59) and group-5 (25.68) increased from group-2. These increased changes in concentration of HDL were in increasing dose dependent manner, with group-5, treated with 72.22mg of nescafe and group-3, treated with 41.67mg of nescafe as highest and lowest respectively. There was no significant difference in all the groups as compared with the Group-1 at $p>0.05$.

The low-density lipoprotein cholesterol (LDL) concentration of CCl_4-induced untreated in Group-2 (141.79) increased from the normal untreated in group-1 (83.73). This increased in concentration of LDL in group-2 was significantly different from the group-1 at $p<0.05$. In the CCl_4-induced treated, the LDL concentrations in group-3 (129.01), group-4 (118.48) and group-5 (86.69) decreased from group-2 (positive control). These decreased changes in concentrations of LDL were in reverse dose dependent manner, with group-5, treated with 72.22mg of nescafe and group-3, treated with 41.67mg of nescafe as lowest and highest respectively. The decreased in concentration of LDL in group-3 were significantly different from group-1 at $p<0.05$.

The very low-density lipoprotein cholesterol (VLDL) concentration in the CCl_4-induced untreated in group-2 (37.51) decreased from the normal untreated of group-1 (26.00). In the CCl_4-induced treated, the concentrations of VLDL in group-3 (36.00), group-4 (33.23) and group-5 (30.42) decreased from the group-2. The decreased changes in concentrations of VLDL in the CCl_4-induced treated groups were in reverse dose dependent manner, with the group-5 (treated with 72.22mg of Nescafe) and group-3 (treated with 41.67mg of Nescafe) as lowest and highest respectively. The groups (2, 3 and 4) were significantly different with the group-1 (Normal untreated) at $p<-0.05$.

Discussion

In the present investigation, administration of CCl_4 to rats causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane.

This work validates the protective ability of nescafe solution on liver damage induced by CCl_4 and the effect on serum lipid profile of the same doses in rats. The damage of the liver caused by CCl_4 was evident by the alteration in serum marker enzymes concentration, beside disruption in protein and lipid metabolism.

The significant elevation in serum maker enzymes activities (aminotransferases and alkaline phosphatase), after the injection of CCl_4 in the rats as shown in table 1 of group-2, is an evidence that, it is injurious to liver/hepatotoxic. AST and ALT are ubiquitous in their cellular distribution and ALP is wider in cellular distribution, increased serum activities of this marker enzymes may be caused by wider variety of disorder involving multiple organs. The majority of sustained AST, ALT and ALP levels are associated with disorders of the liver among others. This opinion in agreement with other studies,[55,56] which declared that any harm in the liver can weaken its functions and cause numerous consequences on health as it is the chief site of intense metabolism, excretion and plays a defining task in excretion plus detoxification (of several exogenous beside endogenous components, and hepatic damage resulted from the distortion of these metabolic functions.

Because of the hepatotoxin used, in the present studies, the hepatocyte is of prime consideration in differential diagnosis as a source of the elevated marker enzymes. These enzymes, found abundantly in the hepatocyte carry out specific functions. Aminotransferases catalyse interconversion of amino acids and oxoacids by transfer of amino group in the liver. The ALP, a group of phosphomoesterase that hydrolyses phosphate esters. The linkage of these enzymes from the liver tissue into the blood will cause elevated activity of these enzymes. The elevated activities are indicative of hepatocyte damage (for ASP and ALP), canalicular membrane damage and obstruction of the liver (ALP) among others. Under normal situation, the activities of the enzymes are low in the

blood. The present finding of significant elevated serum marker enzyme activities indicated that CCl_4 has caused injuries to hepatocytes (Group-2 of table 1). It has demonstrated that injection of CCl_4 to rats induces severe liver damage which is detected due to the increase in the serum levels of AST, ALT, and ALP activities. Increased serum levels of ALT and AST in livers induced with CCl_4 toxicity[57] is an indication of damaged structural and functional integrity of the liver cell membranes since, these cytosolic enzymes are only released into circulation after hepatic cellular damage.[58] The increase in AST and ALT in serum reflects the degree of damage of hepatocytes and the extent of liver injury[59] Lee et al.,[60] reported similar elevation in AST and ALT activities after CCl_4 induced liver damage in animal models. A very high ALT among others is more likely caused by toxic liver injury.[59] In this study, CCl_4-induced liver injury model in rats were successfully established, as shown by the elevated serum ALT AST and ALP levels after CCl_4 treatment (Table 1 in group-2). This has been previous reported.[61]

