IN VIVO AND *INVITRO* ANTIOXIDANT PROPERTIES OF METHANOL EXTRACT OF *STREBLUS ASPER* LOUR

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Running Title

Invivo and invitro antioxidant properties of MESA

Summary

The *in vivo* and *in vitro* antioxidant properties of methanol extract of *Streblus asper* Lour (Family: Moraceae) (MESA) was evaluated. The *in vitro* antioxidant potential determined by performing various assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, lipid peroxidation inhibition assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay, and reducing ability. 3, 5-Di-tert-butyl-4-hydroxytoluene (BHT) was used as a standard. The IC_{50} values of MESA and BHT in DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, lipid peroxidation inhibition, hydroxyl radical scavenging, and nitric oxide scavenging assays were found to be 116.05, 110.07, 130.49, 143.99 µg/ml & 78.87, 89.97, 71.03, 103.77 µg/ml. In reducing ability assay, the Fe^{3+} to Fe^{2+} transformation was established as reducing capacity and the ability increased with increasing the concentration. The phenolic content of the sample was determined using Folin-Ciocalteu reagent and it was found to be 55.28±5.24 mg gallic acid equivalents (GA)/g dry weight. The total flavonoid concentrations, detected using 2% aluminum chloride, amount was 20.57±3.82 mg quercetin equivalents (QE)/g dry weight. In *in vivo* antioxidant, MESA (250 and 500 mg/kg) was administered four days prior to single dose of carbontetrachloride (CCl_4) administration and on the 7^{th} day antioxidant status was measured in the liver. The level of reduced glutathione (GSH) and catalase (CAT) significantly reduced in $CC1_4$ control animal, when compared to normal animal liver. MESA treatment significantly increased the GSH and CAT level. The thiobarbituric acid reactive substances

(TBARS) level significantly reduced in the extract treated groups, when compared to CCl_4 control group. Serum biochemical parameters such as transaminases, phosphataes and total bilirubin level were significantly increased in toxin control groups and it restored to normal by supplementation of MESA and BHT. The obtained *in vitro* and *in vivo* results suggested that MESA possesses a significant antioxidant and hepatoprotective property.

Key words: Antioxidant, BHT, carbon tetrachloride, *Streblus asper*.

Introduction

The role of free radical reactions in disease pathology is well established, suggesting that these reactions are necessary for normal metabolism but can be detrimental to health as well acting on different levels in an oxidative sequences, the antioxidants prevents the risk of several aging related diseases including cancer, cardio vascular disorder, diabetes, neurodegenerative disorders and others [1]. Almost all organisms are well protected against free radical induced oxidative damage by antioxidant enzymes such as superoxide dimutase and catalase or antioxidant compounds such as ascorbic acid, tocopherols, and glutathione. When the protective mechanism becomes unbalanced by exogenous factors (tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides) and endogenous factors (stimulated polymorphonuclear leukocytes, and macrophages and peroxisomes), the effect leads to above mentioned diseases.

However, numerous investigation have proved that the phytoconstituents are responsible for scavenging the free radical and there by exhibiting the various beneficial pharmacological properties such as vasoprotective, anti carcinogenesis, anti viral, anti inflammatory etc [2].

Considering the importantance of this area, *Streblus asper* Lour (Family: Moraceae) from Assam (North eastern India) was evaluated for their *in vitro* and *in vivo* antioxidant profile, while are used by the Assamese community. The seeds of this plant are beneficial in epitaxis, piles, diarrhea etc. The paste form of the seed is applied externally applied in leukoderma. The root of this plant was used in treatment of epilepsy and inflammatory swelling and boiled wounds and juice had astringent and anti septic effect [3, 4]. The phytoconstituents of this plant are mainly glycosides such as kamaloside, iridoside, licknoside, cannodimethoside, asteroside, glucostrebuloside etc. The main and enriched compound is glucostrebuloside [5]. From the above traditional value, the plant was selected for evaluation of this study.

Materials And Methods

Plant material

The aerial part of *Streblus asper* was collected from Jorhat district of Assam, India. The plant material was identified by the Botanical Survey of India, Kolkata, India and the voucher specimen (SAB-2) has been preserved in our research laboratory for future reference. The aerial parts of the plants were collected in the month of May 2006. The plant was dried under shade and powdered with a mechanical grinder. The powdered plant material was then passed through sieve # 40 and stored in an airtight container for future use.

