ORIGINAL ARTICLE

Effects of Green Tea *(Camellia Sinensis)* on Liver Function Tests of Mice on High Fat Diet

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ABSTRACT

Aim: To evaluate the effects of green tea on liver enzymes of mice on high fat diet.
Methods: Sixty adult mice, Balb-C strain were selected and divided into three groups. The control group was given standard laboratory diet throughout the study. In experimental group A, the study was carried out in two phases. In the first phase, hepatic steatosis was induced by high fat diet containing 4 percent cholesterol powder and 40 percent butter fat for six weeks. In the second phase, experimental group was given normal diet with 1 percent green tea over a period of next six weeks. The experimental group B was given high fat diet containing 4 percent cholesterol powder and 40 percent butter fat with 1 percent green tea over a period of twelve weeks. Ten mice in each were sacrificed at six weeks & remaining ten was sacrificed at twelve weeks and liver enzymes were noted.
Results: Results showed that high fat diet for six weeks produced significant hepatic steatosis, evident on biochemical analysis. When experimental group A (induction phase) with high fat diet was compared with the (reversal phase) on normal diet and green tea, statistically significant difference ($p<0.05$) was noted in terms of liver enzymes. Green tea reverted all parameters in experimental group B, which though reduced never reached the control value and remained somewhat elevated.
Conclusion: It is therefore concluded that green tea protects against the development of hepatic steatosis and reduces hepatic injury in mice by decreasing liver enzymes.
Keywords: Camellia Sinensis, alanine aminotransferase, aspartate aminotransferase.

INTRODUCTION

Green tea (*Camellia Sinensis*) is consumed worldwide, especially in the East Asian countries. Green tea research has been extensively conducted only in recent years. People have been prescribing green tea for a number of ailments for hundreds of years, as well as consumed it daily as a refreshing beverage[1].

Green tea contains caffeine and polyphenolic compounds known as catechins. The chief catechins found in green tea are epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin and epicatechin. EGCG is the most abundant catechins found in green tea, and has displayed potent anti-oxidant effects & others cancer combating properties. Green tea contains approximately three times the quantity of catechins found in black tea and one third the amount of caffeine found in black tea. The anti-oxidant effect of green tea is more stronger than vitamin C or E. Though catechins have been found in other plants, those found in green tea have been proven to be among the most effective anti-oxidants known[2].

The American journal of nutrition published report that men on a combination of caffeine and green tea burned more calories than those given only caffeine.Clinical study suggests that green tea may boost metabolism and increase the amount of calories burnt in twenty four hours. In addition to its weight loss effects, there are studies that suggest that green tea consumption may alleviate other metabolic abnormalities related to obesity such as non-alcoholic (NAFLD). Green tea consumption has been inversely correlated with liver damage and with markers of inflammation in humans. Green tea has been offered as a treatment modality for NAFLD, as it prevent build up of fatty deposit in the liver. If the results can be translated to humans, green tea becomes a useful preventative in the development of fatty liver[3].

The persistent intake of diet rich in saturated fats over a long period of time can lead to non alcoholic fatty liver disease-NAFLD. Non-alcoholic fatty liver disease is a broad term encompassing a spectrum of liver diseases ranging from fatty liver and steatosis to non-alcoholic steatohepatitis (NASH), a condition that may progress to end stage liver disease.[4] Non-alcoholic fatty liver disease is the most common cause of chronic liver disease (CLD) and its incidence is rising world wide. NAFLD was first described by Ludwig in 1980, almost up to three decades ago[5].

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The major risk factors for NAFLD include obesity, diabetes mellitis and dyslipidaemias[6]. The other factors contributing to NAFLD & obesity are the changing life style in Pakistani population, eating habits and lack of physical activity.

Non-alcoholic fatty liver disease (NAFLD) is generally asymptomatic at the time of diagnosis, although many patients report vague symptoms. Hepatomegaly is the only physical finding in most patients[7]. Mild to moderately elevated serum level of aspartate aminotransferase, alanine amino-transferase or both are the most common and often the only laboratory abnormality found in patients with NAFLD. The ratio of aspartate aminotransferase to alanine aminotransferase is usually less than 1, but this ratio increases as fibrosis in advances[8].

