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Full Length Research Paper

Hepatoprotective and antioxidant effect of *Sphenocentrum jollyanum* (Menispermaceae) stem bark extract against CCl_4- induced oxidative stress in rats

O. S. Olorunnisola[1], Akintola A. O.[2] and A. J. Afolayan[1]*

[1]School of Biological Sciences, University of Fort Hare, Alice 5700, South Africa.
[2]Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria.

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Methanolic stems bark extract of *Sphenocentrum jollyanum* (SBSJ) was evaluated for hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4) - induced hepatic damage in rats. The extract (50, 100 and 200 mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl_4 (30%, 1.0 ml/kg). *In vitro* antioxidant potential of the extract was evaluated by using superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) radical scavenging assay. At a concentration of 50, 100 and 200 mg/kg, the extract showed a remarkable hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4)-induced liver injury in a concentration dependant manner. This was evident from significant reduction ($P< 0.05$) in serum marker enzymes, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin and lipid peroxidation. It was also observed that the extract of *S. jollyanum* stem bark conferred a significant ($P < 0.05$) protection against CCl_4 – induced total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST) depletion in the liver. The plant extract restored the activities of the marker enzymes to near normal. *In vitro* antioxidant assay showed that SBSJ inhibit superoxide anions and hydrogen peroxide radicals with IC_{50} values of 13.11 and 30.04 µg/ml, respectively. The present study indicated that methanolic extract of *S. jollyanum* possessed hepatoprotective activity against CCl_4- induced liver damage and this effect may be due to its strong antioxidant property.

Key words: Antioxidant, *Spenocentrum jollyanum,* carbon tetrachloride (CCl_4), hepatoprotective.

INTRODUCTION

The liver is one of the most important organs, controlling critical biochemical and physiological activities such as homeostasis, growth, energy provision, reproduction, detoxification, fight against infections and nutrient supply in the body (Palanivel et al., 2008). Hence, its malfunction or damage is usually associated with serious consequences which sometimes lead to terminal illness. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise (Sam et al., 2008). Presently, a few hepatoprotective drugs are available for the treatment of liver disorders. Hence, the search for a hepatoprotective drug becomes imperative. Although the aetiologies of the liver damage are many, free radicals play a major role for the pathophysiological processes. Excessive free radicals generation have been implicated in the pathology of a variety of diseases and degenerative processes such as aging, immunodeficiencies, neurological disorders, and carcinogenesis. They exert their damaging effects by various mechanisms such as causing DNA damage, altering cell-signalling pathways and modulating gene expression (Soobrattee et al., 2006). Carbon tetrachloride (CCl_4) is frequently used in experimental model of hepatic

*Corresponding author. E-mail: aafolayan@ufh.ac.za. Fax: +27866282295.

damage (Muthu et al., 2008). Transient tissue disorders after the administration of CCl_4 is believed to be induced by the trichloromethyl radical (CCl_3). This free radical induces an adverse reaction by forming other free radicals after its administration in the early stage between intracellular uptake and transformation into storage types (Showkat et al., 2010). Many biological substances such as membrane lipids, proteins, and nucleic acids are known to be injured by trichloromethyl radicals (Showkat et al., 2010). The initial event of CCl_4-induced free radical generation is a carbon-halogen bond cleavage, probably through one electron reduction of CCl_4 aided by a particular cytochrome P-450. Chloride ion and the trichloromethyl radical, $\cdot CCl_3$, are the major initial products. $\cdot CCl_3$ is converted into $\cdot CCl_3O_2$ through its reaction with molecular oxygen. Like the aforementioned free radicals, lipid peroxidation is initiated by the interaction of this reactive free radical, CCl_3O_2, with polyunsaturated fatty acids (PUFA) of the membrane lipids (Showkat et al., 2010). Antioxidants can inhibit or delay the initiation or propagation of oxidative chain reaction and thus prevent or repair cell damage caused by reactive oxygen. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are extensively used as antioxidants in order to reduce the damage caused by free radicals. However, the possible toxicity (Subhadradevi et al., 2010) as well as general consumer rejection led to decreasing use of these synthetic antioxidants. In recent years, great interest in finding natural antioxidants from plant materials has drawn more attention. Plant material and products thereof are rich sources of a variety of biologically active compounds such as antioxidant and radical scavenging activities. *Sphenocntrum jollyanum* Pierre (family Menispermaceae) popularly known as Aduro kokoo (red medicine) and Okramankote (dog's penis) in the Akan language of Ghana is a small erect sparsely branched rub which grows up to 1.5 m in height. Different part of the plant has been used extensively for the treatment of various ailments in West Africa Sub-region. Extracts from the root have been used for the relief of constipation, as stomachic as a cough medicine for treatment of sickle cell disease, rheumatism, aphrodisiac and other inflammatory conditions (Iwu, 1993; Moody et al., 2006). Decoction prepared from fruits, together with the fruits of *Piper guineense* and lime juice, is used for relief of cough. The plant is reputed to possess exceptional wound healing properties (Raji et al., 2006). It is also perceived to have unusual hemotatic and stomachic properties as well as an emetic for poisoning by traditional medical practitioners in Ivory Coast (Abbiw, 1990). Some scientific research has been done on this plant in relation to it antiviral and anti-inflammatory activities (Moody et al., 2008) antivirus and anti-angiogenic property (Nia et al., 2004) and also Raji et al. (2006) have shown that methanolic extract of the root of *S. jollyanum* increased the testosterone levels in dose dependant manner and also reduced the count, mortality and viability of spermatozoa in albino rats. In view of the aforementioned medicinal properties of *S. jollyanum*, the present study was carried out to investigate the hepatoprotective and antioxidant potential of methanolic stem bark extracts of *S .jollyanum* carbon tetrachloride induced liver damage in rats

