International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Effect of Methanol Root Extract of *Senna occidentalis* against Carbon Tetrachloride-Induced Hepatic Damage in Rats

Kwalle, Joseph John[1], Bello, M.I [2]

[1]Government Day secondary school Vimtim, Mubi-North local government, Adamawa state, Nigeria

[2]Department of Biochemistry, School of Pure and Applied Sciences, Modibbo Adama University of Technology, P.M.B. 2076 Yola, Adamawa State, Nigeria

Abstract: ***Protective effect of methanol root extract of Senna occidentalis was investigated against carbon tetrachloride (CCl_4) induced hepatic damage in white Wistar strain albino rats. Hepatic damage was induced by injecting a single intraperitoneal dose of 1.0 ml (50% volume/volume) CCl_4 in olive oil. Plant extract (100, 200 and 300 mg/kg body weight) was given for seven days. Hepatic damage was induced and the animals were scarified after 48 hours. The hepatoprotective effect of the extract was monitored by estimating the activities of the serum AST (Aspartate Aminotransferase), serum ALT (Alanine Amino Transferase), serum ALP (Alkaline Phosphatase), Total proteins, unconjugated bilirubin and conjugated bilirubin. This observation could be supported by some Phytochemicals detected in the methanol root extract. Results showed that methanol extract of Senna occidentalis possessed hepatoprotective activity by significantly ($p<0.05$) reversing the damage of the liver indices in the treated experimental animals compared with the CCL_4 treated group. Some of the Phytochemicals component detected in the methanol extract of this plant indicated the presence of Flavonoids (0.8 ± 0.11), Phenols (12.0 ± 0.21), Tannins (5.0 ± 0.26), Saponins (21.5 ± 0.71). Terpenoids (0.28 ± 0.04) and Alkaloids (4.8 ± 0.21).***

Keywords: *Senna occidentalis, in-vitro* antioxidant, hepatoprotective, Phytochemicals screening

1. Introduction

High rate of liver disease has been causing death among the adult population globally today (Kuppuswamy *et al.,* 2003) because of the absence of reliable drugs for the treatment and prevention of liver diseases in modern medicine (Etuk *et al.,* 2009). Traditional medicine is considered as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (WHO report on Traditional medicine in some Asian and African countries 2011). Natural medicinal products are increasingly gaining popularity and used worldwide as complementary alternative therapies (WHO Fifth-Six World Health Assembly report on traditional Medicine; 2003), based on the fact that the raw materials are available naturally and in abundance with an estimated record of 1062–63 potentially beneficial substances. Among such therapeutic preparations are plant-derived phytomedicines, nutraceuticals and cosmeceuticals (Drew, 2000). The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990). It has been shown that $CC1_4$ induced lipid peroxidation can be obstructed by natural antioxidants (Subramanian *et al*., 1999). The identification of naturally occurring inhibitors of peroxidation resulting in cell damage could therefore lead to important new strategies for disease prevention (Subramanian *et al*., 1999). A number of plants have been shown to possess hepatoprotective and hepatocurative effect e.g *Carthamus oxyacantha* (Bukhsh *et al.,* 2012), but there is still lack of scientific proofs to authenticate the hepatoprotective and hepatocurative properties of some plants which are used traditionally to treat liver disorders. An example of such plant is *Senna occidentalis*, which has been used for about hundred years by Fali and Gude people from Mubi in Adamawa state of northern Nigeria as a possible remedy to liver problem. *Senna occidentalis* (cassia occidentalis, L.) is a medium-size flowering shrub belonging to the family caesalpiniaceae, *Senna* is an ancient Arabic name for these plants and *occidentalis* is a Latin word means western, and refer to the origin of this plant (Delachiave and De pincho, 2003). Other local names include mama tasba (fulfulde), Negro coffee, stink weed, rere (Yoruba),akedeagbora and ikpammuo (Ibo) (Bhar *et al.,* 1990). It is known as "coffee *Senna*", since its seed are brewed into a coffee-like beverage for asthma and its flower infusion is used for bronchitis in the Peruvian Amazon and its leaf extract exhibit broad-spectrum antibacterial (Sam and Ignacimuthu, 2000), antifungal activities (Jain *et al*., 1998). While the leave powder and extracts have proved to be in the control of large variety of insects (Dwivedi and Kumar 1998). It has also been used to reduce the number of mosquitoes indoors (Palsson and Janenson 1999).

