ISSN: 2348-8948
Vol: 2; Issue: 8

RESEARCH ARTICLE

EVALUATION OF *IN-VITRO* AND *IN-VIVO* ANTIOXIDANT ACTIVITIES OF ALCOHOLIC EXTRACT OF *CAMELLIA SINENSIS* L.

AJAY SINGH BISHT*, MONIKA BISHT, DIVYA JUYAL

Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun, Uttarakhand, India

Date Received:	Date of Accepted:	Date Published:
30-Apr-2014	20-Aug-2014	26-Aug-2014

Abstract:
Tea (*Camellia Sinensis* L.) is the 2nd most consumed beverage in the world next to water. The tea plant has been used for centuries by ancient cultures for its medicinal values. The antioxidant and free radical scavenging activity of aqueous extract of this plant was investigated both *in -vivo* and *in-vitro* using spectroscopic method against 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), nitric oxide (NO), and the ferric reducing agent. Total phenols, flavonoids, flavonols and proanthocyanidin were also determined to assess their effects on the antioxidant activity of this plant. The plant found to possess strong activity observed against nitric oxide radicals and moderate activity against DPPH and H_2O_2 radical. The reducing power of the extract was found to be concentration dependent. The administration of the 2.5%, 5%, and 10% of extract aqueous solution corresponding to 10 ml/kg body weight to albino mice significantly increased the level of reduced glutathione (GSH). Whereas, lipid peroxidation level in hepatotoxic mice decreased significantly. The plant contains 0.48gm/100 gm of plant material with Gallic acid equivalent, followed by flavonol 0.36gm/ 100 gm of plant material with Rutin equivalent, proanthocyandins 0.29gm/100 gm of plant material with Catechin equivalent., flavonoids 0.22gm/100 gm of plant material with Rutin equivalent. A positive linear correlation was observed between these polyphenols and the free radical scavenging activities.

Keywords: *Camellia sinensis*, free radicals, antioxidant activity, DPPH, oxidative stress, CCl_4

Introduction

There is a generation of reactive oxygen species and nitrogen species (ROS and RNS) during normal biochemical reaction in our body. This get enhanced during pathophysiological condition creating "oxidative stress".[1] Oxidative stress results from imbalance between the formation and neutralization of pro-oxidants[2]. Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite, play a vital role in damaging various cellular macromolecules. This damage may result into many diseases, including diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis[3].

Natural products of plant origin have been proposed as a potential source of natural antioxidants with minimal toxic effects and strong activity. Tea (*Camellia Sinensis* L.), is the 2nd most consumed beverage in the world next to water. The tea plant has been used for centuries by ancient cultures for its medicinal values. Traditionally tea was drunk to improve blood flow, eliminate toxins, and to improve resistance to diseases[9].In the preparation of green tea, the leaves are first steamed and then dried relatively rapidly. This condition stops the enzyme- catalyzed oxidation of tea catechins. The aim of this study was to evaluate the *in-vitro* and *in-vivo* antioxidant and free radical scavenging activity of alcoholic extract of green tea.

MATERIAL AND METHODS

Plant material

Fresh leaves of Green tea leaves were collected in May 2009, from Bhimtal, Uttrakhand, India and authentified by Forest Research Institute (FRI), dehradun, Uttrakhand, India (Accession no- 157030).

Preparation of extract

The leaves were air dried at room temperature. Fine coursed powdered drug (35 g), was packed in a soxhlet apparatus and hot percolated with 95% Ethanol (150 ml). It was subjected to heating mantle (at 70º C) for 48 hours. Finally the solvent was removed under reduced pressure by using Rotary evaporator and brown colored extract (8.6 g) was obtained and stored at 4ºC.

Animals

Albino mice of either sex with a mean weight of 30 g ± 5 were obtained from the animal house of Indian Vet nary Research Institute (IVRI), Izatnagar, U.P, India. Animals were kept in clean metabolic cages placed in a well ventilated housed condition (Temperature 23 ± 1° C: Photo period: 12 h light and dark cycle throughout the experimental period; humidity: 45-50%). The mice were allowed free access to standard food (Pranab Agro-industries limited) and water *ad libitum.* The animal house and breeding facility have been registered with Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India and the CPCSEA guidelines were followed.