Radiological modalities are non-specific, however, ultrasound, computerized tomography and magnetic resonance imaging (MRI) are able to detect hepatic steatosis but are unable to differentiate the non-progressive form of NAFLD (simple steatosis) from potentially progressive form NASH. Despite excellent sensitivity of radiological modalities in detecting significant steatosis, these modalities are not capable of distinguishing between NASH and other forms of NAFLD (Tominaga et al., 1995). Because of the limitations in clinical, laboratory and radiological test, liver biopsy remains the "Gold Standard" for definitive diagnosis of NASH and NAFLD[9].

To date, no single therapy has been approved to directly reduce or reverse liver damage, but it would be desirable to have such a therapy. Out of the various options, the therapy is primarily weight loss followed by drugs and anti oxidants[10]. Improvement in liver function tests is almost universal in obese adults and children after weight reduction. The weight loss however is difficult to achieve and has a poor long term success rate. Several investigators have attempted to explore the potential role of lipid lowering agents in treating patients with NAFLD. Epidemiological data suggests that the consumption of green tea (*Camellia Sinensis*) is associated with reduced mortality from all causes and from cardiovascular disease.[11] However considerable evidence from in vitro, animal and human studies suggests that the protective effect of green tea may be partly mediated through the anti-oxidant properties of its catechins[12]. It has also been reported that green tea protects against the development of hepatic steatosis via multiple mechanisms[13]. The experimental data from rodent models indicate that green tea or its catechins inhibit intestinal lipid absorption.[14] and lowers blood lipids[15]. Moreover, the principal green tea catechins protects against ischaemia/reperfusion induced hepatic steatosis[16] and injury in obese mice by decreasing hepatic lipid accumulation and serum alanine aminotransferase activity[17].

MATERIAL AND METHODS

This analytical experimental randomized control trials study was carried out in the Department of Anatomy, Army Medical College, Rawalpindi, in Collaboration with the department of Pathology, AMC, Rawalpindi and National Institute of Health, Islamabad during twelve weeks. Sixty healthy adult mice, Balb-C strain were obtained, from the animal house of National Institute of Health (NIH), Islamabad (approximate age 8 weeks old, both sexes; weight 20 – 25 grams). All animals were kept under routine animal house conditions at standard room temperature of 18^{o}C to 26^{o}C, for six to twelve weeks. Mice were maintained on 12 hours light/dark cycle.

Sixty adult mice were used in the study, from the animal house of National Institute of Health (NIH), Islamabad. They were randomly divided into three groups of twenty each, control (C), experimental (A) and experimental (B). The control group was given standard laboratory diet throughout the study. In the experimental group A, the study was carried out in two phases. In the first phase, hepatic steatosis was induced by a high fat diet, containing four percent cholesterol powder and 40 percent butter fat (Desi Ghee) over a period of six weeks. In the second phase, the experimental group A was given one percent Green Tea, with the normal laboratory diet for another six weeks. On the other hand the experimental group B, was given high fat diet containing four percent cholesterol powder and forty percent butter fat (Desi Ghee), with one percent Green Tea, throughout the period of twelve weeks.

Ten mice in each group were sacrificed at six weeks and ten were sacrificed at twelve weeks.

Animals were weighed in the beginning-Wi and at the time of sacrifice-Wf.

Blood samples were collected from all groups-4cc each through intra cardiac route at the time of sacrifice (six and twelve weeks) for biochemical analysis. Parameters noted were Serum Alkaline Phosphatase (AP), Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST). Data was entered in a data base using statistical package for social sciences (SPSS) window version 16. "Chi Square" test was used to calculate and compare proportions for qualitative analysis. Results were analyzed and considered significant with P value less than ($p < 0.05$).

