CHAPTER-III

SIGNIFICANCE OF FLUORIDE ON POLYPHENOLS IN GREEN TEA

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Chapter - III

Significance of fluoride on polyphenols in green tea

3.1. INTRODUCTION

The consumption of tea as a popular drink is to be used universally. Tea was discovered in China around 2000 BC (Campbell, 1995). But the main cultivation is in the hilly regions of tropical countries like India, China, Hong Kong and neighboring countries. The tea which we drink is black tea which is a fermented product of tender leaves by the suitable processing. There are also other varieties of tea, and green tea is one among them which is now used in several parts of the world. (Cao et al., 1995; Graham, 1983)

The present work is based on this tea (green tea). Although several studies are carried out with respect to green tea and black tea it is still necessary to investigate the biological aspects of this tea. It is found that tea contain about 700 organic compounds (Bonoli et al., 2003) but the most important ones are mainly polyphenols, Carotenoids, tocoferols, vitamin C. Flavonoids (and their fraction – catechins) are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic et al., 2009; Cai et al., 2002).

The minerals present are mainly aluminium, chromium, manganese, selenium, zinc and surprisingly a lot of fluoride (Fung et al., 1999). The concentration of fluoride in brewed tea is dependent upon the concentration of soluble fluoride in the tea leaves, the level of fluoride in the water used in its preparation and the length of the brewing period (Smid & Kruger, 1985). In 2008 the US Pharmacopedia reviewed the safety of green tea extract. It found 216 case reports, 34 on liver damage, of which 27 were categorized as possible and 7 were categorized as probable. Potential for adverse effects is increased when extracts are used, in particular on an empty stomach (Sarma et al., 2008 Wu et al., 2001). There are few data from which to estimate total exposure to and the bioavailability of fluoride, and there are inconsistencies in reports on the characterization of its adverse effects. The main natural source of inorganic fluorides in soil is the
parent rock (WHO, 1984). During weathering, some fluoride minerals (e.g., cryolite or Na3AlF6) are rapidly broken down, especially under acidic conditions (Fuge & Andrews, 1988). In water, the transport and transformation of inorganic fluorides are influenced by pH, water hardness and the presence of ion exchange materials like clays (Mengel, 2011). Fluoride is usually transported through the water cycle complexes with aluminium (Ares, 1990). Factors that influence the mobility of inorganic fluorides in soil are pH and the formation of aluminium and calcium complexes (Pickering, 1985; Mengel, 2011). Fluoride had been used for decades as effective anti-thyroid medications to treat hyperthyroidism (Galetti, and Joyet, 1958), but it has adversely affected the action of antioxidant enzymes (Szturbal et al., 1998)

From the above information, for the present work, we have used water, methanol and ethyl acetate as solvents for extraction. By changing the solvent according to pH of soil and water, where tea plant is cultivated, the fluoride content in soil and water influence the beneficial effects and high yield of Polyphenol and low fluoride exposure. Our objective is to find out the concentration and effect of polyphenols in the presence of fluoride.

3.2. MATERIALS AND METHODS

3.2.1 Animals

Male albino rats (Wistar strain, weighing 150-200 g) were selected from small animal breeding station, Little Flower Medical Research Centre, Angamaly, Ernakulum and were kept for a week under husbandry conditions (30°C ± 2°C, 60-70% relative humidity, and 12 h: 12 h day-night cycle) and allowed standard pellet rat feed and water ad libitum. The animal experiments were designed and conducted in accordance with the guidelines of the Institutional Animal Ethical Committee (IAEC).

3.2.2 Preparation of green tea extracts

Sampling

Three samples were locally collected from tea estates in Kerala and two were purchased directly from the retail shop, International Airport, Nedumbassery, India.
Fresh tea leaves were collected from three different locations in Kerala. (Munnar, Nelliampathy and Wagomon). A Dragon well (Chinese - L) was chosen because it was one of 8 well-known green tea in China and most roasted green tea (Choi, 2002). Sencha overture (Japanese-L) was selected and are steamed green tea (Kim 1996) for comparative study.