Animal grouping and extract administration

Mice were randomized into five groups consisting of six each. Group 1 served as control and was given distilled water alone (0.5 ml) per day for seven days with the aid of oropharyngeal cannula. Groups 2 animals served as hepatotoxic control, treated with CCl_4 in a single dose of 0.5 ml administered orally for seven days, while the animal in group 3-5 receive 2.5%, 5%, and 10% of extract aqueous solution corresponding to 10 ml/kg of body weight for fifteen days . Again group 3-5 was given 0.5 ml of CCl_4 on the seventh day after 6^{th} hour of extract administration for seven days. All the animals from each group were sacrificed by cervical dislocation after their respective dosing of period. 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 12000 × g for 60 min at 4ºC. The supernatant obtained was used for the estimation of lipid peroxidation (TBARS) and reduced glutathione.

Statistical analysis

The data are expressed as mean ± SEM. Statistical differences between means were determined by one–way analysis of variances (ANOVA), followed by Dunnett t-test. The values of $P< 0.05$ were considered as significant.

Estimation of total phenolic content

Total phenol:

The total phenolic content in the alcoholic extract of *Camellia sinensis* L. was carried out according to modified colorimetric Folin-Ciocalteu reagent using the Wolfe modified method[12]. A volume of 0.5 ml of deionized water and 0.125 ml of a known dilution of the extract were added to a test tube, Folin-Ciocalteu's reagent (0.125 ml) was added to the solution and allowed to react for 6 min. Then, 1.25 ml of 7% sodium carbonate solution was liquated into the test tubes, and the mixture was diluted to the 3 ml with deionized water. The color developed for 90 min, and the absorbance was read at 760nm, r^2= 0.9983, using UV-1 Double beam spectrophotometer. The measurement was compared to the standard curve of prepared Gallic acid solution and expressed as milligrams of Gallic acid equivalents per 100g of the sample extract.

Total flavonoids:

Total flavonoids were estimated by using the method of Ordon[13]. The total flavonoids content of the extract solution were based on the formation of a complex flavonoid-aluminium. A volume of 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ in methanolic solution was added. After one hour at room temperature, the absorbance was measured at 420 nm, using UV-1 Double beam spectrophotometer. Extract samples were evaluated at a final concentration of 0.01 mg/ml. All the determinations were done in triplicate. Total flavonoid content was calculated as Rutin (mg/ml) using the following equation based on the calibration curve: $y = 165.1 xs + 0.069$, $r^2 = 0.998$, where y was the absorbance and x was the Rutin equivalent (mg/ml).

Total flavonols:

Total flavonol content in the plant was determined by adopting the procedure of Kumaran and Karunakaran[14]. To 1.0 ml of sample, 2 ml of 2% $AlCl_3$ in methanol and 3 ml 5% sodium acetate solution were added. The absorbance at 440 nm was read after 2.5 hour at 20°C using UV-1 Double beam spectrophotometer. Extract sample were evaluated at a final concentration of 0.05 mg/ml. Total flavonol content was calculated as Rutin (mg/ml) using the following equation based on the calibration curve: $y = 27.97x + 0.0208$, $r^2 = 0.997$, where y was the absorbance and x the Rutin equivalent (mg/ml).

Total proanthocyanidins

Total proanthocyanidins in the plant were estimated using the method of sun [15]. To 0.4 ml of sample with 3 ml of 4% vanillin-methanol solution and 1.5 ml concentrated hydrochloric acid were added and shake well. The absorbance at 500 nm was read after 15 min at

room temperature using UV-1 Double beam spectrophotometer. Extract sample were evaluated at a final concentration of 0.01 mg/ml. Total proanthocyanidin content was calculated as Catechin (mg/ml) equivalent using the following equation based on the calibration curve: y = 4.8x +0.036, r^2 = 0.974, where y was the absorbance and x the Catechin equivalent (mg/ml).

In vitro antioxidant activity

Determination of reducing power:

The reducing power of the extract was evaluated according to the method of Yen and Chen[16]. A volume of 1.0 ml of the extract prepared in distilled water and BHT, Vitamin C and Vitamin E (0 - 5.0 mg/ml) were mixed individually to the mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%w/v). The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v), which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1 %, w/v). The absorbance was measured at 700 nm against a blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

DPPH radical scavenging assay:

The effect of extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi[17]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes the absorbance of mixture was measured spectrophotometrically at 517 nm Ascorbic acid, Rutin were used as references. The ability to scavenge DPPH radicals was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs $_{control}$ - Abs $_{sample}$) / (Abs $_{control}$)] × 100 where Abs $_{control}$ is the absorbance of DPPH radical + methanol; Abs $_{sample}$ is the absorbance of DPPH radical + sample extract / reference.