2. Material and Methods

Collection of plants materials

Root of Fresh matured *Senna occidentalis* plant was collected from Girei local government area of Adamawa state in the month of October 2014. The plant material was identified by the Botany Department of Modibbo Adama University of Technology Yola.

Experimental animals

Sixty (60) male Wistar strain rats weighing 100-150 g were procured from National Veterinary Research Instituted VOM, Jos plateau state. They rats were housed in plastic cages (5 per cage) in a controlled environment. They were allowed to acclimatize for 2 weeks to obtain a weight gain between 180-200 g. During this period they rats were fed on standard rats-chaw diets ad libitum. The study was carried out based on the guidelines for the use and care for laboratory animals.

Preparation of crude extracts

Root of matured *Senna occidentalis* plant was shed dried under room temperature and made into powder using mortar and pestle. The extract was obtained from the powder (150g) by maceration in 80% methanol for 2 days at 37°C (Abdul *et al*., 2009). The extract was filtered and the excess methanol was evaporated under a reduced pressure in a rotary evaporator at < 50 °c. The dried extract was put in a clean sterile container for further use.

Experimental design

Animals were randomly divided into six (6) groups with five rats per group; group 1 received distilled water only as normal/control group. Hepatic injury was induced in the five remaining groups through injecting a single intraperitoneal dose of 1.0 ml/kg body weight (50% v/v) CCl_4 in olive oil for 48 hours. (Shi *et al.,* 2012), the pre-treatment with extract lasted for 7 days before hepatic damage to group III, IV, V and group VI was treated with sylmarin drugs before inducing hepatic damage.

Biochemical analysis

At the end of the experiment, all animals were anaesthetized with diethyl ether vapor. Blood samples were collected by cardiac puncture into a plain tube. The blood samples were allowed to clot. The clotted blood samples were centrifuged in a bench top centrifuge (3000 rpm for10 min) to obtain serum. This separated serum was used for the following biochemical analyses: Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphotase (ALP), conjugated bilirubin, total bilirubin, albumin and total protein. These tests were assayed using commercially available kits following the manufactures instruction.

Statistical analysis

All data was expressed as mean ± S.E.M (standard error mean). Statistical differences between groups was assessed using the SPSS software, version 20 and Results was statistically analyzed by Student"s„t" test for significant difference between groups.

3. Result

Table 1 and 2 showed the qualitative and quantitative Phytochemicals constituents detected in the methanol root extract of *Senna occidentalis* respectively. Where Saponins 21.5 ± 0.71 mg/g, Tannins, Alkaloids, Phenols, Flavonoids, Glycosides, Terpenoids and Steroids were present in the extract but Anthraquinones was absent.

Table 3 also showed the effect of the methanol extract of *Senna occidentalis* root on serum levels of AST, ALT and ALP in rats. Hepatotoxicity was evidenced by a significant increase ($p < 0.05$) in the serum level of AST, ALT and ALP in the group treated with only CCl_4 when compared with control. Simultaneous administration of the extract with CCl_4 significantly reduced ($p < 0.05$) the level of these enzymes in serum when compared with the group treated with CCl_4 only.