The green tea leaves were collected and dried under shade and it was powdered and extracted using the procedure given in chapter –II (section 2.2.1).

3.2.3 Estimation of fluoride in soil and water

The estimation of fluoride in soil and water from selected places same as in tea leaves collected were carried out by using AAS given in chapter –II (section: 2.2.5).

3.2.4 Determination of pH

5 gm of homogenized samples were dissolved in 10ml of water. The pH was measured with a pH meter (Corning Incorporated, Corning, NY). 5 samples from each source were analyzed. Detailed procedure is given in chapter II (section: 2.2.7).

3.2.5 Estimation of fluoride in tea extract

Principle

The colorimetric method of fluoride determination is based on the reaction of fluoride with the zirconium ion present in red colored SPANDS dye, which results in the formation of a colorless complex (ZrF6²⁻). The extent of bleaching of the red color is proportional to the amount of fluoride present in the water sample.

The procedure for the estimation of tea fluoride is given in chapter II section: 2.2.6

3.2.6 Determination of total Polyphenol content (TP)

Estimation of total Polyphenol content in each green tea extracts was done by the methods explained in chapter –II section: 2.2.6
3.2.7 Determination of green tea polyphenols by HPLC. Section: 2.2.8
Estimation of polyphenols in tea samples

The samples for analysis were prepared following the conditions developed for optimized extraction of tea polyphenols in our laboratory [Vasisht, et al., 2003]. Accurately weighed, about 2g of moderately fine powder of tea sample was taken in a vacuum flask and 100 ml of boiling water was added to it. The flask was stoppered and kept on a rotary shaker for 5 min. The contents were filtered quickly while still hot, using vacuum and were washed with 10 ml of boiling water. The volume of the extract was adjusted to 100 ml with cold water and 1ml of this extract was diluted to 25 ml of mobile phase (water: methanol: acetic acid 70: 30: 0.5). The diluted extract was filtered through a 0.45 µm membrane filter and a constant volume of 5µl was injected for each analysis. The amount of polyphenols in the extract was calculated from the area under the curve corresponding to the respective peaks of two polyphenols and their standard plots.

3.2.8 Invitro antioxidant assay

Lymphocyte culture preparation

HPL’S were cultured in RPMI 1640 (Himedia) media, supplemented with 20% heat inactivated FBS, antibiotics (penicillin and Streptomycin). PHA (Phytoheamaglutin) was used as the stimulant for cell proliferation. The culture was filtered using 0.2µm pore sized cellulose acetate filter (Sartorius) in completely aseptic condition. Fresh plasma was aseptically added to the culture at a concentration of 1×10^6/ml. The culture was then incubated for 72hrs.

In vitro antioxidative effect of green tea Polyphenol on treated HPL’S were assayed by the method given in chapter II, section: 2.2.9

Estimation of reduced glutathione (GSH) was measured spectrometer at 420nm (Moron et al., 1979). Determination of SOD and CAT were measured by NBT (Mishrta et al.,2004) and H2O2 in dichromate calorimetrically 620nm respectively (Singh et al., 1996). Estimation of lipid peroxidation level was by MDA measurement at 535nm spectrometricaly (Okhawa et al.,1979).
3.2.9 Biological assay

Male Wistar albino rats weighing 150g-200g were divided into five groups of 6 animals (housed under same conditions). Aqueous extract of green tea was administered for 3 months at doses of 100, 250, and 500mg/kg B.wt respectively. The control group received standard rat feed and water ad libitum. The fluoride control group received 5mg/kg b.wt (3.2mg NaFl). At the end of experimental period animals were fasted 18hrs then blood was collected by ocular vernic pucture and anesthetized with ether and cut jugular vein. One with EDTA for separate plasma for biochemical estimations, two the other without anticoagulant and was centrifuged at4000rpm at 4³C for 10 minute to obtain the serum. Both the plasma and serum were stored at 20³C until analysed for biochemical parameters. Liver, kidney, lung and heart, spleen and pancreas were dissected out, washed and transferred to an ice-cold saline solution. The organs were weighed and portions of organs were fixed in 10% formalin for histopathological examinations. The biochemical parameters, blood glucose, urea, plasma protein, uric acid, creatinine, TC, TG, PL and FFA were determined. Serum AST and ALT were also determined. Blood fluoride concentration were analysed by Zirconyl SPANDS reagent spectrometricaly(Crosby at al., 1968).