Scavenging activity of nitric oxide:

The method of Garrat was used to determine the nitric oxide radical scavenging activity of *Camellia Sinensis* L[18]. A volume of 2 ml of 10 mM sodium nitroprusside prepared in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract, BHT and Rutin individually at various concentrations (0.025 - 0.5 mg/ml). The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33 % prepared in 20 % glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 540 nm. The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

NO radical scavenging activity = {(Abs control – Abs sample) / (Abs control)} × 100

Where; Abs control is the absorbance of NO radical + methanol; Abs sample is the absorbance of NO radical + sample extract or standard.

Hydrogen peroxide scavenging activity:

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Ruch[19]. 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.

Estimation of lipid peroxidation:

Lipid peroxidation in the liver was estimated by Thiobarbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelsson[20]. In brief, 0.1 ml of liver homogenate (10% w/v) was treated with 2 ml of (1:1:1 ratio) TBATCA- HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and cooled. The amount of Malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm against reference blank and was calculated by using following formula:

n moles of Malonaldehyde/mg protein =

$$\frac{3 \times \text{Absorbance of sample}}{0.156 \times \text{mg protein} / 0.25}$$

Determination of reduced glutathione activity:

Reduced glutathione was determined using the modified method of Ellman[21]. An aliquot of 1.0 ml of supernatant of liver homogenate was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5- dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm. The amount of GSH was calculated using the following formula:

$$\mu \text{ moles of GSH / mg protein} = \frac{3\times \text{absorbance of sample}}{13.6\times \text{mg protein} / 0.25}$$

RESULTS

The polyphenolic contents in the alcoholic extract of *Camellia sinensis* L. are shown in **Figure 1**. The plant possesses to have phenolic content 0.48% with respect to Gallic acid, flavonol 0.36% with respect to Rutin, proanthocyandins 0.29% with respect to Catechins equivalent, flavonoids 0.22% with respect to Rutin equivalent. **Figure 2** Shows total ferric reducing potential of alcoholic extract of *camellia sinensis* L. The antioxidant potential of plant extract was estimated from their ability to reduce Fe^{+++} to Fe^{++}. This was observed from yellow color of test solution that changed to various shades of green and blue depending on the concentration of plant extract. The reducing value of extract was significantly lower than that of Ascorbic acid and significantly higher than that of BHT and vitamin E used as reference compounds in this study. At 0.1 mg/ml, the absorbance of plant extract was still low then that of ascorbic acid but still higher than that of BHT and Vitamin E. **Figure 3** shows the DPPH radical scavenging activity of alcoholic extract of *Camellia sinensis* L. with respect to Ascorbic acid and Rutin, which were used as reference compounds in the study. It was observed from the study that the DPPH radical scavenging activity of plant extract was significantly lower than that of Ascorbic acid and Rutin. At 0.1 mg/ml the scavenging activity was still low as compared to reference drugs. **Figure 4** shows nitric oxide radical scavenging activity of alcoholic extract of *Camellia sinensis* L. with respect to Ascorbic acid and Rutin, which were used as reference compounds in the study. It was observed from the study that the nitric oxide radical scavenging activity of plant extract was significantly lower than that of ascorbic acid but higher than that of Rutin. At 0.1 mg/ml the scavenging activity was still low then that of ascorbic acid but still higher than that of Rutin. **Figure 5** shows the hydrogen peroxide radical scavenging activity of alcoholic extract of *Camellia sinensis* L. with respect to Ascorbic acid and BHT, which was used as reference compounds in the study. It was observed from the study that the hydrogen peroxide radical scavenging activity of plant extract was significantly lower than that of ascorbic acid and BHT. At 0.1 mg/ml the scavenging activity was still low as compared to reference drugs.