MATERIALS AND METHODS

Plant material

A fresh *S. jollyanum* stem bark was collected in November 2008 from University of Ibadan Botanical Garden and authenticated by Professor A. O. Adebisi of Department of Agricultural Science. A voucher specimen of the plant was deposited in the Faculty of Agricultural Sciences, University of Ibadan, Ibadan, Oyo State. Nigeria.

Plant extract

Dried and powder stem bark (500 g) of *S. jollyanum* was exhaustively macerated in 100% methanol (Nia et al., 2005) for 72 h and filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was concentrated under reduced pressure at 35 to 40ºC. 6.47% yield of extract obtained was stored in refrigerator (4°C) till it was needed.

Animals

Healthy adult male albino rats weighing 150 to 180 g were obtained from the Animal Facility Center, Ladoke –Akintola University of Technology, Ogbomosho, Oyo State Nigeria. The animals were kept in a well- ventilated room of 12 h light and 12 h darkness. All the animals were fed with standard rat's cubes from Pfizer Pharmaceutical Plc, Ikeja, Nigeria; while water was provided *ad libitum*. The principles of laboratory animal care were followed, while University Ethical Committee on Animals use approved the study design.

Experimental design

Rats were divided into five groups containing six rats each. The methanolic extract of SBSJ was administered by oral gavage at a concentration of 50, 100 and 200 mg/ml/kg day.

Group I served as control and received olive oil (vehicle) at 1.0 ml/kg-b wt intraperitoneal (ip). Group II served as negative control and received 30% CCl_4 suspended (in olive oil 1.0 ml/kg b wt ip) after every 72 h for 10 days (Shahjahan et al., 2004). Groups III, IV and V received extract of *S. jollyanum* stem bark (50, 100 and 200 mg/kg b wt/day) suspended in olive oil for 10 days and CCl_4 was given as in Group II rats.

At the end of the experimental period, the animals were sacrificed by ether anaesthesia. The liver from each animal was excised, rinsed in ice cold 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4°C (Oyedemi et al., 2010). The supernatant was collected and monitored for oxidative stress parameters such as glutathione (GSH) (Ellman, 1951), glutathione peroxidase (Mantha et al., 1993), catalase (CAT) (Aebieh, 1982), superoxide dismutase (SOD) (Sun et al., 1998), glutathione S-transferase (GST) (Habig et al., 1974) and lipid peroxidation (Yagi,

Table 1. Effect stems bark of *S. jollyanum* (SBSJ) extract on liver marker enzymes in the serum of control and experimental animals.