The potential of Nescafe to protect against chemically induced liver injury was investigated in CCL_4-induced treated groups. CCl_4, metabolized in the liver to toxic-free radical CCl_3, has effect on cellular permeability of hepatocytes that led to elevated levels of serum marker enzymes as mentioned previously. In the CCl_4-induced treated with various doses of Nescafe, the marker enzymes (AST, ALT and ALP) activities were very noticeably reduced (group-3, 4 and 5 of table 1), showing liver quality has improved dramatically after treatment. This marker enzymes are of high concentrated in the liver and are sensitive indicators of hepatocyte damage. Damage to liver cells changes their functional transition, causes membrane permeability, and leads to the leakage of enzymes into extracellular space.[62,63] The prevention of this phenomenon can be considered as hepatoprotective activity and this was observed in the CCl_4-induced treated rats as there were decreased enzyme activities. Normalization of these enzymatic parameters after administration of different doses of Nescafe solution, represent the improvement of normal liver function.[64-66] The fact that no significant difference exists in enzyme activities of the treated and the normal untreated, shows that Nescafe at the doses administered may be efficient in attenuating the increase in enzyme activities caused by CCl_4-induced liver damage. Various authors have reported similar restorations of liver health through decreased enzyme activities among others.[4,67] The most effective dose in the present study was 72.22mg of Nescafe (table 1). This result indicates that Nescafe has the ability to protect against CCl_4-induced hepatocyte injury, which is in agreement with a previous study by Eidi et al.[68] This positive liver health in the treated groups might be due to Nescafe's impressive antioxidant content. Nescafe contains hydrocinnamic acids, polyphenols among others.[38] In a similar result Rajnarayana et al,[69] and Rage et al,[70] had reported, the hepatoprotective role of A. catechu as due to the presence of antioxidant (tannins, cyanidanol, and quercetin).

Liver is the main site of protein synthesis, especially albumin.[71] and bilirubin excretion. Albumin levels decrease in liver diseases; hence serum albumin level is useful test for monitoring liver synthetic activity and it has prognostic meaning in liver disease. A change in serum albumin level may be associated with a decrease in liver functioning mass, although it is not specific for liver disease.[72] Decreased production of albumin is a rare cause of hypoalbuminemia. Significant and severe chronic hepatic impairment is required before a noticeable decrease in plasma albumin concentration is manifested. Serum albumin is a marker of synthetic function of the liver and is a valuable guide to the severity of chronic disease. Sequel to this, serum total protein, albumin, globulin, and total bilirubin were investigated to further ascertain the protective and restorative properties of Nescafe on synthetic and excretory functions of liver, on CCl_4–induced deteriorating liver health. Hepatotoxicity impairs the synthetic and excretory functions of the liver. Albumin, accounting for approximately 55% of plasma protein, is a dominant plasma protein that functions as nonspecific transporter of many molecules, contributes to oncotic pressure of plasma, buffer of hydrogen among others is believed to be solely synthesized by the liver. Serum total protein and albumin decrease in hepatic diseases associate with destruction or loss of parenchymal elements.[73] Chey,[74] illustrated the important of hepatic parenchymal cell integrity, in the synthesis of serum albumin. Although low levels

of serum albumin concentration are frequently multifactorial, with more than one mechanism being responsible but a falling concentration of serum total protein, globulin alongside serum albumin, as seen in the table 2, group-2, of the present research suggests clinically significant deterioration in liver disease. The bilirubin normally is conjugated in the liver with glucuronide which is excreted in bile and exhibits a protective effect against the oxidative stress induced by CCl_4 or other toxicants.

However, in case of liver damage or impaired hepatic clearance, the conjugated bilirubin is inactivated and hence increase the oxidative stress.[75] This liver dysfunction reflected in poor synthesis of protein and weak excretion of bilirubin after treatment with CCl_4. This significant reduction in serum globulin, total protein and concomitant elevation of total bilirubin concentrations in table 2 are due to CCl_4 toxicity and the lower albumin globulin ratio (table 2), indicates, not over production of globulin but under production of albumin, in liver impairment despite good nutrition. Diminution of total protein and albumin induced by CCl_4 is a further indication of liver damage.[8] The Nescafe treated CCl_4-induced groups, showed remarkable increase serum total protein, albumin and globulin concentration. Remarkably, there was no significant difference in CCl_4-induced treated group as compared with the normal untreated group. Interestingly, this might indicate that Nescafe restored liver function. Adeneye et al.,[76] reported on effectively protection of animals against CCl_4-induced hepatic destruction, by increased in total protein, albumin and globulin concentration but decreased bilirubin concentration. The finding of hypoalbuminemia and no other alterations in liver tests virtually rules out the hepatic origin of this abnormality. But the current research has alteration in other function tests. The results of the present study are comparable with studies conducted elsewhere.[77] The decreased in bilirubin may be due to improved excretion from the liver. Liver protection is also associated with control of protein and gene expression. Gao et al.,[78] works on essential oils of Artemisia capillaries is consistent with protective potential of nescafe on liver serum protein, albumin and bilirubin profile.