3.3 STATISTICAL ANALYSIS

Data were analysed by one way analysis of variance (ANOVA) using Bartlett's test (Graph Pad In Stat, Graph Pad Software, San Diego, CA). When significant treatment effects ($p<0.05$) were indicated by GLM, Dunnett's $t$-tests were used to compare each treatment group with the control. If found significant pair wise comparison of treated groups with the control group was done by Dunnett's-t test. All the results were expressed as mean ± SD for six rats in each group and $P<0.05$ was considered significant.
3.4 RESULTS

3.4.1 Estimation of total Polyphenol content and fluoride in different green tea extracts.

We are presenting herewith the results of the various experiments carried out which indicate the presence of polyphenols and fluoride. It is interesting to note that there is inverse relation between Polyphenol content and fluoride in the aqueous extract. The other extracts contain greater amount of polyphenols and absence of fluoride. These are represented by tables (3a).

The extracts were subjected to estimation of Polyphenol and the results are expressed as Polyphenol mg/Gallate/g dry weight.

The results of Polyphenol estimation indicate lesser concentration in aqueous extract as against ethyl acetate and methanol extract samples. This is the case with all samples including Japanese and Chinese tea. It is to be noted that the ethyl acetate and methanol extract samples contain substantial amount of Polyphenol almost two times their value in the aqueous extract, which means more polyphenols are extractable with organic solvents.

Table 3 (a) Levels of Total Polyphenol(mg GAE/g dry weight) in green tea extracts

<table>
<thead>
<tr>
<th>Sample and location</th>
<th>Aqueous Extract of Green Tea</th>
<th>Ethyl Acetate Extract of Green Tea</th>
<th>Methanolic Extract of Green Tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>A loose leaf Dragonwell</td>
<td>48.95± 0.051</td>
<td>106.72± 0.254</td>
<td>163.75± 0.839</td>
</tr>
<tr>
<td>(Chinese L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loose leaf sencha overture</td>
<td>59.80± 0.072</td>
<td>128.58± 0.058</td>
<td>171.78± 0.164</td>
</tr>
<tr>
<td>(Japanese-L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh tea leaves Wagamon,Kerela, India</td>
<td>125.30± 3.493</td>
<td>313.49± 0.227</td>
<td>432.51± 0.457</td>
</tr>
<tr>
<td>Fresh tea leaves Munnar,Kerela, India</td>
<td>97.75± 0.145</td>
<td>293.71± 0.115</td>
<td>410.58± 0.141</td>
</tr>
<tr>
<td>Fresh tea leaves Nelliyampathy,Kerela, India</td>
<td>74.63± 0.066</td>
<td>216.61± 0.228</td>
<td>362.76± 0.220</td>
</tr>
</tbody>
</table>
3.4.2 Quantitative analysis of the polyphenols of the green tea extract by HPLC

Quantitative analysis of the polyphenols of the green tea extract were analysed by HPLC and the results are in agreement with previous study, indicates higher concentration of EGCG in all the extracts as shown by the Figure(3a).

3.4.3 Determination of pH and fluoride content in soil and water.

Polyphenol content of the samples analysed revealed higher concentration from the tea of Wagamon and Munnar (Idukki district, Kerala) as compared to other samples of Nelliyampathy, Japanese and Chinese tea. This is due to the ideal conditions prevalent at Wagamon where soil and water pH 6.39 to 8.21 (near neutral).