Table 1 showed the effect of plant extract on the activities of antioxidant enzymes in the liver of control and experimental mice. There was a marked decreased in the level of reduced Glutathione in carbon tetrachloride treated mice when compared with normal control group. However, the level of reduced glutathione was significantly increased in a dose dependent manner, followed by the oral administration of plant extract at 10 mg/kg body weight of 2.5%, 5% and 10% of aqueous solution of extract. *In vivo* lipid peroxidation study revealed that mice treated with carbon tetrachloride showed a significant increase ($P < 0.05$) in TBARS when compared with normal control group. Treatment with aqueous extract of *Camellia Sinensis* L. for fifteen days was able to lower the rise in TBARS level dose dependently as shown in Table 1.

DISCUSSION

In the present study, it was found that the alcoholic extract of *Camellia Sinensis* L.contains moderate to high level of phenol content that might account for the strong activity observed against nitric oxide radicals. This scavenging activity may be due to the presence of hydroxyl groups attached to the aromatic ring structures and thus help to quench the radicals[23]. On the other hand, the moderate activity depicted in DPPH and hydrogen peroxide radicals may be as a result of lower content of Flavonoids which have been reported to possess high antioxidant activity. In this study the reducing power of *Camellia sinensis* extract was determined by measuring the transformation of Fe^{+3} to Fe^{+2}. The observed result obtained showed that the extract possessed antioxidant activity in a concentration dependent manner. This effect may suggest the ability of *Camellia sinensis* to minimize oxidative damage to some vital tissues in the body[24, 25]. Hydrogen peroxide is highly important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions[26]. This assay shows the ability of *Camellia sinensis* to inhibit hydrogen peroxide in the reaction mixture. The present study revealed that the extract has a moderate activity in scavenging H_2O_2 radical by directly competing with oxygen, nitric oxide and its derivative. This could be due to the presence of phenolic compounds that donate electron to H_2O_2 and thus neutralizing it to water[27]. Nitric oxide (NO) is a reactive free radical generated from sodium nitroprusside in aqueous solution at physiological pH and reacts with oxygen to form nitrite. From the results, it appeared that activities of the plant extract were nearly the same with the reference compounds. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver damage[28]. The hepatotoxic effects of this chemical is mostly based on membrane lipid peroxidation. Consequently, leads to the induction of trichloromethyl radical that resulted into severe cell damage[29]. In this present study, the mice treated with single dose of CCl_4 developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the lipid peroxidation. This indicates the inability of antioxidant defense mechanism in the system to prevent the formation of excessive free radicals. The treatment with alcoholic extract of *camellia sinensis* was able to reduce the level of lipid peroxides in

Table 1. Concentration of Malonaldehyde and Glutathione in carbon tetrachloride induced liver damage in albino mice.

Group	Treatment	Reduced Glutathione µ mol	Lipid Peroxidation nMol MDA/mg protein
Group-I (Control)	Distilled water	73.580 ± 1.224	4.972 ± 0.279
Group-II	CCl_4	28.080 ± 0.897[a]	20.368 ± 1.515[a]
Group-III	CCl_4 + 2.5% aqueous solution of extract	35.688 ± 0.680[b]	16.128 ± 0.686 [b]
Group-IV	CCl_4 + 5% aqueous solution of extract	44.547 ± 1.012[c]	11.235 ± 0.492[c]
Group-V	CCl_4 + 10% aqueous solution of extract	58.262 ± 1.531[d]	9.350 ± 0.915[d]

*Values are mean ± SEM. [a-d] test values carrying superscripts different from the control across each Parameter are significantly different (P< 0.05), results are expressed as concentration.

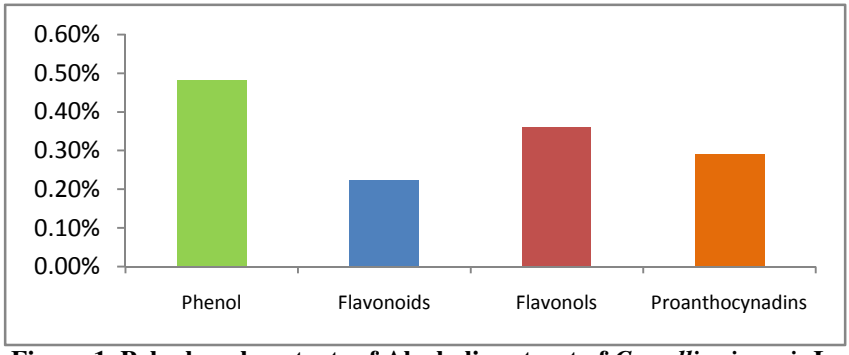

Figure 1. Polyphenol contents of Alcoholic extract of *Camellia sinensis* L.