RESULTS

Serum Markers of Hepatic Injury-ALT,AST and Alkaline Phosphatase

At six weeks- In control group (n=10), mean value of Alanine aminotransferase, Aspartate aminotransferase, and Alkaline phosphatase were 53±1.4u/l,49±1.2u/l and 212±14.1u/l respectively. In experimental group A (induction phase) mean value of ALT, AST and AP were 201±19.7u/l,214±20.1 u/l and 427±32.5u/l respectively. In experimental group B (n=10) mean values were 91±2.1 u/l, 99±1.5 u/l and 295±17.6 u/l. The P-value between control and experimental groups (A & B) at six weeks were statistically significant (P-value< 0.05, Table 1).

At twelve weeks: In control group (n=10), the mean value of ALT, AST and AP were 48±0.79u/l, 49±0.89u/l and 186±7.9u/l respectively. Mean value of these variables in experimental group A (reversal phase) were 62±2.2u/l, 74±1.9u/l and 182±8.4u/l. In experimental group B the mean values of ALT, AST and AP were 77±3.2u/l, 97±1.7u/l and 192±16.7u/l respectively. The P-value between control and experimental groups (A & B) at twelve weeks were highly significant (P<0.05, Table 2). The P-value of ALT, AST and AP between experimental group A (induction phase) and (reversal phase) were 0.000, 0.019 and 0.005 were highly significant (Table 3).

Fig 1: Photograph showing vacutainer tubes after blood sampling of control and experimental groups.

Table 1: Mean biochemcial parameters at six weeks & statistical significance of quantitative difference between control and experimental groups.

Biochemcial Profile	Control Group (C) Mean±S.E (n = 10)	Group (A) Mean±S.E (n=10) Induction	Group (B) Mean±S.E (n=10)	P-value
ALT	53±1.47	201±19.7	91±2.14	P<0.05
AST	49±1.22	214±20.1	99±1.59	P<0.05
AP	212±14.1	427±32.5	295±17.6	P<0.05

Statistical Significance of Mean biochemical parameters between groups is highly significant
C and A p <0.05, C and B p < 0.05, A and B p <0.05

Table 2: Mean biochemical parameters at twelve weeks & Statistical significance of quantitative difference between control and experimental groups.

Biochemical Profile	Control Group (C) Mean±S.E (n=10)	Group (A) Mean± S.E (n=10) Reversal	Group (B) Mean ± S.E (n=10)	P-value
ALT	48±.79	62±2.2	77±3.2	P<0.05
AST	49±.89	74±1.9	97±1.7	P<0.05
AP	186±7.9	182±8.4	192±16.7	P<0.05

Statistical Significance of ALT, AST, TGL and BSR between control & experimental groups
C and A p <0.05, C and B p <0.05, A and B p >0.05

Table 3: Mean biochemcial parameters & Statistical significance of quantitative difference between experimental A (induction phase) and (reversal phase).

Biochemcial Profile	Experimental A (induction phase) Mean ± S.E (n = 10)	Experimental A (Reversal pahse) Mean ± S.E (n = 10)	P-value
ALT	201±19.7	62±2.2	p<0.05
AST	214±20.1	74±1.9	p<0.05
AP	427±32.5	182±8.4	p<0.05

Key: Experimental A (induction phase)
Experimental A (reversal phase)

DISCUSSION

Mice were chosen for the study because they have many genetic and biochemical similarities with humans. Hepatic steatosis was induced in these mice and effects of green tea (*Camellia Sinensis*) were studied on liver enzymes as serum markers of liver injury (ALT, AST and Alkaline phosphatase). The serum activity of these hepatic enzymes was elevated among experimental groups (A&B) on high fat diet. The experimental group A-induction phase demonstrated a four fold increase hepatic enzymes. Similar elevation of hepatic enzymes was seen in studies carried out by Daniel et al. (1999)[18]. The same elevation was seen among human adults in United States as studied by Clark et al. (2001)[19].

In experimental group A-reversal phase the elevated hepatic enzymes reverted back with green tea. The experimental group B however demonstrated a mild increase in hepatic enzymes of one to two fold as compared to control group. This shows that supplementation of green tea is associated with 40% reduction in the above mentioned hepatic enzymes. Bruno et al demonstrated a reduction in hepatic enzymes with different strengths of green tea in genetically obese mice.

