Journal of Animal & Plant Sciences, 2012. Vol. 13, Issue 1: 1725-1730
Publication date: 28/2/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

JAPS

Effects of aqueous extraction of green tea (*Camellia sinensis*) on some biochemical parameters in American white rats

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Keywords: Green Tea, Body Weight, Triglyceride, enzyme activities, serum glucose

1 SUMMARY

This study and experiment was conducted in 2011, was extended into 4 weeks and one week for adaptation for all groups (each group contain 5 rats). The study investigated the effect of oral consumption of aqueous extraction of Green Tea (GT) 0, 3, 6, 9and 12 % (w/v) on body weight, serum glucose, cholesterol, triglyceride level, enzyme activities LDH (Lactate dehydrogenase) and ALP (Alkaline phosphatase) and some minerals (Na, K, Ca and P). The results showed slight loss in body weight and a significant (P ≤ 0.05) reduction in serum glucose, cholesterol, triglyceride level but an increase in the activities of LDH associated with lowering the activities of ALP. There was no change in Na and K, but there was a clear reduction in Ca and P (P≤ 0.05).

2 INTRODUCTION

Green tea (GT) is ranked second as a beverage drink after the water in the worlds (Alan and Iris 2004) It is cultivated in tropical and subtropical areas. The native lands of GT are China, South and southern east of Asia. The scientific name of GT is *Camellia sinensis*. Consumption of GT leads to lowering of different forms of cancers, while the constituents of green tea have cardioprotective, neuroprotective, antidiabetic and antimicrobial activities (FAO, 2009).GT is a good source of polyphenols such as catechins, orgallotannins, flavonols, flavonoids and phenolic acid (Zhen *et al.,* 2002). In addition GT catechins and their derivatives have beneficial health effects such as Antioxidant, Antimutagenic and Anticarcinogenic properties (Johnson and. Williamson, 2003). GT has been found to be useful in the treatment of arthritis and high cholesterol levels, while consumption of green tea has resulted in improved kidney functions in animal models of renal failure (Christiane and Edward 2001). Johnson and Williamson (2003) stated that there are different types of green tea extractions:

- Strong infusions: during the strong infusion the green tea leaves are processed by soaking in the aqueous solution of alcohol (Catechins content is about 2 % w/w)
- Soft extracts: to obtain soft extracts, the solution made by strong infusion beforehand is further concentrated to 20–25% (Catechins content is about 20 % w/w)
- Dry extracts; after the strong infusions have been concentrated to 40–50% solids (Catechins content is above 25 % w/w), tea dried and then becomes dehydrated extract and powder. The leftovers water content, which is less than 5 % w/w, and the extract are usually processed as a powder containing inert processing aids to

become suitable for a variety of uses (tablets, capsules and dry mixes)

- Partly purified extracts: further purification processes, for example, solvent extraction or column chromatography techniques, as well as new techniques such as membrane extraction and separation are utilized in order to acquire a higher content of tea catechins.

Tea can also be called green tea polyphenols (GTP or GTPs). The category includes epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) of which, EGCG accounts for more than 40% of the total content (FAO, 2009). Some clinical study found that men who drink green tea are more likely to have lower total cholesterol than those who do not drink green tea (UMMC, 2011). Results from one animal study suggest that polyphenols in green tea may block the intestinal absorption of cholesterol and promote its excretion from the body. In another small study of male smokers, researchers found that green tea significantly reduced blood levels of harmful LDL cholesterol (Maron, *et al.* 2003). Some scientist reported that the triglycerides and cholesterol can't dissolve in blood, but they circulate throughout body with the help of proteins that transport the lipids (lipoproteins) (Faergeman 2009).Some researchers speculate that substances in green tea known as polyphenols, specifically the catechins, are responsible for the Green tea fat-burning effect (Sara *et al.*, 2007). Nearly every type of cancer, as well as many other diseases, can cause LDH (Lactate dehydrogenase) levels to be elevated, whereas, the normal level of LDH in blood is 200 units/ liter, but LDH markers cannot be used to diagnose a particular type of cancer. LDH levels can be used to monitor the treatment of some cancers such as Testicular cancer, Ewing's sarcoma, Non-Hodgkin's lymphoma, and some types of Leukemia. Holbrook *et al.* (1975) reported that an elevation of LDH levels can be caused by a number of non-cancerous conditions such as heart failure, hypothyroidism, anemia, and lung or liver disease. The optimal range for alkaline phosphatase (ALP) depends on age. A growing adolescent will have a much higher alkaline phosphatase than a full grown adult because his/her osteoblasts are laying down bone very rapidly. For an adult about 50-75 mg/dl is considered a reasonable optimal range (Jeremy and Facp, 2011).