Preparation of extract

Dried and powdered leaves (2kg) were extracted with petroleum ether (60-80ºC), benzene and methanol successively in a soxhlet extraction apparatus. The extract was concentrated until the removal of solvents and kept in under reduced pressure to obtain a dry mass. The yields of the petroleum ether (60-80ºC), benzene and methanol extracts were found to be 6.50, 5.00 and 17.00 % w/w respectively.

Animals

Albino rat weighing 200–250 g were used and separated into five groups each group contains six animals. They were housed in clean polypropylene cages and observed under a 12-hour/12-hour light/dark cycle in a well-ventilated room at 26–27°C. The animals were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. Before starting the experiment the animals were acclimatized for one week and the experiment carried out with the approval of University animal ethical committee.

***In vitro* antioxidant activity**
Determination of DPPH radical scavenging activity

The hydrogen-donating ability of MESA was examined on the basis of the method of Blois (1958) [6] in the presence of DPPH stable radical. Various concentrations of MESA (50-175 µg/ml) and BHT (5-25 µg/ml) had taken for this experiment. The reaction mixtures containing 150µl of DPPH (0.004% DPPH solution in methanol) and one ml containing various concentrations of MESA and BHT were incubated at 37°C for 30 min. Absorbance of the reaction mixture was measured at 515 nm. The percentage inhibition (%) of MESA activity was calculated using the following equation

$$\text{Inhibition } (\%) = \frac{A_o - A_t}{A_o} \times 100$$

Where A_o is absorbance of the control and A_t is absorbance of the test sample at 515 nm.
From the percentage of inhibition the IC_{50} value of the MESA and BHT were expressed.

Lipid peroxidation inhibition assay

Lipid peroxidation induced by Fe^{2+}–ascorbate system in rat liver homogenate was estimated by the method of Ohkawa et al., 1979 [7]. Reaction mixture (0.5 ml) containing rat liver homogenate 0.1 ml (25 % w/v in Tris–HCl buffer (20 mM, pH 7.0); 0.1ml of KCl (30 mM); 0.1ml of $FeSO_4 \cdot 6H_2O$ (0.16 mM); 0.1ml of ascorbic acid (0.06 mM) and 0.1 ml of various concentrations of MESA (175 to 50 μg/ml) and BHT (5-25ug/ml) were incubated at 37° C for one hour. After the incubation period, reaction mixture was treated with 0.2 ml SDS (8.1%); 1.5 ml TBA (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4 ml by distilled water and then kept in a water bath at 95–100°C for 30 minutes. After cooling, 1.0 ml of distilled water and 5.0 ml of *n*-butanol and pyridine mixture (15:1, v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was removed and its absorbance was measured at 532 nm. The IC_{50} of the MESA and BHT was calculated from the dose inhibition curve.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was evaluated by studying the competition between deoxyribose and MESA for hydroxyl radicals generated from the Fe^{3+}-ascorbate-EDT-H_2O_2 system. The hydroxyl radical attacks deoxyribose and produce MDA which measured by Ohkawa et al., 1979 [8].

Reaction mixture (1 ml) containing deoxyribose (2.8 mM), $FeCI_3$ (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), H_2O_2 (1 mM), phosphate buffer (20 mM, pH 7.4) and various concentrations of MESA (175 to 50 μg/ml) and BHT (5-25 μg/ml) were incubated at 37°C for one hour. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated.

Nitric oxide scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions which can be estimated with Griess reagent [9, 10]. The reaction mixture containing 2ml of (10mM) sodium nitroprusside, 0.5 ml of phosphate buffer and various concentrations of MESA (175 to 50 μg/ml) and BHT (5-25 μg/ml) (0.5 ml) were incubated at 25^0C for 150 min. The IC_{50} value was estimated as concentration of sample required to scavenge 50% nitric oxide radical

Reducing ability

Total reducing power was determined as described previously [11]. 10 mg of MESA (in 1 ml of distilled water) mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%); the mixture was then incubated at 50°C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. The same protocol was followed for BHT (10 μg) and increased absorbance of the reaction mixture indicated increased reducing power.

