ABSTRACT:

OBJECTIVES:
The objective of the study was to evaluate the effects of green tea on blood sugar 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. The duration of study was twelve weeks.

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

RESULTS:
The result 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 biochemical markers (Blood Glucose). Green tea affected all the biochemical parameters, 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, reduces hepatic injury and significantly lower the blood glucose.

KEY WORDS:
*Camellia Sinensis*, Blood Glucose.

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 mammary 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

The major risk factors for NAFLD include obesity, Diabetes mellitis and dyslipidaemias.⁵

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

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 though the 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 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.¹¹

The experimental data from rodent models indicate that green tea or its catechins inhibit intestinal lipid absorption and lowers blood lipids and glucose.¹²-¹⁴

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° C to 26° 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. 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, Blood Glucose, Serum total Cholesterol and Serum Triglycerides. 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

At six weeks- The mean values of blood glucose in control, experimental group A (induction phase) and experimental group B were 6.7±0.18 mmol/l, 12.7±0.58 mmol/l and 9.7±0.16 mmol/l respectively, with (P-value<0.05), (Table-1). The P-value of blood sugar random (BSR) between experimental group A (induction phase) and (reversal phase) showed significant results (P<0.05, Table-3).

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

<table>
<thead>
<tr>
<th>Biochemical Profile</th>
<th>Control Group (C)</th>
<th>Experimental Group (A)</th>
<th>Experimental Group (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E (n = 10)</td>
<td>Mean ± S.E (n = 10)</td>
<td>Mean ± S.E (n = 10)</td>
<td></td>
</tr>
<tr>
<td>BSR</td>
<td>6.7±0.18</td>
<td>12.7±0.58</td>
<td>9.7±0.16</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

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

At twelve weeks- The mean values of blood glucose in control, experimental group A (reversal phase) and experimental group B were 5.8±0.20 mmol/l, 8.9±0.19 mmol/l and 9.8±0.22 mmol/l respectively with a (P-value<0.05), which is highly significant(Table-2).

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

<table>
<thead>
<tr>
<th>Biochemical Profile</th>
<th>Control Group (C)</th>
<th>Experimental Group (A)</th>
<th>Experimental Group (B)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>BSR</td>
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<td>8.9±0.19</td>
<td>9.8±0.22</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Statistical Significance of 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 biochemical parameters & Statistical significance of quantitative difference between experimental A (induction phase) and (reversal phase).

<table>
<thead>
<tr>
<th>Biochemical Profile</th>
<th>Experimental A (Induction phase) Mean ± S.E (n = 10)</th>
<th>Experimental A (Reversal phase) Mean ± S.E (n = 10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSR</td>
<td>12.7 ± 0.58</td>
<td>8.9 ± 0.19</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

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 blood glucose (BSR). The conditions coexisting with a fatty liver can be Hyperlipidemia, Obesity and Diabetes Mellitus and these are known risk factors for hepatic steatosis as shown by Assy et al. (2000). The other frequently coexisting condition with NAFLD is Diabetes Mellitus. In this study blood glucose showed a significant increase in experimental group A-induction phase. This was lowered by green tea in the reversal phase by 56%. However this did not come back to the pre-induction phase value (Table 1, 2 & 3). The experimental group B, (high fat diet and green tea) demonstrated an elevation in blood sugar. This leads to the outcome that green tea reverses hyperglycemia by 55% as compared to 40% when taken with high fat diet.

Angulo (2002) also noted hyperlipidemia and hyperglycemia as two coexistent conditions frequently associated with non alcoholic fatty liver disease (NAFLD). This was reiterated by other researchers as Adler and Schaffiner (1979), Lee (1989), Powel and Cooksley (1990) and Bacon and Farahvash (1994). These findings are in conformity with the research carried out by Daskalopoulou et al. (2004). Mousumi Bose et al (2008) also demonstrated a twenty five percent reduction in blood glucose. Similar results were seen by Tsuneki et al. (2004), Wolfram et al. (2006).

CONCLUSION:

The study provides evidence that green tea has a role against the development of hepatic steatosis, reduces hepatic injury and significantly lower blood glucose. The findings suggest that green tea may be used as a potential dietary strategy for preventing NAFLD and hyperglycaemia.

REFERENCES:


