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RENAL AND HEPATO PROTECTIVE EFFECTS OF GREEN TEA (*Camellia sinensis*) EXTRACT ON WISTAR RATS TREATED WITH SODIUM OXALATE

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ABSTRACT

Nowadays different foods and vegetables are contaminated with oxalates of sodium and calcium, present in pesticides and chemical fertilisers. Even many drugs contain sodium oxalate, which is a causative agent of urolithiasis and hepatic cell damage. Herbal plant supplements may reduce this drug induced toxicity. Renal and hepatoprotective effects of the aqueous extracts of green tea (*Camellia sinensis*) were studied in sodium oxalate treated rats. Green tea extract was administered orally to Wistar rats for 20 days, exposed to sodium oxalate in different doses (100 mg/kg body weight and 200 mg/kg body weight. It was administered at a dose of 100 mg/kg body weight, which caused hepatic and renal cell damage. As a result, the different stress marker enzyme activity (Aspartate Transaminase, Alanine Transaminase and Alkaline Phosphatase) were increased. Green tea extract have an ameliorative effect on sodium oxalate induced damage and can also reduce the tissue cholesterol level in both liver and kidney.

KEYWORDS: Green tea extract, Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Urolithiasis.

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INTRODUCTION

The liver represents the body's major detoxification system as it inactivates and eliminates waste products and toxic substances that have been ingested, such as food additives, harmful minerals, toxic medications, and toxic metabolites resulting from the cellular break down, and transforms them to less toxic products, so that they can be excreted by the intestines or kidneys; [1] The kidney is the main excretory organ of the body. Kidneys fulfill the important task of purifying the blood from harmful substances, such as toxic medications, poisonous chemical substances and toxic metabolites, by filtering them out of the blood and excreting them in the form of urine. The liver and kidneys automatically detoxify and excrete many toxic materials including metabolic wastes. Sodium oxalate causes renal toxicity by inducing oxidative stress in the kidney, resulting in DNA damage and cell apoptosis.[2] It is crucial to reduce the load of toxic metabolites and toxins accumulated in the liver and kidney tissues, and thus enhance their power of detoxification. Herbal medicines and plant extracts are being increasingly used to treat a wide variety of clinical diseases. More attention is being paid on the medicinal plants to avoid drug induced toxicity in the cell. Tea (*Camellia sinensis*), is the most common beverage used in the world next to water.[3] It belongs to the family *Theaceae.* The family contains about 520 species and are placed in 28 genera.[4] It has a large number of antioxidant components, which help to prevent the free radical induced damage in the cell. The chemical composition of tea is complex: polyphenols, alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates and trace elements. Among these, the polyphenols exhibit the most potent antioxidant and chemo protective properties *in vitro* and *in vivo* which help to increase thermogenesis and whole body fat oxidation, reduce blood pressure and improve body mass index ratio.[5, 6]Lipid peroxidation, a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of processes.[7] Sodium oxalate, a strong dicarboxylic acid and potent oxidative stress inducer is present in many foods such as cocoa, chocolates, almonds, pasta noodles etc.[8] It is produced in the body by metabolism of glyoxylic acid or ascorbic acid. It is not metabolised in the body, but is excreted in urine. High levels of intracellular oxalate exposure in renal and hepatic cells causes' oxidative cell injury, as assessed by increased Lactate Dehydrogenase (LDH) leakage from renal and hepatic cells and also increased lipid peroxidation in these tissues. Conversely, the GSH (Glutathione peroxidase) and Catalase enzymes are depleted due to the administration of oxalate in the renal epithelial cells. Significant oxidative cell injury occurred within 24 hours after oxalate exposure.[9] Oxalate binds with the membrane proteins of the cell. In this regard, it may be said, that, oxalate induces lipid peroxidation in the tissue through oxalate – enzyme interaction due to its chelating activity.[10]The present study was conducted to evaluate the antioxidant effects of Green Tea Extract (GTE) on sodium oxalate induced oxidative damage in rat liver and kidney.

