Original Article

EFFECTS OF GREEN TEA (CAMELLIA SINENSIS) ON LIVER HISTOLOGY OF MICE ON HIGH FAT DIET- A MORPHOMETRIC STUDY

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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 in collaboration with the Department of Pathology, Army Medical College, Rawalpindi and National Institute of Health, Islamabad. Twelve weeks study.

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 group were sacrificed at six weeks & remaining ten were sacrificed at twelve weeks.

RESULTS:

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, Morphometry.

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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.

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.[2]

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.[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.[4]

The major risk factors for NAFLD include obesity, Diabetes mellitis and dyslipidaemias.[5] The other factors contributing to NAFLD & obesity are the changing life style in Pakistani population, eating habits and lack of physical activity.[6,7]

NAFLD seems to be a major public health concern in Asia Pacific region. In all Asian pacific countries, where estimation of NAFLD Prevalence has been made, the magnitude of the problem is comparable to western countries.[8] In Pakistan, fatty liver disease has been reported with some frequency. In Pakistan, data regarding NAFLD is lacking, but with increasing awareness and understanding about this disease, gradual rising trend is seen.[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. An attempt at gradual weight loss along with appropriate metabolic control is a useful first step.[10]

The experimental data from rodent models indicate that green tea or its catechins inhibit intestinal lipid absorption and lowers blood lipids.[11-13] Moreover, the principal green tea catechins protects against ischaemia/ reperfusion induced hepatic steatosis and injury in obese mice by decreasing hepatic lipid accumulation and serum alanine aminotransferase activity.[14,15]

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.

The general architecture of the liver was noted. The number of hepatocytes per high power field were counted, number of hepatocytes with fatty change per high power field (40x) were counted, percentage of hepatic steatosis calculated, and graded into mild, moderate and severe.

The study was used to assess the diameter of fat globules in H & E stained liver sections with steatosis. Three fields were used around portal tract, central vein or lobule. The diameter of fat globules was obtained (all measurements in micrometers). The mean diameter of fat globule was calculated under 40X objective. The diameter was measured with eye piece micrometer.

Fat globules were graded microvesicular steatosis when mean diameter of fat globules was less than 15µm. While mean diameter of fat globules equal to or larger than 15µm was termed as macrovesicular or mixed steatosis respectively.

For the calculation of diameter of fat globule, ocular micrometer was used, which was calibrated with the stage micrometer. The stage micrometer is a slide with I millimeter long scale etched on the surface. This 1 millimeter was divided into 100 divisions, so that each division was equal to 0.01mm (10µm). The stage micrometer was placed on the stage of the micrometer and focused under 40X objective. The number of divisions of ocular micrometer corresponding with that of stage micrometer were noted and then the value of one division of ocular micrometer was calculated. The ocular micrometer scale was superimposed on the fat globules and the mean diameter of the globule was calculated.

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 were analyzed and considered significant with P value less than ($p<0.05$).

RESULTS:

Mean diameter of fat globules:

- There were no fat globules in the control group. The mean diameter of fat globules in experimental group A-induction phase was 14.8 ± 1.05µm. In experimental group B the mean diameter of fat globules was 9.1 ± 0.37µm at six weeks, which was statistically significant with a P-value of <0.05. At twelve weeks the mean diameter between control, experimental A and experimental group B were nil, 5.9 ± 0.84µm and 11.2 ± 0.53µm respectively. P-value was determined which was statistically significant (Table 3).

The type of steatosis was determined in each group using zaitoun's grading system.[23] 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 1: Photograph showing mice liver of experimental group A (induction phase) and control group, immersed in 10% buffered formalin for fixation.

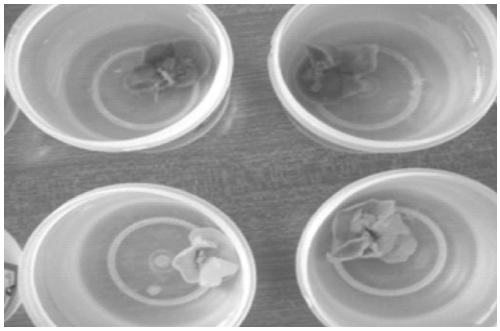


Fig2: Photomicrograph showing grades of steatosis in experimental group A (induction phase, (1) mild steatosis (2) moderate steatosis (3) severe steatosis. H&E stain. Bar 12µ m.

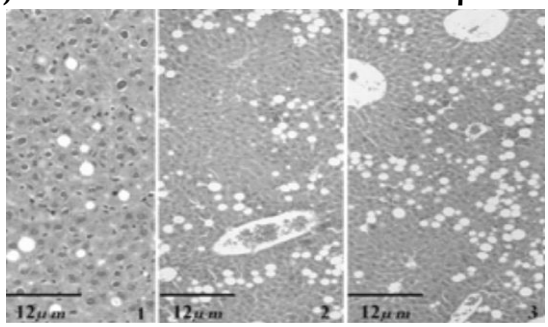


Fig3: Photomicrograph of mice liver, experimental group A (induction phase) showing micro vesicular steatosis. H & E stain. Bar 24µ m.