The objectives of this study are to assess the effect of different concentration 0, 3, 6, 9 and 12 % of an aqueous extraction of green tea on body weight, glucose, cholesterol, enzymes activity (LDH and ALP) and some minerals (Na, K, Ca and P).

3 MATERIAL AND METHOD

3.1 Experimental design: Twenty five Adult male albino rats (weighing 130–200 g) were fed with a standard pellet diet and watered for one week before the start of the experiment. All animals were kept under same conditions that prevented them from experiencing unnecessary pain and discomfort according to guidelines approved by the ethical committee. These 25 males rat were divided into five groups, each group contained five rats. G_1 (control) received standard diet without GT extraction, G_2 got the standard diet + 3 % GT extract, G_3 received standard diet + 6 % GT extract, G_4 received standard diet + 9 % GT extract and finally G_5 received standard diet + 12 % GT extract. Different concentrations of the aqueous extraction of GT were given for oral consumption in each group.

3.2 Preparation of sample: The concentration of green tea (3, 6, 9 and 12 %) was prepared according to method described by (Sara *et al.,* 2007). Sample of eye blood was collected from each groups and kept in specific containers containing EDTA. The plasma was separated by centrifugation at 5000 rpm. The prepared sample was kept until start the biochemical analysis.

3.3 Estimation of glucose, Cholesterol and triglyceride (mg/dl): Glucose was determined according to method described by Teuscher and richterich (1971), Cholesterol was determined according to method described by Allain *et al.,* (1974) and triglyceride was determined according to method described by Mc Gowan *et al*., (1983).

3.4 Estimation of enzymes activity (LDH and ALP) (u/L): Activity of LDH and ALP were measured according to method described by Friedman and Young (1997).

3.5 Estimation of minerals (K, Na, Ca and P) mg/dL: Sodium (Na) was determined according to method described by Henry (1974) and Potassium (K) was determined according to method described by Tietz (2006), Calcium (Ca) and Phosphorus (P) were determined according to method described by Tietz (1999),

3.6 Statistical analysis: Data obtaining were assessed by analysis by ANOVA as described by Gomez and Gomez (1984).

4 RESULTS AND DISCUSSION

Table 1 showed that the body weight of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 200, 174, 163, 190 and 171 g, respectively. The results showed slight loss in body weight in all concentrations of GT extracted compared with the control.

Table 1: Effect of GT extracted (0, 3, 6, 9 and 12%) on body weight and serum parameters of American experimental white rats.

Groups	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**
Body weight (g)	200	174	163	190	171
Glucose (mg/dl)	126	85	75	68	66
Cholesterol (mg/dl)	377	269	193	166	151
Triglyceride (mg/dl)	100	81	81	78	68

Each value is average of five replicates.

These findings are partial agreement with some previous studies in human and animal Imai and Nakachi (1995). The glucose level of oral consumption of the GT extraction 0, 3, 6, 9 and 12 % is 126, 85, 75, 68, and 66 mg/ dl, respectively. The cholesterol level of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 377, 269, 193, 166 and 151 mg/ dl, respectively. While the triglyceride level of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 100, 81, 81, 78 and 68 mg/ dl, respectively. There is loss of body weight in the different groups associated with lowering of serum glucose, cholesterol and triglycerides. These findings are similar to those results obtained by Raederstoff *et al.,* (2003). The suppressive effect of GT Catechins on the postprandial level of triglycerides and cholesterol has also been reported in humans (Unno *et al.,* 2005). These observations may be responsible for body weight loss and in lowering the incidence of cardiovascular diseases (Kao *et al.,* 2000). The GT consumption increased glucose metabolism in adipocytes whereas, hepatic glucose production was reported to be inhibited by GT, which led to lower blood glucose (Broadhurst *et al.,* 2000). Table 2 indicated that the activity of LDH level of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 402, 494, 612, 1033 and 1143 u/L , respectively.