Determination of total phenolic compounds in the extracts

The total phenolics content was determined using the Folin-Ciocalteu method [12]. The reaction mixture was composed of 1.0 ml of MESA (10 mg), 10.0 ml of distilled water, and 1.5 ml of the Folin-Ciocalteu reagent. After a period of 5 min, 4.0 ml of 20% sodium carbonate solution was added and made up to 25 ml with distilled water. This mixture were shaken and allowed to stand for 30 min. The absorbance was measured at 765 nm. The percentage of total phenolics was calculated from the calibration curve of gallic acid plotted and total phenolics were expressed as mg GAE (gallic acid equivalents)/g dry extract.

Determination of total flavonoid content in the extracts

The total flavonoid content was determined spectrophotometrically according to Lamaison and Carnat [13]. The reaction mixture was composed of 0.5 ml of 2% aluminum chloride ($AlCl_3$) ethanol and 0.5 ml of MESA (1 mg/ml). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). The total flavonoid content was expressed as mg of quercetin equivalents/g of dry extract).

***In vivo* antioxidant activity**

The animals were randomized in five groups (n=6). Group I served as control and received normal saline (10 ml/kg b.w. p.o). Group II served as CCl_4 control. Group III & IV received daily doses of MESA (250 and 500 mg/kg b.w. p.o) and vitamin E (50mg/kg b.w. p.o) was administered to group V. All the groups except vehicle control (Group I) were administered

with $CC1_4$ (0.5 ml/kg) on the fifth day of experiment. At the end of the 7^{th} day, all the rats were anaesthetized by ether anesthesia and blood and liver samples were collected.

Determination of serum biochemical parameters

After 7 days of treatment, collected blood samples were, kept for 1 hr and centrifuged at 3000 rpm for 30 min at 37^0C to obtain sera. The levels of serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) [14], serum alkaline phosphatase (SALP) [15], serum bilirubin and [16] and protein [17] estimation were measured.

Lipid peroxidation levels in liver

Lipid peroxidation was quantified based in MDA production by the Ohkawa et al (1979) [7] using the cytosolic fraction of liver homogenizes as an enzyme source. The absorbance was read at 532 nm against an appropriate blank. The levels of lipid peroxides were expressed as μmoles of TBA reacting substances (TBARS)/mg of wet liver tissue using molecular extinction co-efficient of 1.56×10^5 M^{-1} cm^{-1}.

Reduced glutathione levels in liver

Reduced glutathione was estimated by spectrophotometrically by determination of dithiobis (2-nitro) – benzoic acid (DTNB) reduced by SG groups, as described by Mulder et al (1995) [18] and expressed as μg/mg of wet liver tissue. The absorbance was read within (2-3 min after the addition of DTNB) at 412 nm against a reagent blank.

Catalase levels in liver

Catalase activity was measured based on the ability of the enzyme to break down H_2O_2 [19]. Test sample was taken in tubes containing H_2O_2 in phosphate buffer. Time required for 0.05 optical density change was observed at 240nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer. Catalase activity is expressed in the amount of $H_2\ O_2$ consumed /min/mg of wet liver tissue of any concentration in 100 sec at 25°C.

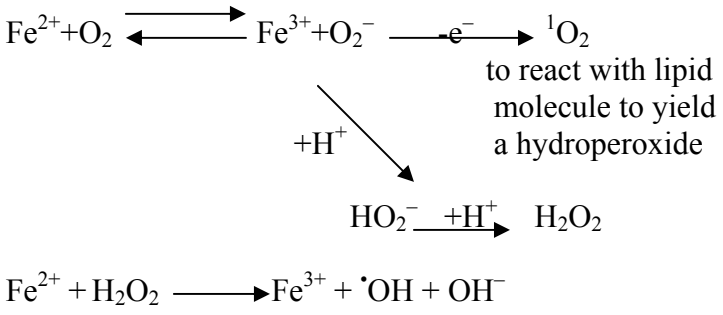
Results and discussion

DPPH Radical Scavenging Activity

DPPH radical is a stable free radical in an aqueous or methanol solution. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). The antioxidant of activity measured by the capacity odd electron of the radical becomes paired off in the presence of extract (hydrogen donor). When it becomes paired off, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured [6]. This reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts. In this test, MESA significantly inhibited the DPPH radical as compare to control ($p<0.001$). At the same time BHT had better activity than the MESA and the free radical scavenging activities of MESA and BHT were dose dependent manner (Figure 1 & 2). The IC_{50} values of MESA and BHT were found to be 116.05 & 78.87 µg/ml respectively. These results indicated that MESA has a noticeable effect on scavenging free radical.