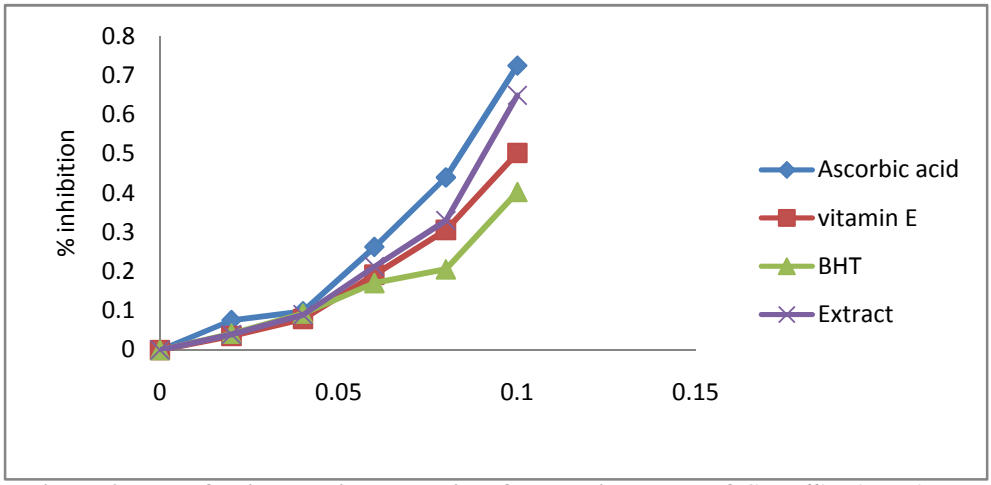

Figure 2. Total ferric reducing potential of alcoholic extract of *Camellia sinensis* L.

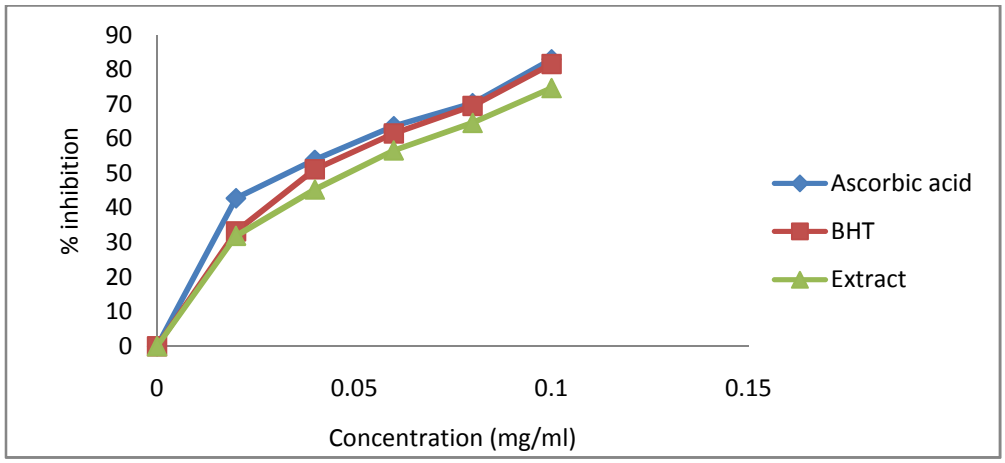

Figure 3. DPPH radical scavenging activity of alcoholic extract of *Camellia sinensis* L.