MATERIALS AND METHODS

Chemicals

Assay kits for the estimation of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Cholesterol were purchased from SPAN Diagnostic Limited, Surat (India). Purified sodium oxalate was purchased from EMerck Limited, Mumbai (India). 1g of sodium oxalate was dissolved in 40ml of 0.9% saline to obtain 25mg/ml sodium oxalate solution. The solution was administered intraperitoneally at a concentration of 100 mg/kg body weight in rats to induce lipid peroxidation.

Animals

24 male Wistar rats weighing 150±10g were used in the experiment. They were maintained in a 12 hour light/ dark cycle at 25±2^{o}C. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) of Presidency University and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India (Approval Number 796/2011/CPCSEA/MS-18 /10.09-14).

The composition of the normal diet for the total period (20days) was as follows

- Bengal gram powder:- 3kg
- Milk powder:- 750g
- Sliced bread:-Three and half pounds.
- Bengal gram:-750g

Methods

Preparation of Green Tea Extract (GTE)

Commercially available green tea was purchased from the local market. A stock tea solution was prepared by the following method:-

10g of tea leaves were added to 100ml boiling water and was soaked for 15 minutes. The infusion was cooled at room temperature and then filtered. Then the infusion was boiled with 20ml of water again and the volume was made upto 100ml to obtain 100mg/ml concentration of tea extract. The tea extract was diluted to give the desired concentration to be fed into groups of rats. Administration of tea was through the oral route.[11, 12]

Treatment of Animals

All the rats were divided randomly into four groups (Group A to D) with 6 rats in each group. Rats in Group A (Control) were given distilled water orally along with the normal diet. Rats in Group B (Experimental) were given 100mg/kg body weight of sodium oxalate intraperitoneally along with normal diet and sacrificed after 12 hours. Rats in Group C (Supplement low dose) were fed100mg / kg body weight of tea extract daily. After 20 days all animals were intraperitoneally administered sodium oxalate and sacrificed after 12 hours. Rats in Group D (Supplement high dose) were fed 200mg / kg body weight of tea extract daily. After the first 20 days all animals were intraperitoneally administered sodium oxalate and sacrificed after 12 hours.

Preparation of Tissue Extract

After the treatment period was over, the animals were anesthetised by ether. The abdomen was opened, the liver and kidneys were removed and placed in a beaker containing ice-cold phosphate buffer (pH 7.4). 1.5g of each tissue was minced into small pieces on ice in separate watch glasses and homogenised with two fold weight of ice-cold phosphate buffer in a glass homogenizing tube equipped with a Teflon-pestle. The preparation was centrifuged at 4000 rpm for 10 minutes. The supernatant was decanted and collected in another test tube. Then 0.1ml of supernatant was further diluted with 4.9ml of phosphate buffer solution.[13] The resultant homogenate was used for assessment of different biochemical parameters.

Assessment of Biochemical Parameters

Transaminase (SGOT, SGPT) activity was measured by the method of Reitman and Frankel[14] and that of Alkaline Phosphatase (ALP) was done by the method of Kings and Kings[15] and the serum cholesterol was measured by CHOD PAP method.[16]

Statistical analysis

All data was expressed in terms of mean ± SD (n=6). Multiple co-relation and One-Way ANOVA was performed to determine the level of significance. p values less than 0.05 were considered as moderately significant and 0.01 were as highly significant.

RESULTS

Effect of tea on Aspartate transaminase (AST) activity in the liver and kidney of sodium oxalate treated rats

Table 1

Effect of tea consumption on Aspartate transaminase (AST) activity in the liver and kidney of sodium oxalate treated rats:-

GROUP OF ANIMALS	LIVER (IU/L)	KIDNEY (IU/L)
1. Group A (No treatment)	12.83±0.68	10.66±0.25
2. Group B (100mg/kg body weight sodium oxalate treated)	42.33±0.68	31.33±0.68
3. Group C (100mg/kg body weight tea extract+100mg/kg body weight sodium oxalate treated)	19.33±0.93	13±0.44
4. Group D (200mg/kgbody weight tea extract +100mg/kg body weight sodium oxalate treated)	14±0.44	10.66±0.68

Values are mean ± S.D. p<0.01 (considered for all the groups)

Figure 1

Bar diagrams showing the effect of consumption of tea in different doses for 20 days on the AST activity of liver and kidney of sodium oxalate treated rats

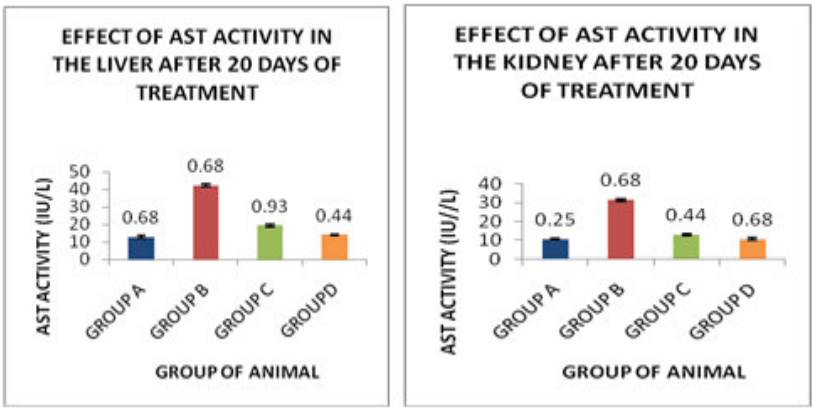

Figure 1 (i) **Figure 1 (ii)**

Group A, B, C and D animals treated as described in table 1

AST activity in the liver and kidney of rats consuming tea before sodium oxalate treatment is presented in **Table 1**. Sodium oxalate treatment produce statistically significant ($p<0.01$) increase in the AST levels in the liver and kidney compared to the control group. Consumption of GTE at doses (100mg /kg body weight and 200mg/kg body weight) for 20days produced a statistically significant decrease in AST activity in the kidney and liver when compared to the experimental group. The increased AST level in the liver and kidney caused due to sodium oxalate treatment was restored to almost to the normal level after 20 days of GTE treatment [Table 1, Fig 1(i), 1(ii)].

Effect of tea on Alanine transaminases (ALT) activity in the liver and kidney of sodium oxalate treated rats

Table 2

Effect of consumption of tea in different doses on the Alanine transaminases (ALT) activity in the liver and kidney of sodium oxalate treated rats:-

GROUP OF ANIMALS	LIVER (IU/L)	KIDNEY (IU/L)
1. Group A (No treatment)	20±0.44	6.75±0.59
2. GroupB (100mg/kg body weight sodium oxalate treated)	144.5±1.18	68.5±0.44
3. Group C (100mg/kg body weight tea extract +100mg/kg body weight sodium oxalate treated)	43.33±0.93	50.9±0.71
4. Group D (200mg/kgbody weight tea extract+ 100mg/kg body weight sodium oxalate treated)	24.16±0.93	9.5±0.59

Values are mean ±S.D. p<0.01(considered for all the groups)

Figure 2

Bar diagrams depicting the effect of consumption of tea in different doses on the ALT activity of the liver and kidney of sodium oxalate treated rats

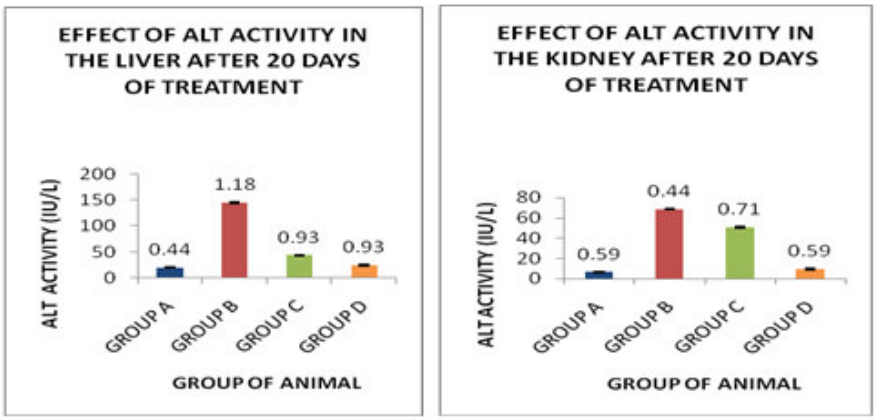

Figure 2 (i) **Figure 2 (ii)**

Group A, B,C and D animals treated as described in table 2

Effect of consumption of tea extract for 20 days at doses of 100mg/kg body weight and 200 mg/kg body weight, on the tissue (liver and kidney) levels of ALT activity in sodium oxalate treated rats is presented in **Table 2**. Administration of 100mg/kg body weight of sodium oxalate to experimental (Group B) group of rats produced a statistically significant rise in the level of ALT as compared to control (Group A) group of rats ($p<0.01$). Prolonged consumption of tea (for 20days) remarkably decreased the level of ALT. [Table 2, Fig 2 (i), 2 (ii)].

Effect of tea on the Alkaline Phosphatase (ALP) activity in the liver and kidney of sodium oxalate treated rats

Table 3

Effect of consumption of tea on the Alkaline Phosphatase (ALP) activity in the liver and kidney of sodium oxalate treated rats

GROUP OF ANIMALS	LIVER(IU/L)	KIDNEY(IU/L)
1. Group A (No treatment)	1.06±0.03	3.33±0.09
2. GroupB (100mg/kg body weight sodium oxalate treated)	3.37±0.06	10.23±1.15
3. GroupC (100mg/kg body weight tea extract + 100mg/kg body weight sodium oxalate treated)	1.27±0.02	1.95±0.11
4. GroupD (200mg/kg body weight tea extract + 100mg/kg body weight sodium oxalate treated)	0.52±0.37	1.26±0.02

Values are mean ±S.D. p<0.01(considered for all the groups)

Figure 3
Bar diagrams showing the effect of consumption of tea in different doses on the ALP activity of liver and kidney of sodium oxalate treated rats

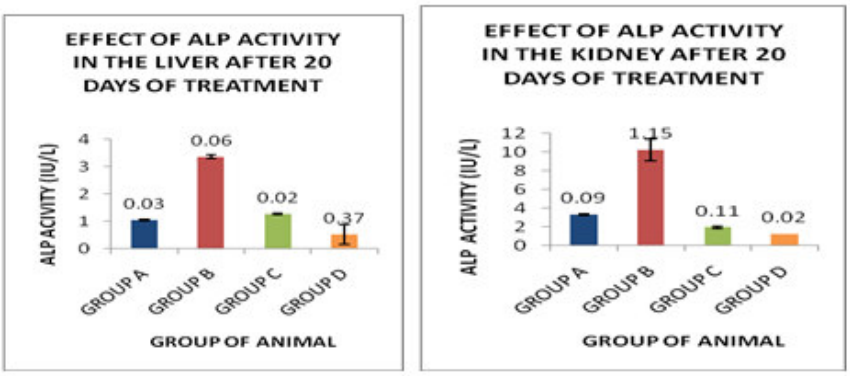

Figure 3 (i) **Figure 3 (ii)**
Group A, B, C and D animals treated as described in table 3

The effect of consumption of tea extract for 20 days on the level of Alkaline Phosphatase in sodium oxalate treated rats is presented in Table 3. After consumption of tea for 20days, the level of ALP in control (Group A) group rats was significantly lower ($p<0.01$) compared to rats treated with sodium oxalate (Group B). Consumption of low concentration of tea (100 mg/kg body weight) for 20days produced a statistically significant lowering of ALP activity ($p<0.01$). Prolonged tea consumption at higher concentration (200 mg/kg body weight) produced about 83% protection in liver and 87% protection in kidney from sodium oxalate effect. There was a significantly pronounced increase in the rate of production of ALP in both the liver and kidney homogenates of the rats treated with sodium oxalate. [Table 3, Fig 3 (i), 3 (ii)].

Effect of consumption of tea on the Cholesterol levels in liver and kidney of sodium oxalate treated rats

Table 4
Effect of consumption of tea extract in different doses on the Cholesterol levels in liver and kidney of sodium oxalate treated rats

GROUP OF ANIMALS	LIVER(mg/dl)	KIDNEY(mg/dl)
1. Group A (No treatment)	83.49±2.27	62.62±2.39
2. Group B (100mg/kg body weight sodium oxalate treated)	89.89±3.25	69.35±2.75
3. Group C (100mg/kg body weight tea extract + 100mg/kg body weight sodium oxalate treated)	43.27±2.50	25.25±1.80
4. Group D (200mg/kg body weight tea extract + 100mg/kg body weight sodium oxalate treated)	8.41±1.88	14.47±3.65

