ORIGINAL ARTICLE

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

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ABSTRACT

Objectives: To evaluate the effects of green tea on liver histology of mice on high fat diet.
Study Design: Analytical experimental randomized control trial.
Place & Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi and National Institute of Health, Islamabad. Twelve weeks from May 2009 - July 2009.
Material & 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 were sacrificed at twelve weeks.
Results: Showed that high fat diet for six weeks produced significant hepatic steatosis, evident on histological 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 histology. 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.

Key Words: *Camellia Sinensis*, Histomorphology of liver.

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 stronger than vitamin C or E. Anti-oxidant properties protect the cells against the damaging effect of reactive oxygen species such as singlet oxygen, super oxide and hydroxyl radical. 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]

Green tea catechins have also being linked to helping fight bacterial infection, as an anti-viral agent, regulator of cholesterol and have proven useful in the prevention of major conditions like diabetes, cancers (duodenum, lung, liver and mammory gland) and heart diseases.[3]

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 fatty liver disease (NAFLD). 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.[4] The persistent intake of diet rich in saturated fats over a long period of time can lead to non alcoholic fatty liver disease-NAFLD.[5]

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.[6]

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.[7]

The major risk factors for NAFLD include obesity, Diabetes mellitis and dyslipidaemias.[8]

The other factors contributing to NAFLD & obesity are the changing life style in Pakistani population, eating habits and lack of physical activity.

NAFLD accounts for high proportion of liver diseases world wide and is a reversible condition, and if not managed in time will lead to non alcoholic steatohepatitis (NASH). This is irreversible and can progress to chronic liver disease, cirrhosis and hepatocellular carcinoma [(9)].

At present there are no well established treatments for hepatic steatosis beyond weight management.[10]

Therefore the prevention of hepatic steatosis of limiting dietary approaches may reduce the incidence of progressing towards more severe forms of NAFLD.[11]

Epidemiological data suggests that the consumption of green tea (*Camellia Sinensis*) is associated with reduced mortality from all causes and from cardiovascular disease.[12]

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.[13]

It has also been reported that green tea protects against the development of hepatic steatosis via multiple mechanisms. The experimental data from rodent models indicate that green tea or its catechins inhibit intestinal lipid absorption.[14]

Moreover, the principal green tea catechins protects against ischaemia/reperfusion induced hepatic steatosis (Day and James, 1998) and injury in obese mice by decreasing hepatic lipid accumulation.[15,16]

MATERIAL AND METHODS

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.

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, and the histological parameters (qualitative and quantitative) were noted.

Qualitative parameters, General Architecture, Quantitative parameter number of Hepatocytes per high power field, Number of hepatocytes with fatty change per high power field (40X), grading of steatosis, and type of stealosis. Data was entered in a data base using statistical package for social sciences (SPSS) window version 16. Significance was calculated by applying one way “ANOVA” test. “Chi Square” test was used to calculate and compare proportions for qualitative analysis.

RESULTS

The control group, exhibited normal hepatic architecture – consisting of organized plates of hepatocytes, connective tissue stroma and

sinusoidal capillaries. There was no histological evidence of hepatic steatosis. In experimental group A-induction phase, exhibited hepatocytes both with and without fatty change. Large and small vesicles of fat were present within the hepatocytes. This parenchyma was compared to the reversal phase of the same group, where the hepatocyte showed much improvement from steatosis. The experimental group B presented a normal hepatic parenchyma admixed with hepatocytes with fatty change. Mean values of both normal hepatocytes and hepatocytes with fatty change were taken and there p-values between control and experimental groups (A & B) at six and twelve weeks which were highly significant ($p < 0.05$).

Table 1: Mean Number of Hepatocytes at six weeks and Statistical significance of quantitative difference between control and experimental groups

Hepatocytes/HPF	**Control Group (C) Mean ± S.E (n = 10)**	**Experimental Group (A) Mean ± S.E (n = 10)**	**Experimental Group (B) Mean ± S.E (n = 10)**	**p- Value**
No of hepatocytes	230±2.5	225±2.1	221±1.8	p>0.05
No of hepatocytes with fatty change	Nil	79±7.3	31±3.6	p<0.05

Table 2: Mean Number of Hepatocytes at twelve weeks and Statistical significance of quantitative difference between control and experimental groups

Hepatocytes/HPF	**Control Group (C) Mean ± S.E (n = 10)**	**Experimental Group (A) Mean ± S.E (n = 10)**	**Experimental Group (B) Mean ± S.E (n = 10)**	**p- Value**
No of hepatocytes	229±2.4	225±2.1	219±1.8	p<0.05
No of hepatocytes with fatty change	Nil	17.7±2.7	31±3.6	p<0.05

Table 3: Mean Number of Hepatocytes & Statistical significance of quantitative difference between experimental A (Induction phase) and (reversal phase)