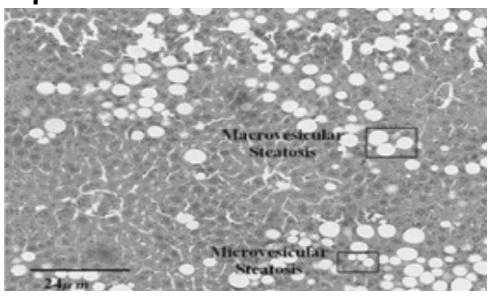


Fig4: Photomicrograph of mice liver experimental group B showing micro vesicular type of steatosis. H & E stain. Bar 24µ m.

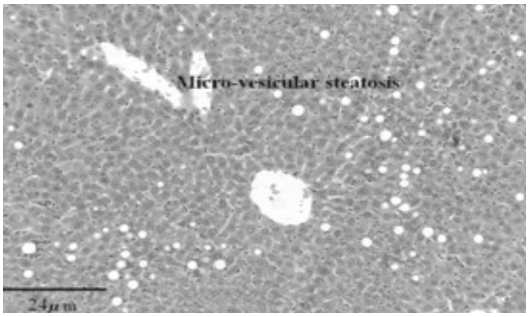


Table 1: Mean diameter of fat globules at six weeks & statistical significance of quantitative difference between control and experimental group.

	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
Mean Diameter (µm)	Nil	14.8±1.05	9.1±.37	P<0.05

Table 2: Mean diameter of fat globules at twelve weeks & statistical significance of quantitative difference between control and experimental groups

	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
Mean Diameter (µm)	Nil	5.9+.84	11.2+.53	P<0.05

Table 3: Mean diameter of fat globules and statistical significance of quantitative difference between experimental A (induction phase) and (reversal phase)

	Experimental A (induction phase) Mean ± S.E (n = 10)	Experimental A (reversal phase) Mean ± S.E (n = 10)	P- Value
Mean Diameter (µm)	14.8±1.0	5.9±.84	P<0.05

Fig 4, Bar Chart showing type of steatosis (micro vesicular, macro vesicular and mixed) at six weeks between control and experimental groups

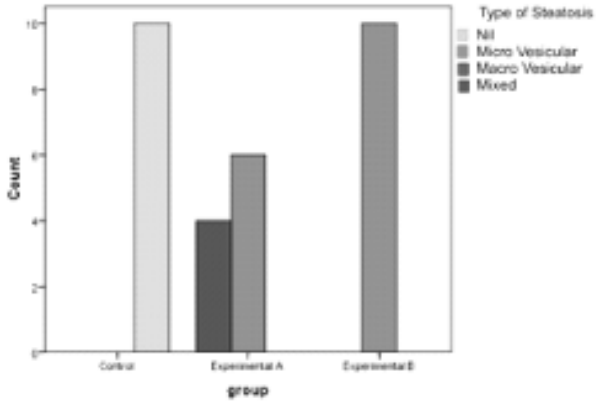
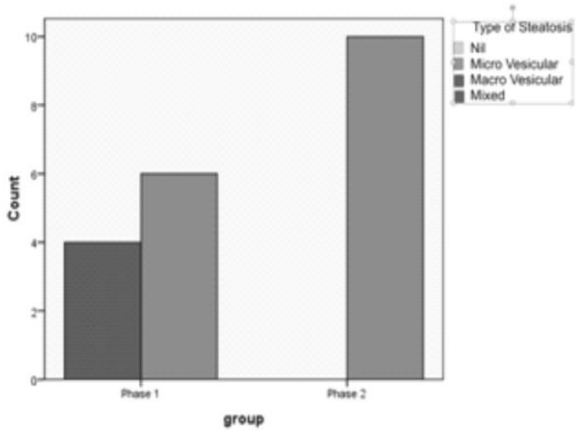


Fig 5, Bar Chart showing type of steatosis Experimental group A (induction phase) and (reversal phase)



DISCUSSION:

Histological evaluation is regarded as "gold standard approach" to evaluate the presence and severity of non-alcoholic fatty liver disease.[16] 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.[24]

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 (1995).[17,18]

The development of NAFLD is significant as it progresses to non-alcoholic steatosis to hepatitis (NASH), which can result in cirrhosis and hepatocellular carcinoma.[19]

Chalasani and Colleagues investigated histologic features that define NASH in 331 liver biopsy specimens, with 5 percent or greater steatosis.[20]

Kleiner and Colleagues reviewed 364 biopsies and confirmed a unique type of NASH in children, with a significant zone 1 steatosis.[21]

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.[22]

CONCLUSION:

The study provides evidence that green tea plays a protective role against the development of hepatic steatosis and reduces hepatic injury.

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