Fluoride analysis of soil and water in various locations were collected and analysed by AAS and the results are presented in the Figure (3b). Increased amount of fluoride found in Nelliyampathy soil and water, probably due to acidic pH (5.44 for soil and 3.93 for Water). The soil and water samples of Munnar and Wagamon showed a relatively low concentration of fluoride, which can be explained in terms of a higher pH 6.35 and 7.66 in Munnar and 6.37 and 8.21 in Wagamon(Figure 3c). The aqueous extract of green tea leaves were analysed for fluoride content and the results are presented in table (3b). Fresh tea contains lesser amount and the processed teas have higher concentration probably due to manufacturing conditions. There is no much significance in these results.
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Figure 3(a) Quantitative analysis of the polyphenols of the green tea extract were analysed by HPLC.

Typical HPLC chromatograms

Fig. 3(a) Mixed Catachin Standard

Fig. 3(a) Green Tea Leaf Extract
Figure 3(b) Level of fluoride content in soil and water of specified study area.

Figure (3 c) Analysis of pH of water and soil in specified study locations
Table 3 (b) levels of Fluoride in aqueous extract of green tea (mg/kg)

<table>
<thead>
<tr>
<th>Type</th>
<th>Fluoride (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese L</td>
<td>5.05</td>
</tr>
<tr>
<td>Japanese L</td>
<td>3.55</td>
</tr>
<tr>
<td>Leaves from Munnar</td>
<td>0.95</td>
</tr>
<tr>
<td>Nelliyamabathi</td>
<td>3.15</td>
</tr>
<tr>
<td>Wagomon</td>
<td>1.1</td>
</tr>
</tbody>
</table>

There was no significant fluoride content observed on both Methanolic Extract of Green Tea and Ethyl Acetate Extract of Green Tea in all samples.

3.4.4 Antioxidative effects of different green tea extracts on HPLs invitro.

It is already reviewed that lower concentration of green tea extracts exhibit strong antioxidative mechanisms against different cells. The antioxidative capacity of green tea extract was assessed by using FeCl₃/H₂O₂ to enhance oxidative stress in the cells and antioxidant enzymes and markers were taken into consideration.

Reduced glutathione

Glutathione (GSH) was quantifying in cell lysate of HPLs treated with FeCl₃/H₂O₂ at a concentration which can exhibit a potent oxidative stress in the cells. The retention of antioxidative polyphenols in green tea in a cell system may reduce the oxidative stress by improving antioxidant status. The results were obtained from the current study showing maximum effects on ethyl acetate and methanol extracts of green tea than aqueous extract: Figure 3 (d1). Similar results are obtained in other antioxidant enzymes such as Catalase and SOD they are represented in figure 3(d2) and figure 3 (d3).
Lipid peroxidation

Excessive formation of ROS can lead to oxidative stress which can damage cellular macromolecules including nucleic acids, proteins and lipids. Biological membrane containing unsaturated fatty acids are prone to oxidation. This oxidation of lipids results in the formation of lipid peroxidation products which further propagates free radicals.

Thiobarbituric acid reactive substance was determined by the methods of Ohkawa et al., 1979, using malondialdehyde standard. The results showed in figure 3(d4), that the lipid peroxidation have been less in both methanolic and ethyl acetate extract of green tea while aqueous extract have lesser activity on lipid peroxidation may be due to low level of polyphenols and fluoride in this extract, which can inhibit the natural antioxidant enzymes.

Figure 3( d1) Antioxidative effect of different green tea extract on Reduced Glutathion – HPL

On Y axis: optical density at 420nm.
Figure (3 d$^2$) Antioxidative effect of different green tea extract on Catalase – HPL

On Y axis: optical density at 620nm.

Figure (3 d$^3$) Antioxidative effect of different green tea extract on SOD – HPL

On Y axis: optical density at 600nm.
Figure (3 d) determination of lipid peroxidation protective activity of different green tea extract on HPL

On Y axis: optical density at 535nm.