Administration of CCl_4 alone to rats caused a significant increase ($p < 0.05$) in serum level of conjugated bilirubin and unconjugated bilirubin in Table 4 when compared with controls. Simultaneous administration of 100, 200 and 300 mg/kg body weight methanol root extract with CCl_4 to rats significantly reduced ($p < 0.05$) the level of unconjugated bilirubin in serum to the range of the control value. A significant decrease ($p < 0.05$) in concentration of albumin in serum of rats treated with CCl_4 only when compared with control was observed (Table 4). The change effected by the administration of CCl_4 only was significantly reversed ($p < 0.05$) by the simultaneous administration of CCl_4 with 100, 200 and 300 mg/kg body weight of methanol root extract. Treatment of rats with CCl_4 alone significantly reduced ($p < 0.05$) the concentration of total protein in both the liver and the serum when compared with control (Table 4). Simultaneous administration of rats with CCl4 with 100, 200 and 300 mg/kg body weight of methanol root extract significantly reversed the change ($p < 0.05$).

Table 4.1: Qualitative Phytochemicals composition of *Senna occidentalis* root extract.

Phytochemicals	Inference
Saponins	+
Tannins	+
Alkaloids	+
Steroids	+
Phenols	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Athraqiunones	–

Key: + = Presence of Phytochemicals
- = Absence of Phytochemicals.

Table 2 Quantitative Estimation of Phytoconstituents of *Senna occidentalis* root methanol extract (g/100g)

Phytochemicals	Percentage composition
Saponins	6.0 ± 0.27
Flavonoids	3.5 ± 0.26
Terpenoids	0.4 ± 0.06
Alkaloids	10 ± 0.68
Tannins	7.8 ± 0.38
Phenols	9.9 ± 0.12
Values are expressed as mean ± SEM: n=3	

Table 3: Effect of Methanol Root Extract of *Senna occidentalis* on CCL_4 Induced Hepatic Damage in Rats

Treatment	AST (IU)	ALT (IU)	ALP (IU)
Normal control	16.91 ± 1.45	21.31 ± 3.02	60.77 ± 4.21
CCL_4(1.5 ml/kg) control	210.44 ± 11.61[ab]	134.00 ± 10.04[ab]	213.07 ± 12.41[ab]
100mg/kg bwt + CCL_4	179.11 ± 7.15[c b]	120.25 ± 3.14[c]	199.154 ± 9.16[c]
200mg/kg bwt + CCL_4	78.45 ± 5.59[c b]	94.44 ± 6.78[c]	132.74 ± 5.81[c]
300mg/kg bwt + CCL_4	33.74 ± 4.30[c]	32.78 ± 3.09[c]	97.87 ± 5.92[c]
Sylmarin bwt +CCL_4	18.28 ± 2.86[c]	28.2 ± 1.03[c]	82.97 ± 6.07[c]

Values are expressed as mean ± SEM: n=5
[a] significantly higher than normal control $p<0.05$
[c] Significantly lower than CCL_4 control
[b] Significantly higher than the sylmarin control

Table 4: Effect of Methanol Root Extract of *Senna occidentalis* on CCL_4 Induced Hepatic Damage in Rats

Treatment	TB (mg/dl)	CB(mg/dl)	TP (g/L)	ALB (g/l)
Normal control	0.89 ± 0.06	0.28 ± 0.10	43.15 ± 3.71*	39.36 ± 2.42*
CCL_4 (1.5 ml/kg bwt) control	3.72 ± 0.14[ab]	2.58 ± 0.54[ab]	30.25 ± 0.42	21.34 ± 0.65
100mg/kg bwt + CCL_4	1.63 ± 0.21[c b]	0.94 ± 0.03[c b]	32.47 ± 0.67	32.42 ± 0.68
200mg/kg bwt + CCL_4	1.15 ± 0.26[c b]	0.54 ± 0.084[c b]	39.30 ± 0.17*	36.34 ± 1.87*
300mg/kg bwt + CCL_4	0.90 ± 0.015[c]	0.41 ± 0.07[c b]	41.31 ± 0.87*	38.34 ± 0.44*
Sylmarin +CCL_4 bwt	0.72 ± 0.04[c]	0.15 ± 0.014[c]	44.63 ± 0.23*	40.63 ± 0.42*

