ORIGINAL ARTICLE

Effects of Green Tea (Camellia Sinensis) on Liver Morphology and Body Weight of Mice on High Fat Diet

*FARHANA SAJJAD, LIAQAT ALI MINHAS
Department of Anatomy, Allama Iqbal Medical College, Lahore

ABSTRACT

Objective: The objective of this study was to evaluate the effects of green tea on liver morphology and weight 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 were sacrificed at twelve weeks.

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 body weight and morphology / gross 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 and body weight 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 epigallocatechingallate(EGCG), epicatechingallate, 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).

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].

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.[5]

Non-alcoholic fatty liver disease is the most common cause of chronic liver disease (CLD) and its incidence is rising world wide.

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. A cross-sectional study was conducted at DHQ Teaching hospital, D.I.Khan, by Hussein et al.(2006) showed that 33% obese people were using oil-rich foods while 30% were taking food more than three times a day (2006). Similar results were seen in other studies by Jaffar et al.(2006) from AKU (2006) and Aziz et al.(2009) from SIUT.[7,8,9]

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.[12]

MATERIAL AND METHODS

The study was carried out in the department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. The duration of study was 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 animal house of 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.

Mice were euthanized by ether anaesthesia. Mice were dissected, and liver was removed enbloc by cutting the ligaments of liver. ross parameters were noted were colour & appearance of the liver, texture of the liver, weight of the liver. The body weight of the animal in the beginning Wi and at the time of sacrifice-Wf.

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

All animal survived and remained active / healthy throughout the duration of experimental period. The food intake remained steady in control and experimental group (A & B) as assessed by 24 hours left over food. But the revered phase mice looked quit thin, compare with the induction phase mice.

In the control group (n = 20), all the liver samples exhibited a healthy look. The livers were reddish brown in colour, had glistening capsule. The texture were entirely smooth and had a firm consistency. In experimental group A-induction phase (n = 10) all liver samples presented a waxy, greasy and flabby look. Almost all samples were yellowish brown in

colour and had a dull capsule. The textures were smooth, but friable and difficult to handle. In mice from experimental group A-reversal phase (n = 10) livers presented a healthy look. The liver were reddish brown in colour and had a normal healthy looking shinning capsule. The textures were entirely smooth and had a firm Consistency, which was similar to the control group livers. In experimental group B (n -=20), all liver samples appeared healthy. Sixteen liver samples presented a reddish brown colour and a glistening capsule. Four liver samples exhibited a yellowish hue. The textures were completely smooth and had a firm consistency.

The weight of the liver at six weeks in different study groups were compared and mean values calculated. The mean values of weight of liver at six weeks between control group, Experimental group A and Experimental group B were 0.77+0.02g,1.31+0.04g and 0.96+0.04g respectively, with a P-value<0.05, which is highly significant (Table-1). The mean values of weight of liver between control, experimental group A (reversal phase) and experimental group B at twelve weeks were 0.78+0.02g,0.89+0.04g and 0.97+0.04g respectively with a P-value<0.05 which is statistically significant (Table-2).

The P value of weight of liver between exPerimental grouP A (induction Phase and (reversal Phase) showed statistically insignificant results (Table-3)

The Mean initial weight (wi) at six weeks, experimental group A (induction phase) & experimental group B were 23.20+0.32g, 22.60+0.47g and 22.30+0.33g respectively. At the end of six weeks, the mean final weight (wf) of mice in control group, experimental group A (induction phase) & experimental group B were 36.60+1.21g, 43.20+1.69g and 37.80+1.49g respectively. The difference between the mean body weight at six weeks between groups (C, A & B) was statistically significant (P<0.05, Table).

The Mean initial weight (wi) at twelve weeks in the three groups-control, experimental A (reversal phase) and experimental group B were 23.20+0.32g , 22.60+0.47g and 22.30+0.33g respectively. Mean final weight (wf) of mice in the three groups-control, experimental A (reversal phase) and experimental B were 36.20+1.3g, 35.30+1.1g and 37.10+1.5g respectively. The difference between the mean the body weight at twelve weeks between groups (C, A & B) was statistically in- significant (p>0.05, Table).