Lipid peroxidation inhibition and hydroxyl radical scavenging assay

Both ferrous and ferric ions induce lipid peroxidation through various mechanisms involving reactive species. Most of the antioxidants inhibit iron-induced lipid peroxidation [20]. Hence MESA was tested for its effect on lipid peroxidation induced by ferric ions (Fig 1) in deoxyribose sugar, it showed concentration dependent inhibition of peroxidation, but less active than BHT. Hydroxyl radical scavenging effect of MESA was measured by studying the competition between MESA and deoxyribose for the hydroxyl radical generated from the ferric-ascorbate-EDTA-H_2O_2 system and can inhibit deoxyribose degradation depending on its concentration and rate constant for reaction with hydroxyl radical. The hydroxyl radical attacks deoxyribose and sets off a series of reactions that eventually result in TBARS formation. When a molecule scavenges a hydroxyl radical, it decreases TBARS formation. Figure 1 depicts the MESA and BHT significantly scavenged the hydroxyl radical produced in this system. The IC_{50} values of MESA and BHT in this test were found to be 130.49 & 71.03 µg/ml respectively. In the lipid peroxidation inhibition assay, TBARS formation induced by ferrous ions and it was comparatively higher than the TBARS formation in hydroxyl radical scavenging assay. Fe^{2+} exposed to air can react with molecular oxygen to produce superoxide and can slowly oxidize to Fe^{3+}. Superoxide can be dismuted to form hydroperoxides and hydrogen peroxide at same time Fe^{3+} reduced to Fe^{2+}[21].



The fenton chain reaction can be continued resulting in the formation of hydroxyl radicals by reaction of H_2O_2 with Fe^{2+}. These reactions may operate as a cycle, so that even trace amounts of metal ions may be effective in generating radicals in Fe^{2+}-ascorbate system. Figure 1 shows antioxidant activity of MESA and BHT in the Fe^{2+}-ascorbate system and ferric-ascorbate-EDTA-H_2O_2 system. The percentage inhibition of MESA significantly ($p<0.05$) when compare to control. The percentage inhibition and IC_{50} values of MESA and BHT (110.07 & 89.97 µg/ml) indicates that they were effectively inhibited the superoxide and hydroxyl radical.

Nitric oxide scavenging assay

Nitric oxide (NO), a short lived free radical generated endogenously, exerts influence on a number of functions including vasodilation, neurotransmission, synaptic plasticity and memory in the central nervous system [22]. Besides mediating normal function, NO has been implicated in pathophysiological states, eg. DNA fragmentation, cell damage and neuronal cell death. The present study demonstrates that MESA and BHT are potent NO radical scavengers. NO generated from sodium nitropruside in aqueous solution at physiological pH reacts with oxygen to form nitrite ions. MESA significantly inhibited ($p<0.05$) nitrite formation by competiting with the oxygen atom to react with NO. The IC_{50} values of MESA and BHT were found to be 143.99 & 103.77 µg/ml respectively.

Reducing Power

Several methods have been developed to measure the efficiency of antioxidants. These methods focus on different mechanisms of the oxidant defense system that is, scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions. In most cases, irrespective of the stage in the oxidative chain in which the antioxidant action is assessed,

most nonenzymatic antioxidative activity (scavenging of free radicals, inhibition of lipid peroxidation, etc.) is mediated by redox reactions. The reducing power of MESA to reduce ferric ions was determined in this study. MESA had significant reducing power and also was in dose dependent manner.

Determination of total phenolic and total flavonoid content in the extract

It has been already reported that phenolic compounds plays important role in scavenging of free radicals. The above mentioned *in vitro* studies, the free radical scavenge activity of MESA may be due to the presence of phenolic compounds. Our results in this experiment also agreed the hypothesis by the presence phenolic compounds. The percentage of total phenolics in the MESA was expressed 55.28±5.24 mg gallic acid equivalents (GA)/g dry weight of extract. Along with total flavonoids content also measured and it was 20.57±3.82 mg quercetin equivalents (QE)/g dry weight of extract.