3.4.5 Biological Assay

These investigations were carried out using Albino (Wister) rats six in each batch as per protocol given below. Different parameters are estimated.

**Treatment**

- **Group I** - Normal
- **Group II** - Animals were given fluoride (NaF) with solution every day for three months at a dose of 5mg/Kg b. wt.
- **Group III** - Aqueous Extract of Green Tea(for three months at a dose of 100mg/kg body wt).
- **Group IV** - Aqueous Extract of Green Tea for three months at a dose of 250mg/kg body wt.
Group V - Aqueous Extract of Green Tea for three months at a dose of 500mg/kg body wt

3.4.5.1 Estimation of fluoride concentration mg/dl in serum.

Fluoride is a compound present in tea which is being used. However the amount taken is not much to produce excess concentration leading to fluorosis and similar symptoms. It can happen if excessive amount of tea is used daily. In our analysis the amount of fluoride content in experimental animals we have found at 0.436 mg/dl in normal and have increased amount of fluoride found in experimentally treated groups (1.405mg/dL and 0.83 mg/dL) only in higher dose. The values are expressed in Figure 3 (e). It may be noted that concentration of polyphenols depends on fluoride.

3.4.5.2 Effects of aqueous extract of green tea on body weight.

The body weight changes of control and aqueous extract treated rats are presented in Figure 3 (f). There were significant (p<0.001) difference in the body weight gain between the control and aqueous extract treated groups. Moreover no lethality was recorded for any dose upto the maximum of 500mg/kg.b. wt during the experimental period.

3.4.5.3 Effects of aqueous extract of green tea on glucose.

The significant increase in serum glucose level by 205mg/dl and 197mg/dl (p<0.001) both in fluoride control and aqueous extract of green tea treated group at the dose of 500mg/kg.b.wt. respectively. The results are represented in figure 3 (g).

3.4.5.4 Effects of aqueous extract of green tea on total protein.

Effects of aqueous extract on total protein in plasma were decreased in fluoride control group significantly (p<0.001). All other groups treated with aqueous extract in varying concentration were increased significantly (p<0.001) and reach to normal level. The values are presented in figure 3(h).
3.4.5.5 Effects of aqueous extract of green tea in different dose on NPN.

Effects of aqueous extract on NPN are presented in figure.3 (i). Serum urea, uric acid, and creatinine significantly (p<0.001) increased in group-II (fluoride control treated). Similarly urea and creatinine were increased significantly (p<0.001) in aqueous extract (500mg/kg b. Wt) with 16.50± 0.25 and 0.74±0.001 respectively, while other two groups (aqueous extract with 100mg, 250mg/kg.b.wt) were not altered significantly (p>0.05).

3.4.5.6 Effects of aqueous extract on AST, ALT

In serum AST and ALT were increased significantly (p<0.001) both in fluoride control group and aqueous extract treated at 500mg/kg b. Wt. Dose. Other two groups with aqueous extract treated at 100mg and 250mg/kg.b.wt. were normal limit and is presented in figure.3(j)

3.4.5.7 Effects of aqueous extract on total cholesterol, LDL, HDL and VLDL in serum

The aqueous extract of green tea was given to experimental animals at a dose of 100mg, 250mg, 500mg equivalent dry weight of extracts and the results were significant with respect to higher concentrations. The lower concentration did not respond to this, while the higher concentration influenced the total cholesterol and HDL-C, LDL-C, and VLDL lipoproteic cholesterol. The results, as expressed in the table (3c), thus the higher concentration, experiment produced higher level total cholesterol (253 ± 1.31 and 210 ± 4.42), HDL-C (66.6 ± 0.172 and 68.3 ± 0.530), and LDL-C (41.3 ± 0.147 and 28.0 ± 0.698).

3.4.5.8 Histopathology for liver, kidney and heart

There are no significant pathological changes seen in tissues (under study). In liver tissue mild fatty infiltration were observed.
Figure 3(e) Level of serum fluoride on green tea extract treated experimental animals.