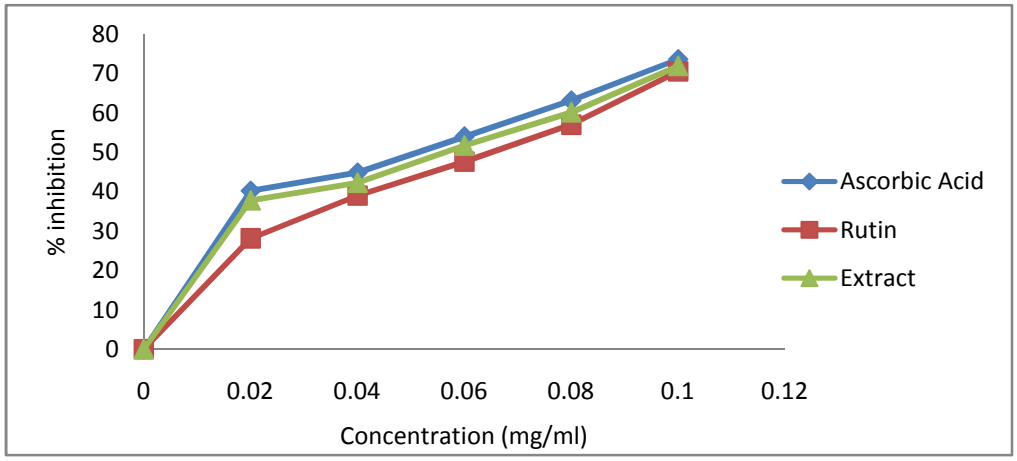

Figure 4. Nitric oxide radical scavenging activity of alcoholic extract of *Camellia sinensis* L.

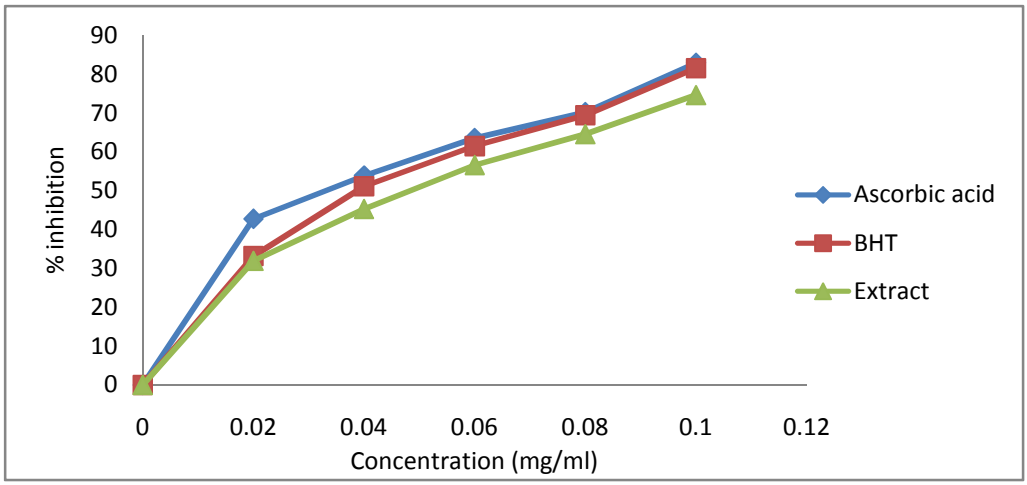

Figure 5. Hydrogen peroxide scavenging activity of alcoholic extract of *Camellia sinensis* L.

a dose dependent manner as compared with the hepatotoxic group.

Reduced glutathione (GSH) is a tripeptide, non enzymatic biological antioxidant present in the liver. It protects cellular proteins against reactive oxygen species generated from exposure to carbon tetrachloride[30]. Decreased level of GSH is associated with increase lipid peroxidation which is also confirmed in this study. The ability of plant extracts to reactivate the hepatic Glutathione reductase was reflected by decreasing the level of lipid peroxidation.

CONCLUSION

In conclusion, present results demonstrate that alcoholic extract of leaves of *Camellia sinensis* has both *in-vivo* and *in-vitro* antioxidant activities due to the presence of phenolic compounds. Moreover, the ability of this plant to prevent the process of initiation and progression of liver diseases may be attributed in future to the observed result.