Fig 1. *In vitro* Antioxidant potency of methanol extract of *Streblus asper*

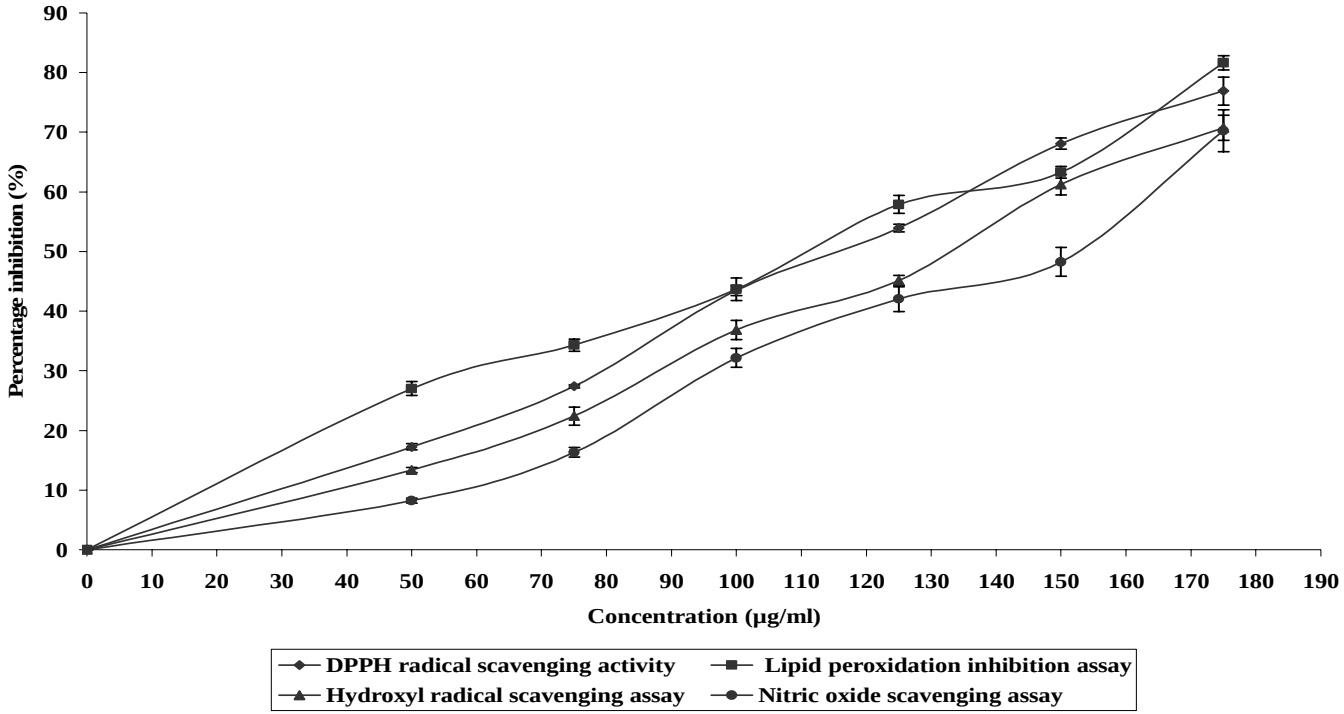


Fig 2. In vitro Antioxidant potency of Butylated hydroxy toluene (BHT)