Fig 1: Photograph showing experimental group A (induction phase) Laboratory mice.

Table 1: Mean weight of Liver at six weeks & Statistical significance of quantitative difference between control and experimental groups

	Control Group (C) Mean + S.E	Experimental Group (A) Mean + S.E	Experimental Group (B) Mean + S.E	P-Value
Weight of liver (g)	.77+.02	1.31+.04	.96+.04	P<0.05

Statistical Significance of Mean Liver Weight difference between groups in highly significant
C and A P<0.05
C and B P<0.05
A and B P<0.05

Table 2: Mean Weight of Liver at twelve weeks & Statistical significance difference between control and experimental groups

	Control Group (C) Mean + S.E	Experimental Group (A) Mean + S.E	Experimental Group (B) Mean + S.E	P-Value
Weight of liver (g)	.78±.02	.89±.04	.97±.04	P<0.05

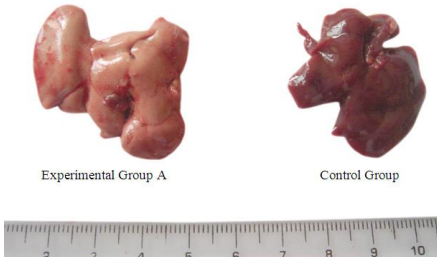

Fig 3: Photograph showing gross difference of colour & appearance of mice liver between experimental group A (induction phase) and control group.

Table 3: Mean Weight of liver & Statistical significance of quantitative between experimental A (induction phase) and (reversal phase)

	Experimental A (Induction phase) Mean ± S.E	Experimental A (Reversal phase) Mean ± S.E	P- Value
Weight of liver (g)	1.31±.04	.89±.04	P>0.05

Key: Experimental A (induction phase)

Experimental A (reversal phase)

Table 4: Mean Body Weight at six weeks & Statistical significance of quantitative difference between control and experimental groups

Group	Control (n = 10)	Experimental A (n = 10)	Experimental B (n = 10)	P- Value
Initial Weight- wi Mean± S.E	23.20±.32	22.60±.47	22.30±.33	P>0.05
Final Weight-wf Mean± S.E	36.60±1.21	43.20±1.69	37.80±1.49	P<0.05

Table 5: Mean Body Weight at twelve weeks & Statistical significance of quantitative difference between control and experimental groups

Group	Control (n = 10)	Experimental A (n = 10)	Experimental B (n = 10)	P- Value
Initial Weight- wi Mean± S.E	23.20±.32	22.60±.47	22.30±.33	P>0.05
Final Weight-wf Mean± S.E	36.20±1.3	35.30±1.1	37.10±1.5	P>0.05

Table 6: Mean Body Weight & Statistical significance of quantitative difference between experimental A (induction phase) and (reversal phase)

Group	Experimental A (induction phase) (n = 10)	Experimental A (reversal phase) (n = 10)	P- Value
Initial Weight- wi Mean± S.E	22.60±.47	22.60±.30	P>0.05
Final Weight- wf Mean± S.E	43.20±1.6	35.30±1.9	P<0.05

Key: Experimental A (induction phase)

Experimental A (reversal phase)

DISCUSSION

This study was conducted in adult mice to observe the morphological changes in the liver of three different groups of mice and body weight of mice. 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) on morphology and body weight were studied.