Furthermore, lipid profile was investigated on CCl_4-induced liver damage. The liver plays a crucial role in the synthesis, secretion, catabolism, and storage of lipids and lipoproteins. Therefore, the serum lipids and lipoproteins concentrations in liver diseases could change.[79-82] Carbon tetrachloride poisoning can damage the endoplasmic reticulum, resulting in a significant reduction in protein synthesis (Table 2), while the TG generated in liver tissue is excreted in the form of very low-density lipoprotein (VLDL)-TAG. The administration of CCl_4 to rats led to a significant increase in the TAG, and LDL cholesterol level, VLDL cholesterol and decrease in HDL cholesterol level in respect to normal control (Table 3). The injection of CCl_4 also increase non significantly total cholesterol (Group-2, in Table 3). This is not unconnected with a derangement in lipid metabolism which forms the bedrock for the altered membrane functions and integrity. The decrease in protein synthesis as observed in the present research might be involved in abnormal lipoprotein levels. Increase in the cholesterol levels might be due to the increased esterification of fatty acids, inhibition of fatty acid β-oxidation, and decreased excretion of cellular lipids.[83] CCl_4 stimulates the transfer of acetate into liver cells (probably by increasing access to acetate) and leads to an increase in cholesterol synthesis. It also increases the synthesis of fatty acids and triglyceride from acetate and enhances lipid esterification.[84] Again, when apolipoprotein synthesis is insufficient, VLDL is insufficient to transport TAG, as a result, T-Chol will also accumulate in the liver.[85]

Interestingly the Administration of Nescafe to CCl_4-induced treated groups modulated lipid profiles. Treatment with Nescafe, decreased cholesterol, triglyceride, and LDL levels and increased HDL level. This may be attributed to phenolic among other contents in Nescafe, although, this was not investigated. Among the antioxidant compounds, phenolic compounds are the strongest in inhibiting lipid peroxidation.[86] By increasing HDL cholesterol production and decreasing LDL cholesterol production, it facilitates the breakdown of acetate by liver. The findings of this study were consistent with the findings of other studies.[86-88] VLDL and LDL are sometimes called "bad" cholesterols because they can contribute to the build-up of plaque in the arteries. Low HDL cholesterol seen in

group-2 (Table 3), in unhealthy liver is an indication of poor prognosis, increasing the risk of cirrhotic death.[89] Whereas larger sizes of HDL particles are thought to be atheroprotective.[90-92] In this respect, an increase in total cholesterol concentration and specifically LDL cholesterol, is an atherogenic lipid marker, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome and probably involves independent risk.[93] The decrease in concentrations T-Chol, TAG, LDL, VLDL and increased HDL in the different doses of nescafe treated groups showed that the nescafe could alleviate CCl_4-induced liver injury. Our study demonstrated a strong association between T-Chol, TAG, lipoprotein profiles and liver health. This association was more prominent between the CCl_4-induced liver damage untreated and CCl_4-induced treated with nescafe as the serum levels of total cholesterol, TG, LDL-C, and VLDL-C showed remarkable increases in CCl_4-treated rats. Jurgoński, et al.,[94] and Palíková, et al.,[95] reported on the ameliorated abnormal lipid and glucose metabolism in rats and exhibited hepatoprotective by blue honeysuckle, High serum levels of cholesterol are associated with testicular dysfunction96 and decreased sperm quality.[97] Although, this was not investigated.

Conclusion

The explanations of coffee's apparent beneficial effects have been greatly debated. With nescafe growing in popularity its documented health benefits are also growing. With the benefits of coffee consumption ranging from liver enzyme laboratory test improvement to improved mortality from cirrhosis. The biochemical results in this present research demonstrated that nescafe protected the liver against CCl_4-induced hepatotoxicity in rats, through liver function and lipid parameters amelioration properties. The findings indicated that the protective effect resulted due to decreased in the serum level of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin and increased in serum total protein, albumin in the CCl_4 treated groups. These results are indicators of hepatocyte dysfunction, cellular leakage and loss of functional integrity of the cell membrane in the liver, as previously reported.[98] There was also improvement in serum lipid profile. All these led to restoration of structural integrity of hepatocyte cell membrane, and regeneration of damaged liver cells. Further studies are recommended on the histopathological studies of the liver.

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