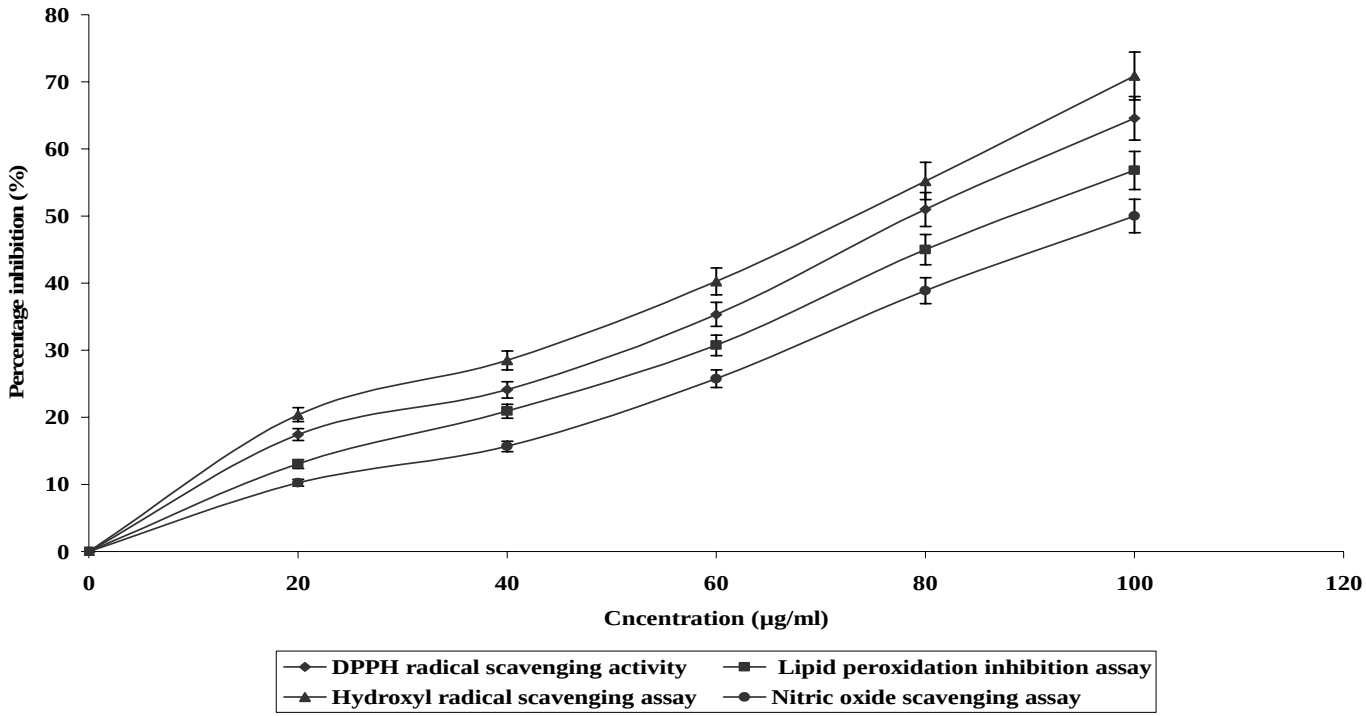
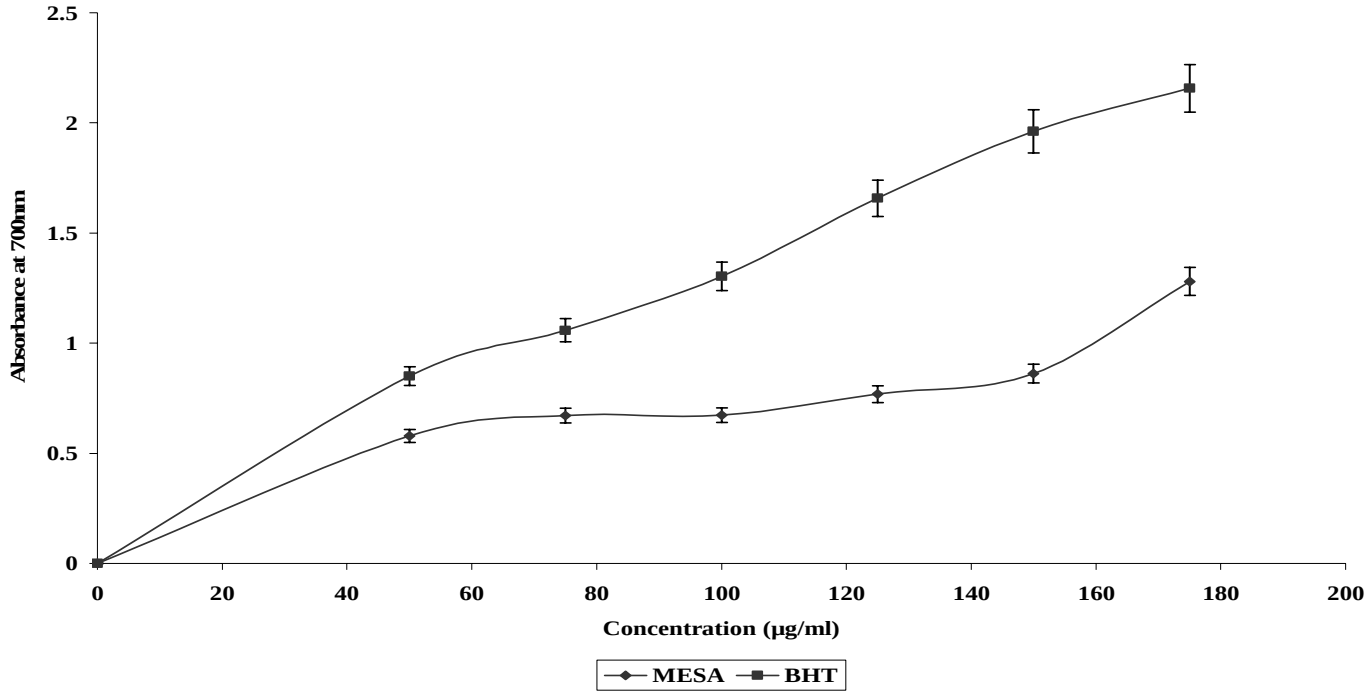