Hepatocytes/HPF	**Experimental A (induction phase) Mean ± S.E (n = 10)**	**Experimental A (reversal phase) Mean ± S.E (n = 10)**	**P- Value**
No of hepatocytes	225±2.1	225±2.1	p<0.05
No of hepatocytes with fatty change	79±7.3	17±2.7	p<0.05

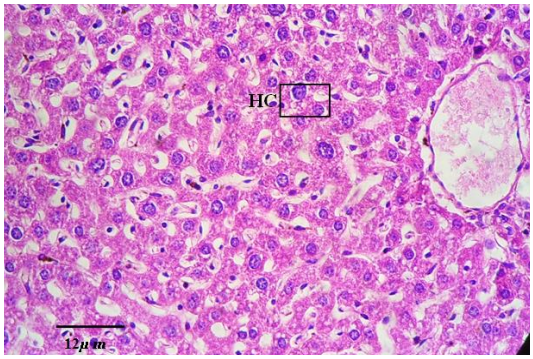

Fig 1: Photomicrograph showing section of mice liver: control group, animal no.5, showing magnified view of parenchyma, box showing column of hepatocytes. H & E stain. Bar 12 µ m.

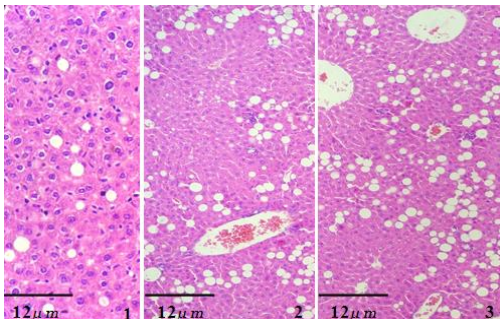

Fig 2: Photomicrograph showing grades of steatosis in experimental group A (induction phase), animal no.8, (1) mild steatosis (2) Moderate steatosis (3) severe steatosis. H&E stain. Bar 12µ m.

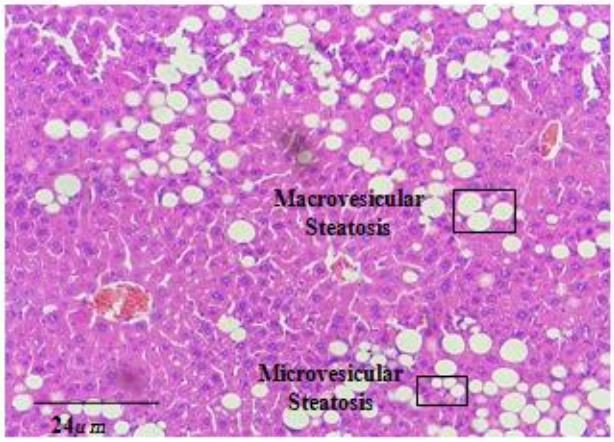

Fig 3: Photomicrograph of mice liver, experimental group A (induction phase) showing micro vesicular steatosis. H & E stain. Bar 24µ m.

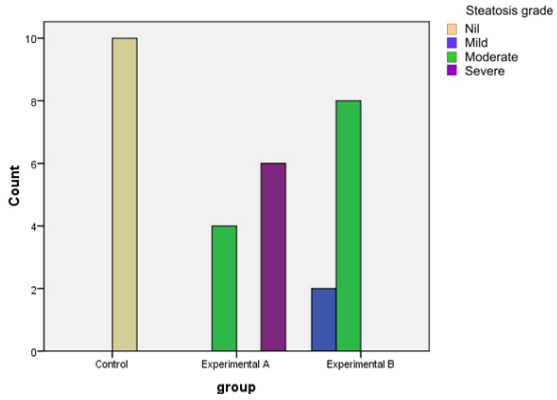

Fig 4: Bar Chart showing grades of steatosis at six weeks between control and experimental groups

Percentage of steatosis i.e. the percentage of total heapatocytes volume affected by fat was calculated. The percentage of each sample was averaged and graded following Brunt's grading system To the percentage of steatosis in control group, Experimental groups (A & B) chi-square test was applied which gave statistically significant results ($p<0.05$) both at six and twelve weeks.

There were no fat globules in control group. In experimental group A (induction phase) showed micro vesicular steatosis in 60% samples, and a mixed steatosis in 40%. In experimental group A (reversal phase) showed micro vesicular steatosis in 100%. Experimental group B showed 95% micro vesicular and 5% showed mixed steatosis, chi-square test was applied to the type of steatosis between control and experimental groups (A & B), showed significant results ($p<0.05$).

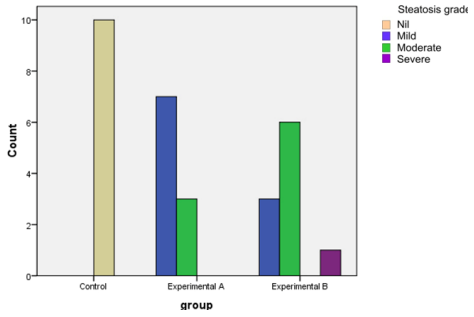

Fig 5: Bar Chart showing grade of steatosis at twelve weeks between control and experimental groups.