Regarding the gross study of the liver, the liver samples in the control group presented a healthy look (Appendix IX). The healthy liver was reddish brown in color and had a glistening capsule. In experimental group A-induction phase all the livers appeared waxy and greasy, they were friable and difficult to handle. In the reversal phase of the same group, green tea administration resulted in most of the liver samples presenting with a healthy look and firm consistency. In experimental group B, all samples presented a healthy look. Hence these results pertaining to colour, appearance and texture of liver in various groups represents a protective effect of green tea on the liver. A similar spectrum of gross appearance of liver has been described by Matteoni et al. (1998).[13]

In this study, six to twelve weeks dietary green tea treatment significantly decreased body weight in mice on high fat diet as evident from both experimental groups (Table 4, 5 & 6). In experimental group A, high fat fed mice, were significantly heavier after six weeks than green tea treated reversal phase mice. Mice

body weight of experimental group A (induction phase) was 43.2 ±1.6g and that of reversal phase mice was 35.3 ±1.9g, the p - value < 0.05 for this parameter was statistically significant (Table 3). In experimental group B, mean mice body weight after twelve weeks of high fat diet and green tea were comparable to that of control group.

This demonstrated the role of green tea (camellia sinensis) treatment in reduction of body weight. Similar results were obtained in previous studies. This was also evident in a study carried out by Klaus et al., 2005.[16] Another study carried out by Wolfram et al., 2005 showed that sixteen weeks EGCG treatment significantly decreased body weight in rodents by reducing adipose tissue mass.[17] The same observations were made by Richard Bruno and colleagues. They noted that obese mice given different strengths of green tea weighed twenty three to twenty five percent less than that in the control group.[18] This was comparable to our study where green tea in experimental group A reversal phase induced 35% weight loss.

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. Pettigrew, J. (2004). The Tea Companion: A Connoisseur's Guide. Philadelphia: Running Press.
2. 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
3. Singaravel, S., Srinivasan, D. and Jothivel, N. (2008). Hepatoprotective Activity of Camellia sinensis and its Possible Mechanism of Action Iranian Journal of Pharmacology & Therapeutics. IJPT., 7:9-14,
4. 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.
5. Angulo, P., Hui, J.M., Marchesini, G. and Bugianesi. (2007). The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology., 45:846–54.
6. 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: 37-9.
7. Hussain, M.K., Khan, H., Sarwar, G., Iftikhar, B., Jan, A., Naimat-ullah, M. and Gul, A. (2006). Study of Obese Persons Profile At D.I. Khan, NWFP, Pakistan.
8. Jafar, T.H., Chaturvedi, N. and Gregory, P. (2006) Prevalence of overweight and obesity and their association with hypertension and diabetes mellitus in an Indo-Asian population. CMAJ., 175(9): 1071–1077.
9. Aziz, S., Noorualin, W., Zaidi, U.R., Hossain, K., Siddiqui I.A. (2009). Prevalence of overweight and obesity among children and adolescents of affluent schools in Karachi. JAMA.,84(1): 84-91.
10. Ayyad, C. and Andersen, T. (2000). Long-term efficacy of dietary treatment of obesity. Obes. Rev., 1:113–9.
11. Angulo, P., Hui, J.M., Marchesini, G. and Bugianesi. (2007). The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology., 45:846–54.
12. 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.
13. Matteoni, C.A. and Younossi, Z.M. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity.Gastroenterology.116:1413-19
14. Ross, M. H., Kaye, G. I and Pawlina, W. (2003). Digestive system III: Liver, Gall bladder and Pancreas In: Histology, A Text and Atlas. Lippincott Williams & Wilkins, Philadelphia. pp.532-37.
15. Murase, T., Nagasawa, A., Suzuki, J., Hase, T., Tokimitsu, I. (2002). Beneficial effects of tea catechins on diet-induced obesity: stimulation

of lipid catabolism in the liver. Int. J. Obes Relat Metab Disord., 26:1459–64.
16. Klaus, S., Pultz, S., Thone-Reineke, C., Wolfram, S. (2005). Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int. J. Obes. (Lond)., 29:615–23.
17. Wolfram, S., Raederstorff, D., Preller, M., Wang, Y., Teixeira, S.R., Riegger, C., Weber, P. (2006). Epigallocatechin gallate supplementation alleviates diabetes in rodents. J. Nutr.,136:2512–8.
18. 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 utritional Sciences and Pathobiology and Veterinary Science, University of Connecticut, Storrs., 06:269-4017