The mechanism leading to the development of hepatic steatosis remains poorly understood, it is often characterized by excess lipid accumulation, hepatic injury and dyslipidemias. The studies have suggested that liver is vulnerable to secondary insult such as those mediated by oxidative stress, which would accelerate the progression of hepatic steatosis towards more debilitating and advanced stages of NAFLD.

CONCLUSION

This study provides evidence that green tea protects against the development of hepatic steatosis and reduces hepatic injury in mice. The findings suggest that green tea may be used as a potential dietary strategy for preventing NAFLD.

REFERENCES

1. Anand, B. P. V., Sabitha, K. E. and Shyamala Devi, C. S. (2006). Green tea extract impedes dyslipidemia and development of cardiac dysfunction in streptozotocin diabetic rats. Clin. Exp. Pharmacol. physiol., 33: 1184-6.
2. Angulo, P. (2002). Nonalcoholic fatty liver disease. N. Engl. J. Med., 346:1221–31.
3. Bruno, R.S., Christine, E., Dugan, Joan, A., Smyth, Dana, A. and Sung, I. (2008). Green Tea Extract Protects Leptin-Deficient, Spontaneously Obese Mice from Hepatic Steatosis and Injury. *Departments of Nutritional Sciences and Pathobiology and Veterinary Science, University of Connecticut, Storrs., 06:269-4017*
4. Chitturi,S., Abeygunasekera, S. and Farrell,G.C. (2002).NASH and insulin resistance insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology.,*35: 373–9.
5. Daniel, S., Ben-Menachem, T., Vasudevan, G., Ma, C.K., Blumenkehl, M. (1999).Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. Am. J. Gastroenterol., 94:3010-3014.
6. Daniells, S. (2008). Green tea shows benefits against fatty liver. Journal of Nutrition., 138: 323.
7. Day, C.P. and James, O.F. (1998).Steatohepatitis: a tale of two "hits"? Gastroenterology., 114:842–5.
8. Farrell,G.C. and Larter,C.Z. (2006).Nonalcoholic fatty liver disease: from Steatosis to cirrhosis. *Hepatology.,*43: 99–112.
9. Fiorini, R. N., Donovan, J. L. and Rodwell,D.(2005). Short term administration of (-) epigallocathingallate reduces hepatic steatosis and protects against hepatic ischemia / re-perfusion injury in steatotic mice. Liver transplant., 11: 298-304.
10. Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., Tsubono, Y., Tsuji, I. (2006). Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAM, 296:1255–65.
11. Lotito, S.B. and Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic. Biol. Med., 41:1727–46.
12. Mathieson, N.L., Franzen, L.E., Fryden, A., Fuberg, U., Bodear, G. (1999). The clinical significances of slightly to moderately increased liver treansaminase values in asymptomatic patients. Scand. J. Gastroenterol., 34: 55-91.
13. Palmer, M. and Schaffner, F. (1990).Effect of weight reduction on hepatic abnormalities in over weight patients.Gastroenterology., 99: 1408-13.
14. Pettigrew, J. (2004). *The Tea Companion: A Connoisseur's Guide.* Philadelphia: Running Press.
15. Wang, S., Noh, S.K. and Koo, S.I. (2006). Green tea catechins inhibit pancreatic phospholipase A(2) and intestinal absorption of lipids in ovariectomized rats. J. Nutr. Biochem., 17:492–8. :2512–8.
16. Yang, S.Q., Lin, H.Z., Lane, M.D., Clemens, M., Diehl, A.M., (1997). Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc. Natl. Acad. Sci., 94:2557-2562.
17. Daniells, S. (2008). Green tea shows benefits against fatty liver. Journal of Nutrition. 138: 323.
18. Clark, J.M., Brancati, F.L. and Diehl, A.M. (2003). The prevalence and etiology of elevated aminotransferase levels in the United States. Am. J. Gastroenterol., 98: 960–7.