Fig 3. Reducing power of methanol extract of *Streblus asper* and Butylated hydroxy toluene



***In vivo* antioxidant activity**

The *in vivo* antioxidant activity of MESA was measured by its protection against CCl_4 induced liver damage in rats. Liver damage induced by carbon tetrachloride are best characterized system of xenobiotic-induced hepatotoxicity and is a commonly used model for the screening of *in vivo* antioxidant and hepatoprotective activity of drugs [23]. CCl_4 is first metabolized by cytochrome P450 2E1 in the liver endoplasmic reticulum to the highly reactive $^{\bullet}CCl_3$ radical. One of the principal causes of CCl_4 induced liver injury is lipid peroxidation by free radical derivatives of CCl_4. CCl_4 is less harmful when compare to the its metabolite. The antioxidant status was measured by lipidperoxidation level, enzymatic and non enzymatic antioxidant level. The lipid peroxidation was induced $^{\bullet}CCl_3$ radical and the level lipid peroxidation renowned by malondialdehyde, which forms a MDA-TBA adduct (LPO marker) with thiobarbituric acid. The lipid peroxidation reduced by the treatment of antioxidant, which may acts as reduce the metabolism of CCl_4 by inhibit cytochrome P450 2E1 or scavenge the $^{\bullet}CCl_3$ radical. Our results supports the hypothesis because a good correlation was found as TBARS level in MESA (500mg/kg) treated groups (201.17±5.47) were significantly ($p<0.001$) reduced when it is compare to toxin control (437.33±7.97) and inhibition was also dose dependent manner (Table 2).

The next important factor in the measurement of *in vivo* antioxidant status was non enzymatic antioxidant e.g GSH. GSH plays the important role in the detoxification of the reactive toxic metabolites of CCl_4 and liver cells damage when the GSH depleted. GSH forms adduct with the toxic metabolites of CCl_4 and it is mediated through the activity of glutathione-S-transferase. Moreover GSH contributes the detoxification of CCl_4, the experimental shows that GSH level was significantly reduced in the CCl_4 control groups (207.33±6.07) when it is compare to normal animals (315.83±7.07) and it was restored to normal by

supplementation with MESA (500mg/kg) and vitamin E (50mg/kg) (305.33±2.75 & 316.83±5.24 respectively). The increase of GSH level may be due to de novo GSH synthesis or GSH regeneration.

The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is accomplished by set of endogenous enzymatic antioxidant such as superoxide dismutase and CAT. These enzymes cause mutually supportive team of defense against reactive oxygen species (ROS) [24]. In CCl_4 induced hepatotoxicity, the balance between ROS production and protection from ROS may be lost, 'oxidative stress' results, which through series of events deregulates the cellular functions leading to hepatic necrosis. The reduced activity of CAT (15.27±7.02) observed point out the hepatic damage by administration of CCl_4. But the MESA (500mg/kg) and vitamin E (50mg/kg) treated groups (23.23±0.77 & 28.98±0.38) showed significant increase in the level of this enzyme

In addition to antioxidant, the ability of hepatoprotective action of MESA was assessed through measuring the level of biochemical enzyme. The lowering of the enzyme level is a definite indication of hepatoprotective action of the MESA. Protection of hepatic damage caused by carbontetrachloride administration was observed recording SGOT, SGPT, SALP, total bilirubin and total protein level in drug treated, toxin control and normal groups because serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of hepatic damage [25]. Table 1 shows that SGOT, SGPT, SALP and total bilirubin level in toxin treated animals (37.33±2.62, 45.00±3.09, 155.83±3.26 & 3.70±0.19 respectively) were significantly ($p<0.001$) elevated when compare with normal animals (22.33±2.01, 23.50±2.32, 117.17±4.63 & 1.15±0.08 respectively). This disturbance in the transport function of the hepatocytes as a result of hepatic injury, cause the leakage of enzymes from cells due to altered permeability of membrane. This results decrease the biochemical enzymes in the liver and elevated in serum [26]. This elevation significantly ($p<0.001$) inhibited by the treatment of MESA (500mg/kg) and the level of SGOT,

SGPT, SALP and total bilirubin was 17.50±1.38, 18.00±1.46, 100.66±1.84 & 1.10±0.09 respectively.