Parameter	Normal (Group 1) 1.0 ml/kg olive oil	CCl_4-induced (Group II)*	CCl_4+SBSJ (50mg/kg) (Group III)**	CCl_4+SBSJ (100 mg/kg) (Group IV)**	CCl_4+SBSJ (200 mg/kg) (Group v)**
AST (U/L)	41.32 ± 3.21	112.3 ± 5.23	78.20 ± 1.20	61.10 ± 7.21	55.41± 6.10
ALT (U/L)	54.21 ± 1.63	121.6 ± 3.33	90.2 ± 2.10	76.20 ± 3.76	69.3 ± 2.15
ALP (U/L)	51.30 ± 3.76	140.1 ± 4.54	102 .7 ± 1.11	92.13 ± 2.21	61.22± 6.23
TB (mg %)	0.89 ± 2.25	3.10 ± 2.40	2.12 ± 1.32	1.83 ± 7.89	1. 25 ± 3.95
TSP (mg %)	9.34 ± 8.10	5.20 ± 2.01	6.23 ± 2.01	7.12 ± 2.23	8.89 ± 9.23

Values represent the Mean ± SD; number of rats used in each group = 6; $P < 0.05$ compared with the Normal treated group, $P<0.05$ compared with CCl_4 treated group.

1984).

Blood samples collected were allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters such as phosphatase (ALP) (Kind and king, 1954) and total bilirubin (TB) (Mallay and Evelyn, 1937) and total serum protein (TSP) (Lowery et al., 1951).

In-vitro antioxidant activity

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Ruch et al. (1989). Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

H_2O_2 radical scavenging activity = {(Abs control – Abs sample)/ (Abs control)} × 100

where, Abs control is the absorbance of H_2O_2 radical + methanol; Abs sample is the absorbance of H_2O_2 radical + sample extract or standard.

Superoxide anion scavenging activity

The superoxide anion scavenging activity was done by the method described by Beissenhirtz et al. (2004) and it is based on the reduction of cytochrome *c*. One millilitre of the extract was mixed with 1 ml of the solution containing 0.07 units per ml of xanthine oxidase, xanthine (*c* = 100 µ mol/l), and cytochrome *c* (*c* = 50 µ mol/l). After incubation at 20°C for 3 min, the absorbance at 550 nm was determined.

Superoxide anion scavenging activities were calculated as follows:

Scavenging activity (%) = $(A_0 – A_1/A_0) \times 100$

where A_0 is absorbance of the control (without the sample), and A_1 is absorbance of the mixture containing the sample

Statistical analysis

The values were expressed as mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) and Student's 't' test was used for statistical significance between groups. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results (Table 1) of the assessment of hepatoprotective properties of stem bark *S. jollyanum* (SBSJ) extract in CCl_4-induced liver damage in rats showed that the extract significantly ($P < 0.05$) restored the elevated enzyme level (AST, ALT and ALP), total bilirubin and decrease total serum protein in dose dependent manner when compared with control. CCl_4 is one of the commonly used hepatotoxin in experimental study of liver diseases (Shenoy et al., 2001). The toxicity of CCl_4 is largely due to it active metabolite, and trichloromethyl radical ($^{\cdot}CCl_3$) (Shawkat et al., 2010). These activated radicals elicit lipid peroxidation by binding covalently to cell membrane and organelles (Mohammed et al., 2010). This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 (Wegwu et al., 2005) and this is evidenced by the elevated level of serum maker enzymes (AST, ALT and ALP) total bilirubin and decreased total serum protein (TSP) (Muthu et al., 2008). The reversal of increased serum enzymes in the extract treated group may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. 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 (Thabrew and Joice, 1987; Wolf, 1999). The decrease in TSP observed in CCl_4 treated rats (Table 1) may be associated with the decrease in the number of hepatocytes which in turn, may result into the decreased hepatic capacity to synthesize protein, however, the restoration of the level of TSP after the administration of SBSJ confirmed the hepatoprotective nature of *S. jollyanum.* In this study a significant elevated level of lipid peroxidation (LP) ($P< 0.05$) and a decline in the activities of intracellular antioxidant enzymes such as SOD, CAT, GPx, GST and level of GSH were observed in CCl_4 treated Group II when compared with the control group

Table 2. Effect of stem bark of *S. jollyanum* extract (SBSJ) on the antioxidant status of liver in the control and experimental rats.