Values are mean ± S.D., p<0.01(considered for all the groups)

Figure 4
Bar diagrams showing the effect of consumption of tea in different doses on the cholesterol levels of liver and kidney of sodium oxalate treated rats

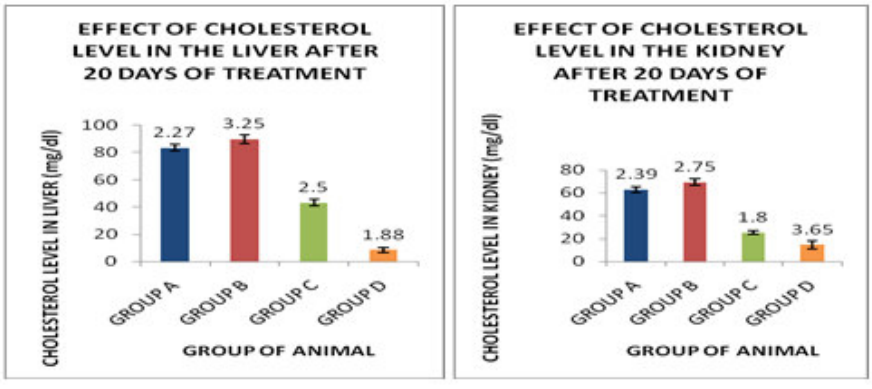

Figure 4 (i) **Figure 4 (ii)**
Group A, B,C and D animals treated as described in table 4

The level of cholesterol in the liver and kidney of Group A, Group B, Group C and Group D rats consuming tea after sodium oxalate treatment is presented in Table 4. From the results, it is clearly evident that sodium oxalate does not have any direct effect in increasing the tissue cholesterol level in both liver and kidney. But prolonged treatment with GTE (20 days) can lower the tissue cholesterol level significantly when compared to control group ($p<0.01$). So the cholesterol levels of GTE treated animals at both doses (100mg/kg body weight and 200 mg/kg body weight) have much lower value than the control group [Table 4, Fig 4 (i), 4 (ii)].

DISCUSSION

Sodium oxalate is a potent stress inducer and damages the hepatic and renal cells exposed to it. But the exact mechanism through which the latter acts as a stress inducer is not clearly known. A few recent studies have attributed the toxic effects of oxalate to the inhibition of the catalase activity in the cell, as a result of which lipid peroxidation is enhanced. Sodium oxalate administration results in hyperoxaluria. The latter is a major risk factor for urolithiasis. As a result, the AST, ALT, and ALP activity is increased in the cell.[17]Some cell culture experiments suggest that hepatic and renal cells exposed to oxalates of sodium and calcium, formed crystals which stimulates early response of gene expression, cytoplasmic reorganisation, and possibly cell proliferation associated with mitogenesis and enhances expression of certain growth factors which augments kidney fibroblast proliferation and hepatic necrosis.[18, 19] Supplementation with GTE may reduce cholesterol levels of liver, kidney and serum. Recently, Thomas J. et al studied the effect of green tea extract on obesity triggered hepatic steatosis in rats, where the hypo-lipidemic effects of green tea extract were shown. A significant decrease was observed in serum triglycerides, cholesterol, VLDL, LDL except HDL levels.[20] The mechanism of action of how green tea can reduce tissue cholesterol levels is not exactly known, but it is suggested that 'flavonoids', the active principle of green tea acts in the aqueous phase, perhaps on the surface of the lipoprotein particle. Some studies (Hsu et al) also suggest that 'catechin' which is another active principle of green tea normalizes the plasma cholesterol level which correlates with our study.[21, 22]

CONCLUSION

From the present study, it may be concluded that sodium oxalate induced hepatic and renal tissue damages occurring in the body can be ameliorated by green tea extract. The tissue cholesterol levels may also be reduced by treatment with green tea extract. Since these models of renal and hepatic tissue damage in the rat simulates many features of human liver and renal pathology, natural antioxidants and scavenging agents in GTE might be effective as renal and hepato – protectors and thus may have some therapeutic implications.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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