Conclusion

This experiment discussed about the *in vitro* and *in vivo* antioxidant effect of the methanol extract of *Streblus asper*. From the *in vitro* studies, the antioxidant activity may be due to inhibit the formation of radicals or scavenge the formed radical and it may be due to the presence of the phenolic compounds. To find out antioxidant effect in the intact system, MESA was experimented in the CCl_4 induced hepatotoxic rats. These results also support the *in vivo* antioxidant effect of MESA. These results concluded that MESA has promising antioxidant effect in both *in vivo* and *in vitro* and it also acts as hepatoprotective.

Table 1. Effect of methanol extract of *Streblus asper* lour and vitamin E on serum biochemical parameters in carbontetrachloride intoxicated rats

(Each value represents the mean ±SEM, six rats in each group)

Biochemical parameters	Normal animals (2%DMSO, 5ml/kg)	CCl_4 Treated (1 ml/kg)	CCl_4 + MESA (250 mg/kg)	CCl_4 + MESA (500 mg/kg)	CCl_4 + Vitamin E (50 mg/kg)
SGOT (IU/L)	22.33±2.01	37.33±2.62 [a*]	21.33±2.56 [b*]	17.50±1.38 [b*]	20.16±1.56 [b*]
SGPT (IU/L)	23.50±2.32	45.00±3.09 [a*]	27.66±2.02 [b*]	18.00±1.46 [b*]	24.66±1.71 [b*]
ALP (IU/L)	117.17±4.63	155.83±3.26 [a*]	105.00±3.23 [b*]	100.66±1.84 [b*]	105.83±3.27 [b*]
Total bilirubin (mg/dl)	1.15±0.08	3.70±0.19 [a*]	2.55±1.29	1.10±0.09 [b#]	1.32±0.08 [b#]
Total protein (mg/dl)	5.42±0.14	3.38±0.14 [a*]	5.13±0.14 [b*]	5.35±0.19 [b*]	5.22±0.28 [b*]

All values represent the mean ± SEM. *P* values calculated by ANOVA followed by Dunnett's post hoc test.
[a*] significance $p < 0.001$ compare with normal group עs CCl_4 control group;
[b*] significance $p < 0.001$ compare with CCl_4 control group עs all treated groups;
[b#] significance $p < 0.05$ compare with CCl_4 control group עs all treated groups;

Table 2. Effect of methanol extract of *Streblus asper* lour and vitamin E on biomarker enzymes and lipid peroxidation in carbontetrachloride intoxicated rats

(Values are mean± SEM, 6 rats in each group)

Treatment	Lipid peroxides (µM of MDA/mg of wet liver tissue)	GSH (µg /mg of wet liver tissue)	Catalase (µM of H_2O_2 consumed /min/mg of wet liver tissue)
Normal animals (2%DMSO, 5ml/kg)	132.83±7.35	315.83±7.07	31.15±0.58
CCl_4 treated (1 ml/kg)	437.33±7.97[a*]	207.33±6.07[a*]	15.27±7.02[a*]
CCl_4 + MESA (250 mg/kg)	232.50±4.89[b*]	260.67±6.97[b*]	19.30±0.59[b*]
CCl_4 + MESA (500 mg/kg)	201.17±5.47[b*]	305.33±2.75[b*]	23.23±0.77[b*]
CCl_4 + Vitamin E (50 mg/kg)	142.33±6.42[b*]	316.83±5.24[b*]	28.98±0.38[b*]

All values represent the mean ± SEM. *P* values calculated by ANOVA followed by Dunnett's post hoc test.
[a*] significance $p < 0.001$ compare with normal group עs CCl_4 control group;
[b*] significance $p < 0.001$ compare with CCl_4 control group עs all treated groups.

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