Parameter	Normal (Group I) 1.0 ml/kg olive oil	CCl_4-induced (Group II)*	CCl_4+SBSJ (50 mg/kg) (Group III)**	CCl_4+SBSJ (100 mg/kg) (Group IV)**	CCl_4+SBSJ (200 mg/kg) (Group v)**
GSH	16.20 ± 0.26	11.03 ± 0.32	13.23 ± 0.43	15.39 ± 0.60	16.01 ± 1.48
LP	116.8 ± 9.05	202.2 ± 4.56	185.3 ± 9.21	149.3 ± 5.43	119 ± 2.16
GPx	18.09 ± 0.13	15.35 ± 0.12	16.05 ± 0.76	17.25 ± 0.98	17.98 ± 0.45
CAT	45.21± 0.09	37.34 ± 0.06	38.70 ± 0.03	40.21 ± 0.08	43.56 ± 0.01
SOD	3.52 ±1.02	1.98 ± 0.07	2.05 ± 0.08	2.98 ± 0.05	3.01 ± 0.08
GST	0.58 ± 0.05	0.12 ± 0.06	0.25 ± 0.08	0.39 ± 0.05	0.54 ± 0.02

Values represent the Mean ± SD; number of rats used in each group = 6; *P < 0.05 compared with the normal treated group. ** P<0.05 compared with CCl_4 treated group.

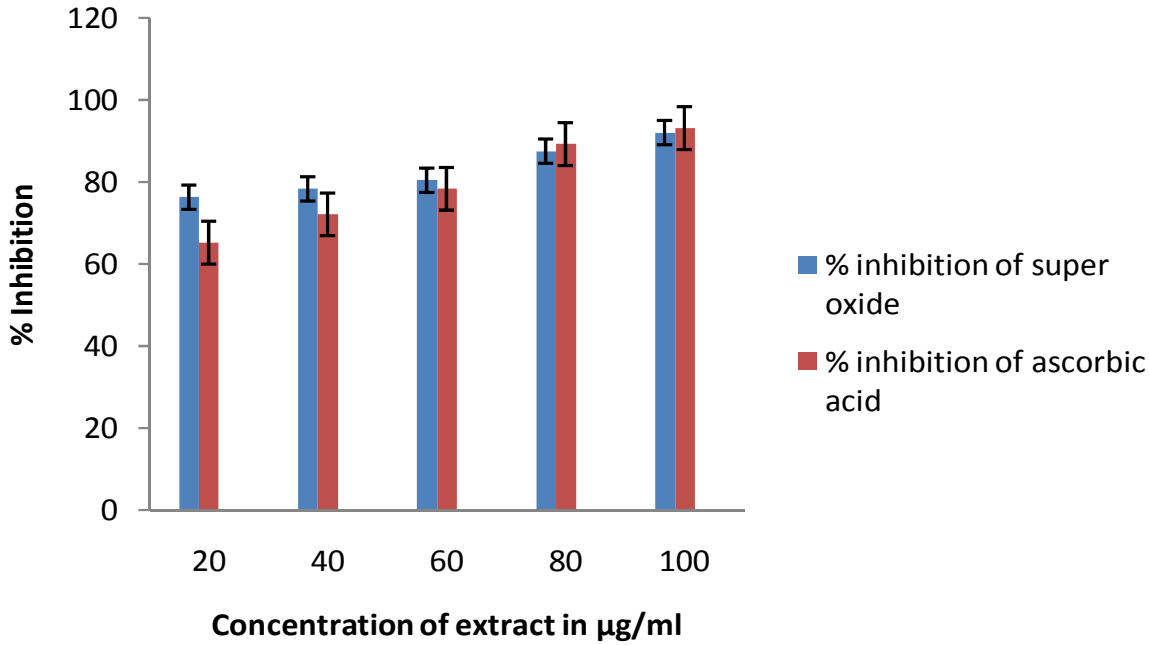

Figure 1. Superoxide scavenging activity of methanolic extract of SBSJ and ascorbic acid.