Table 4: Percentage, Grade and type of steatosis at six weeks & Statistical significance between control and experimental groups

Brunt's Grading System:

*Mild Steatosis <10%of hepatocytes.

**Moderate Steatosis-10 to 30% of hepatocytes.

***Severe Steatosis >than 30% of hepatocytes.

	Control Group (C) (n = 10)	Experimental Group (A) (n = 10)	Experimental Group (B) (n = 10)	P- Value
Percentage of Steatosis	Nil	35%	15%	p<0.05
Grades of Steatosis	Nil	Severe	Moderate	p<0.05
Type of Steatosis	Nil	Mixed	Micro vesicular	P<0.05

Table 5: Percentage, Grade and type of steatosis at twelve weeks & Statistical significance between control and experimental groups

	Control Group (C) (n = 10)	Experimental Group (A) (n = 10)	Experimental Group (B) (n = 10)	p- Value
Percentage of Steatosis	Nil	9%	15%	p<0.05
Grades of Steatosis	Nil	Mild	Moderate	p<0.05
Type of Steatosis	Nil	Micro Vesicular	Micro Vesicular	p<0.05

Table 6: Percentage, Grade and type of steatosis & its Statistical significance between experimental A (induction phase) and (reversal phase)

	Experimental A (indcution phase) (n = 10)	Experimental A (reversal phase) (n = 10)	p- Value
Percentage of Steatosis	35%	9%	p<0.05
Grades of Steatosis	Severe	Mild	p<0.05
Type of Steatosis	Mixed	Micro Vesicular	p<0.05

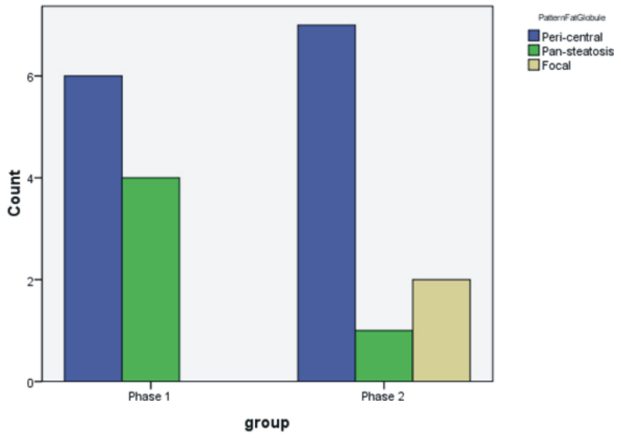

Fig 6: Bar Chart showing pattern of steatosis between Experimental group A (induction phase) and (reversal phase)

DISCUSSION

Histological evaluation is regarded as "gold standard approach" to evaluate the presence and severity of non-alcoholic fatty liver disease.[17] The effect of green tea on liver histology was evaluated to assess the extent to which green tea averted the development of hepatic steatosis. The number of hepatocytes, (with and without fatty change), were counted per high power field. Percentage steatosis was based on percentage of total hepatocyte volume affected by fat. The percentage of each sample was averaged and graded by Brunt's grading system, into mild, moderate and severe. Steatosis was assessed quantitatively.

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The mice in the control group exhibited normal hepatic architecture. There was little or no histological evidence of hepatic steatosis. In contrast, in the experimental group A-induction phase, moderate to severe steatosis was observed, with micro vesicular fat mostly in centri-lobular distribution. A marked reduction in the degree of steatosis was noted in the livers from mice, in the reversal phase. Steatosis scores were lower in the mice, from experimental group B, on high fat diet and green tea for twelve weeks.

Similar effect of green tea consumption on hepatic steatosis was demonstrated by Farrell and Larter (2006) and Imai and Nakachi.[18,19]

The development of NAFLD is significant as it progresses to non-alcoholic steatosis to hepatitis (NASH), which can results in cirrhosis and hepatocellular carcinoma.[20]

Chalasani and Colleagues investigated histologic features that define NASH in 331 liver biopsy specimens, with 5 percent or greater steatosis.[21]

In this cohort, there was statistically significant relationship between severity and lobular inflammation, zone 3 fibrosis and presence of NASH.[22] Kleiner and Colleagues reviewed 364 biopsies and confirmed a unique type of NASH in children, with a significant zone 1 steatosis [(23)].

Epidemiological data suggests that the consumption of green tea *(camellia sinensis)* is associated with reduce mortality from all causes and from cardiovascular disease. However considerable evidence from in vitro, animal and human studies suggest that the protective effect of green tea may be partly meditated through the anti-oxidant properties of its catechins.[24]

CONCLUSION

The study provides evidence that green tea has a role against. The development of hepatic steatosis and reduces hepatic injury in mice.

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