Values are expressed as mean ± SEM: n=5
Keys: [a] significantly higher than normal control $p<0.05$
[c] Significantly lower than CCL_4 control
*Significantly higher than CCL_4
[b] Significantly higher than the sylmarin control

4. Discussion

The activity of AST, ALT and ALP are normally found in the cytoplasm (Mohammed *et al.,* 2009), which are released into the blood circulation after cellular damage (Sallie *et al.,* 1991). In this research, treatment of animals with single dose of CCl_4 caused significant ($P < 0.05$) elevation in the levels of serum ALT, AST, ALP, unconjugated bilirubin and conjugated bilirubin as well as significant decreased in level of serum total protein and albumin compared to the normal control. This current study which showed a similar rise in the levels of ALT, AST, ALP and bilirubin after injecting rats subcutaneously with CCl_4 to induce liver injury as early reported by Bahar *et al.* (2003) a marked elevation in the serum levels of ALT, AST and ALP in CCl_4 treated animals compared to that of the normal control animals. this findings also showed a significant elevation in serum values of ALT and AST in rats exposed to a single toxic non-fatal dose of CCl_4 as early reported by El-Dosuky *et al*., (1982), Anupam *et al*. (1995) and all this changes is due to free radicals which been reported as the predominant mechanism of hepatotoxicity (Gregus and Klaassen, 1995). The decreased in serum total protein and albumin serum level may be due to the interaction of CCl_4 with protein molecules leading to an impairment of cellular processes. The same observation was earlier suggested by Chung *et al*., 201).The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990).

The liver cells damage was evidenced by the significant increase ($p < 0.05$) in the level of unconjugated bilirubin in the serum of the group treated with only CCl_4 when compared with the normal control group. Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced (Dennis and Mark 1996), this result from bilirubin being the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile (Van *et al.,* 2012).

Subsequently the normalization of these enzymes suggests that *Senna occidentalis* extract root methanol was capable of regeneration parenchymal cells, thus protecting against membrane fragility consequently and minimizing the leakage of liver enzymes into blood circulation. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Anusha *et al.,* 2011). It is therefore, a clear manifestation of hepatoprotective effect of the extracts which may be due antioxidant activity of phenolic and Flavonoids present in extracts (Stankovic *et al*., 2014).

Significant ($p<0.05$) decrease in conjugated bilirubin and unconjugated bilirubin levels in animals treated with 200 and 300 mg/kg body weight compared to CCL_4 control group was observed and this reduction of the conjugated and unconjugated bilirubin levels by the methanol root extract suggests that the extract might have activated the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver (Saini *et al*., 2004). The primary function of CAR in the bilirubin clearance pathway is to direct a coordinate response to elevated levels of bilirubin by increasing the hepatic expression of each component of the pathway (Saini *et al*., 2004).

They results also showed significant increase ($p<0.05$) in serum albumin and total protein followed the same trend; it thus implicates the same mechanism by which the extract exerts its effect on these liver indices. The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins such as albumin in the liver. Administration of CCl_4 to rats already treated with 200 and 300 mg/kg body weight methanol root extract which significantly reversed ($p < 0.05$) these changes might have interfered with protein synthesis. This observation suggested that hepatoprotective activity of *Senna occidentalis* root extract possessed or exhibited protective activity against liver injury cause by CCL_4 administration. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells (Hayato and Shin 2012).

It has been shown that $CC1_4$ induced lipid peroxidation can be obstructed by natural antioxidants (Subramanian *et al*., 1999). In the present study *Senna occidentalis* methanol root extract possessed *In vivo* hepatoprotective activity as suggested by the observation so far. The hepatoprotective activity of *Senna occidentalis* methanol root extract may be due to its various bioactive compounds contained in the extract.

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