Figure 3 (f) Level of bodyweight on green tea extract treated experimental animals.
Figure 3(g)  Level of serum glucose on green tea extract treated experimental animals.

Figure 3(h)  Level of serum total protein on green tea extract treated experimental animals.
Figure 3(i) Level of serum Urea, Uric acid, creatinine on green tea extract treated experimental animals.

Figure 3(j) Level of serum AST, ALT on green tea extract treated experimental animals.
Table (3c) Effects of aqueous extract of green tea on lipid profiles in treated (at different doses) and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol Mg%</th>
<th>HDL-cholesterol Mg %</th>
<th>LDL cholesterol Mg %</th>
<th>VLDL Mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>124±1.21</td>
<td>84.4±0.256</td>
<td>26.48±0.213</td>
<td>30.4±0.040</td>
</tr>
<tr>
<td>Control</td>
<td>253±1.31</td>
<td>66.6±0.172</td>
<td>41.3±0.147</td>
<td>30.5±0.063</td>
</tr>
<tr>
<td>Aqueous extract of green tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100mg</td>
<td>145±1.89</td>
<td>87.6±0.14</td>
<td>24.4±0.136</td>
<td>29.3±0.081</td>
</tr>
<tr>
<td>250mg</td>
<td>142± 1.16</td>
<td>89.6±0.075</td>
<td>23.2±0.213</td>
<td>32.3±0.154</td>
</tr>
<tr>
<td>500mg</td>
<td>210± 4.42</td>
<td>68.3±0.530</td>
<td>38.0±0.698</td>
<td>31.3±0.711</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n = 6 animals;

a. P<0.001 Comparison between normal verses control group and control verses other treated groups;
b. P<0.01 Comparison between normal verses treated groups; and different fractions.
c. P<0.05
d. P>0.05

3.5 DISCUSSION

Widely used drink like tea may have some effect on human beings in view of the organic compounds present in tea, however we have not found any report in which drinking of tea can cause any serious changes in human metabolism. There is no doubt that there can be alterations possible in view of the
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constituent present in tea. Extract of the tea with solvents like water (ordinary tea) and organic solvent like methanol and ethyl acetate gave interesting results in the concentration, mainly polyphenols and fluoride. Fluoride is found to be present in the tea leaves, the soil and water of the area. In the soil it is present mainly as $\text{AlF}_3$ and this is absorbed by the tea plants, while the neighboring other plants do not entertain (Fung et al., 1999) resulting in lesser concentration of fluoride in them. Systematic study of the various extracts contains higher fluoride in water while the other extracts contain no significant amount of fluoride. There are many reports describing the antioxidant activity of teas, but the result varies depending on the preparation and assay method. Their results showed that different teas had widely deferent in vitro antioxidant power and that the antioxidant capacity was strongly correlated with total polyphenols content of tea. Another interesting factor is the higher concentration of polyphenols in the organic extracts as against the water extract. This is an agreement with the previous work (Yao et al., 2004) in which methanolic extracts contain more of polyphenols. Ethyl acetate extract is also found to have similar concentration. This has happened due to a number of reasons. Here we assume that polyphenols can remain in the tea plant as water soluble one and organic soluble one. The water soluble extract may be free one or a compound one or a conjugated form with a carbohydrateportion similar to glycoprotein or glycompounds (Clifford et al., 2000). We can also have increase in water solubility due to a glucoronide combination similar to bilirubin diglucoronide. Further, the synthesis of polyphenols take place in the metabolically active tender leaves, the biosynthesis and transportation affected by the presence of fluoride (Jayman and Sivasubramaniam, 1980). The synthesis can proceed till the leaves become completely ripen (the tea leaves are plucked before ripening for the manufacturing of tea). The higher concentration of polyphenols in organic extracts are mainly due to the organic soluble form of the polyphenols. It is also assumed that they are not extractable with water. The fact is that insoluble in water or soluble in organic solvents indicates a different variety of polyphenols. This polyphenols may be in a form which may conjugate with lipid like compound and are insoluble in water. The consumption of ordinary green tea can
have only water soluble components which are biologically active in human body. There is another possibility of a polymerization type reaction which take place to form a compound conjugated together and may be physically insoluble in water but soluble in organic solvent (Farhoosh et al., 2007). In commercial green tea available the tea leaves are subjected to heat treatment and further processing. During this process the cells are ruptured and the polyphenols oxidase present in cells may leach out when the temperature is reduced. The reaction processed may be a biosynthesis or degradation. It has been reported that the enzyme Polyphenol oxidase may act on polyphenols to quinoines which is irreversible. Therefore this polyphenols are lost (Mayer, 1987). On the other hand synthetic pathways are possible, that can generate more phenols. Heat processing can also give rise to extraction of polyphenols which are not possible with hot water as the process give rise to rupture of the cell membrane exposing the polyphenols in water extraction. The lower polyphenol content of green tea extract (aqueous extract) may be due to thermal destruction of polyphenols during heat treatment that inactivatepolyphenol oxidase enzyme despite the fact that the content and composition of tea polyphenols are strongly influenced by various factors such as variation and leaf variety, harvesting season, climate, processing method and analytical method (Gramza and Korczak 2005).