REFERENCES

1. Devasagayam T P A and Sainis K B. Immune system and antioxidants, especially those derived from Indian medicinal plants. *Indian J Exp Biol* .2002;42. 639-655.
2. Hazra B, Santana B, Nripendranath M, Antioxidant and free radicals scavenging activity of *Spondias Pinnata*. *J. BMC.* 2008; 8. 63.
3. Polterat O, Antioxidants and free radical Scavengers of natural origin. *Current Org. Chem* .1997; 1. 415-440.
4. Chandra A, Samali S, Orrenius S. Triggering and modulation of apoptosis by oxidative stress, *Free radical Biol Med* .2000;29. 323-333.
5. Knight J A, Review: Free radicals, antioxidants, and the immune System, *Ann Clin Lab Sci*. 2000; 30.145.
6. De La Fuente M and Victor V M, Anti-oxidants as modulator of immune function, *Immunol Vell Biol* .2000;78. 49.
7. Robak J, Marcinkiewicz E, Scavanging of reactive oxygen species as the mechanism of drug action, *Pol. J. Pharmacol Pharm*. 1995; 47. 89-98.
8. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB, The relative activities of plant-derieved polyphenolic flavanoid, *Free radic. Res*. 1995; 22. 375-383.
9. D.A. Balentine, S.A. Wiseman, L.C. Bouwens, the chemistry of flavonoids, *Crit Rev Food Sci Nutr*. 1997;37.693–704.
10. Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL, Antioxidative activities of oolong tea, *J Agric Food Chem* . 2002;50.6929-6934.
11. Gramza A, Khokhar S, Yoko S, Gliszczynska-swiglo A, Hes M, Korczak J, Antioxidant activity of tea extracts in lipids and correlation with polyphenol content, *Eur J Lipid Sci Technol*. 2006;108. 351-62.
12. Wolfe K, Wu X, Liu RH, Antioxidant activity of apple peels, *J Agric Food Chem*. 2003; 51,.609-614.
13. Ordon Ez AAL, Gomez JD, Vattuone MA, Isla MI, Antioxidant activities of *Sechium edule* (Jacq), *Food chem.* 2006; 97. 452-458.
14. Kumaran A, Karunakaran RJ, *In vitro* antioxidant activities of methanol extracts of *Phyllantus* species from India, *Lebens-Wiss technologie* .2007; 40. 344-352.
15. Sun JS, Tsuang YW, Chen JJ, Huang, WC, *et al*, An ultra-weakchemiluminescence study on oxidative stress in rabbits following acute thermal injury, *Burn.* 1998; *24*.225-231.
16. Yen G, Chen H, Antioxidant activity of various tea extract in relation to their antimutagenicity, *J Agric Food Chem*. 1995; 43. 7-32.
17. Liyana-Pathiranan CM, Shahidi F, Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L) as affected by gastric pH conditions, J *Agric Food Chem*. 2005;53.2433-2440.
18. Garrat DC, The quantitative analysis of drugs, *Chapman and Hall ltd Japan*. 1964; 3. 456 - 458.
19. Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea, *Carcinogen* 1989;10.1003-1008.
20. Niehius WG, Samuelson B, Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation, *Eur J Biochem*. .1968; 6. 126-130.
21. Ellman GL, Tissue sulfhydryl groups, Arch *Biochem Biophys* 1951; 82, 70-77.
22. Duh PD, Tu YY, Yen GC, Antioxidant activity of water extract of Harng Jyur (*Chrssanthemum morifolium* Ramat), *Lebnesmittel-Wissenschaft und technologie*. 1999; 32.269-277.
23. Vinson JA, Yong H, Xuchui S, Zubik L, Phenol antioxidant quantity and quality in foods: vegetables, *J agric Food Chem* .1998; 46. 3630-3634.
24. Kojic G, Vlahonic P, Ravloure D, The possible importance of the cation binding site for the oxidative modification of liver nucleolidase, *Arch Physiol Biochem* .1998;106.91-99.
25. Weighand MA, Laipple A, Plascke K, Concentration changes of malondialdehyde across the cerebral vascular bed and shedding of L selectin during carolide endarterectomy, *Stroke* .1999 ;30.306-311.
26. Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI, Screening of anti- oxidant and antimicrobial activities of anise (*Pimpinella anisum* L) seed extracts, *Food Chem*. 2003; 83.371 - 382.
27. Mathew S, Abraham TE, In vitro antioxidant activities and scavenging effects of *Cinnamomum*

verum leaf extract assayed by different methodologies, *J Food Chem Toxicol* .2006. 44.198-206.

28. Lee KS, Lee SJ, Park HJ, Chung JP, Han KH, *et al*, Oxidative stress effect on the activation of hepatic stellate cells, *J Med (Yonsei)* .2001; 42. 1- 8.
29. Johnson DE, Kroening C, Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes, *Pharmacol Toxicol* .1998;83.231-239.
30. Arivazhagan S, Balasenthil S, Nagini S, Garlic and neem leaf extracts enhance hepatic glutathione and glutathione dependent enzymes during N-methyl- N nitrosoguanidine (MNNG) - induced gastric carcinogenesis, *Phytother Res* .2000;14. 291-293.