(Table 2). Similar observation has equally been reported by Showkat et al. (2010). The decreased activity of SOD in the liver tissues in CCl_4 treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes (Showkat et al., 2010). Treatment of *S. jollyanum* stem bark at the doses of 50, 100 and 200 mg/kg resulted in a significant increase of SOD, catalase, GPx and GST, GSH level and a reduction in LP when compared to CCl_4 treated rats. Interactions between the aforementioned antioxidant enzymes adequately protect the integrity of the liver cells. For example, superoxide dismutase (SOD) a sensitive index in hepatocellular damage ((Muthu et al., 2008) scavenge superoxide anion to form hydrogen peroxide (Muthu et al., 2008) and thus diminishing the toxic effect caused by this radical. Catalase (CAT) on the other hand decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Oyedemi et al., 2010) while GPx, glutathione and GST in a redox cycle protect the cell against hydrogen peroxide, superoxide radicals and maintains membrane protein thiols (Pedram et al., 2009). We might suggest that the plant extract decreased CCl_4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. Hence it is likely that the mechanism of hepatoprotection of *S. jollyanum* stem bark is due to its antioxidant effect. *In vitro* antioxidant activities of the extract reveal that it had a scavenging activity on the superoxide radicals and hydrogen peroxide in a dose dependent manner (Figures 1 and 2). When compared to ascorbic acid, the % superoxide and hydrogen peroxide

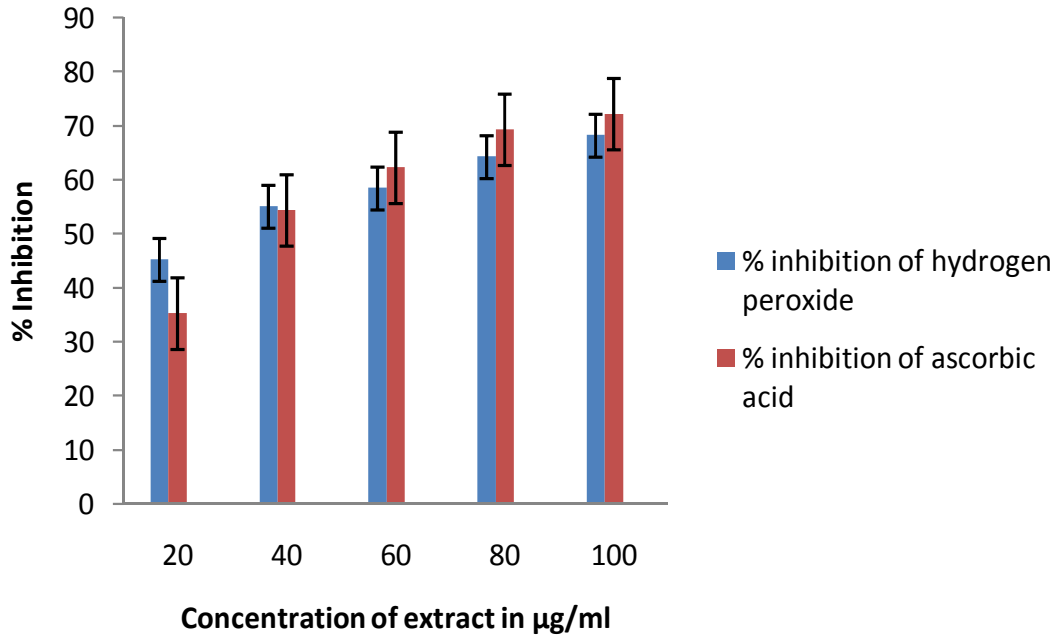

Figure 2. Hydrogen peroxide scavenging activity of methanolic extract SBSJ and ascorbic acid.

Table 3. Effect of methanolic extract of SBSJ on different radicals and ascorbic acid standard.

IC_{50} value of SBSJ			
Superoxide radical scavenging activity	**Ascorbic acid**	**Hydrogen peroxide radical scavenging activity**	**Ascorbic acid**
13.11	15.34	30.04	35.44

SBSJ: Stem bark of *S. Jollyanum* (Values in µg/ml).

scavenging activity of the extract was found to be higher at low concentrations (Table 3) but gradually decrease as the concentration increases with respect to the standard (Figures 1 and 2). Furthermore, the IC_{50} value of the extract on superoxide and hydrogen radical scavenging activity was found to be lower than the value obtained for ascorbic acid (Table 3) or *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L., a plant which has been reported to possess low IC_{50} (Abalaka et al., 2011). The low IC_{50} value of SBSJ indicates high free radical scavenging ability. The radical scavenging power of the extract could be due to the presence of reactive concentration of bioactive constituents and mixture of other nutrients in the extract. Further work need to be carried out to investigate the active compound(s) in the plant extract and the mechanism(s) of it hepatoprotective activity.

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