We have collected samples of soil and water surrounding the tea along with collection of the leaves for analysis. We found a difference in the concentration of fluoride and polyphenols. From the table we can see that fluoride concentration is inversely proportional to pH. i.e. if pH is lowered the fluoride content is high; when the fluoride is high polyphenols are low. This may be due to inhibitory effect of fluoride in the biosynthesis of polyphenols.

Tea grows in acidic soil with pH ranges from 3.5 to 5.6. Tea plants take up a large quantity of fluoride from acidic soils. Fluoride is accumulated mainly in the leaves and increase with age in the leaves. The highest fluoride content found in fallen leaves branches and roots (Fung et al., 1999). Fluoride is accumulated in old leaves 2000mg/Kg and young leaves ranging from 250mg/Kg to 360mg/Kg (Fung et al.,1999). The shedding of mature leaves contain more fluoride that can also cause higher concentration of soil and water.
which result in greater absorption of fluoride. The commercial tea preparation contains fluoride and this fluoride at the normal consumption of tea will not result in fluorosis or any other disorders. It has been reported that large consumption of tea may result in fluorosis.

The fluoride is required for the human body 1ppm (WHO). Tea leaves accumulates more fluoride than other edible plants (Meiers et al., 1984) fluoride in tea gets absorbed by the body similarly as the fluoride in drinking water (Ruh, 1968). In the experimental animals tea aqueous extracts were given at concentration of 500mg /Kg body weight showed an increase in the total cholesterol, LDL-C and blood glucose. This is in comparison with a standard fluoride (5mg/Kg b.wt.) which also shows similar results. The glycolytic pathway is affected by the inhibition of enolase due to the increased concentration of fluoride causing hyperglycemia and related symptoms. This hyperglycemia may be caused for elevation of cholesterol (Chlubek et al, 2003; Grucka et al., 2004; Rupal et al., 2010)

Polyphenols have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li et al., 2003). Polyphenols serve as health promoting compound as a result of its anion radicals (Hausteen, 1983). Raised NPN level in blood have been observed with impaired renal function or in acute renal failure (Varley, 1964). In the present study, a significant increase (p<0.001) in fluoride control and aqueous extract (500mg/kg) at the highest dosage indirectly manifests the hazardous effects. It may be due to high concentration of fluoride elimination and have demonstrated necrosis of the proximal and distal renal tubules (Lim et al., 1978; Whitford, and Taves, 1971).

Transaminases are good indices of liver, kidney damage respectively (Martin et al., 1981). There were no deleterious changes found in the levels of transaminases in serum, elevated transaminases reflected hepatic injury (Singh et al., 2002)