Table 2: Effect of GT extracted (0, 3, 6, 9 and 12%) on LDH and ALP of American experimental white rats.

Groups	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**
LDH u/l	402	494	612	1033	1143
ALP u/l	32	23	13	6	2

Each value is average of five replicates. LDH = Lactate dehydrogenase, ALP = Alkaline phosphatase

The increasing of LDH activity can be caused by a number of non-cancerous conditions such as heart failure, hypothyroidism, anemia, and lung or liver disease (Mitra and Thakur, 2007).. While activity of ALP in oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 32, 23, 13, 6 and 9 u/L, respectively. The decreasing of ALP activity may be due to Zinc deficiency, Hypothyroidism, Vitamin C deficiency /

Scurvy, Folic acid deficiency, Excess Vitamin D intake, Low phosphorus levels (hypophosphatasia), Celiac disease, Malnutrition with low protein assimilation (including low stomach acid production / hypochlorhydria), Insufficient Parathyroid gland function, Pernicious anemia and Vitamin B_6 insufficiency (Holbrook *et al.*, 1975). Table 3 illustrated that Na for oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 137, 144, 121, 134 and 139 mg/dL, respectively.

Table 3: Effect of GT extracted (0, 3, 6, 9 and 12%) on Na, K, Ca and P of American experimental white rats.

Groups	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**
Sodium (mg/dl)	137	144	121	134	139
Potassium (mg/dl)	14	11	32	24	16
Calcium (mg/dl)	10	7	0.6	0.5	0.4
Phosphorus (mg/dl)	2	6	6	6	5

Each value is average of five replicates.

In the present study, the data on Na level indicates there was no significant change at level $P \leq 0.05$. Therefore, Oral consumption of GT has no effect on the Na level. K level for oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 14, 11, 32, 24 and 16 mg/dL, respectively. Data of K level indicates there was significant ($P \leq 0.05$) difference in oral consumption of GT extracted 6 and 9 % compared with control. 0ral consumption of GT extracted 12 % has no significantly ($P \leq 0.05$) difference, but oral consumption of GT of 3 % has clear reduced in K level. Therefore, oral consumption of GT extracted 3, 6 and 9 % has effected on the K level compared with control ($P \leq 0.05$). Ca level of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 10, 7, 0.6, 0.5 and 0.4 mg/dL, respectively. The results indicated there is significant difference in Ca in all concentrations compared with control ($P \leq 0.05$). P level of oral consumption of GT extraction 0, 3, 6, 9 and 12 % is 9, 6, 6, 6 and 5 mg/dL, respectively. The results indicated there is significant difference in 3 % and 6 % compared with control ($P \leq 0.05$), but there was no significant difference in 9 and 12 % compared with control ($P \leq 0.05$). Data of Ca and P level indicates there is a high significant ($P \leq 0.05$) difference compared with control. It is clear that there was reduction in Ca and P level with the concentration of oral consumption of GT. Therefore, metabolism of calcium increased with increasing oral consumption of GT. While inorganic phosphorus is an essential component of intermediary metabolism: energy conservation as ATP, biosynthesis of cellular membrane as phospholipids and other important biomolecules such as NAD, NADP and various RNAs. The decrease in the serum inorganic phosphorus may suggest its over-utilization. We propose that GT polyphenols and other constituents can cause specific adaptive changes in the cellular metabolism of certain tissues and thus improve their function. GT contains characteristic polyphenolic compounds such as (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3- gallate (ECG) and (–)-epicatechin (EC). The most important function of these polyphenols is the antioxidant property due to their capacity to sequester metal ions and to scavenge reactive oxygen species (Weisburger, 1988). This reaction is responsible for antitumerogeneic effect of G T(Zhu, 1999).. These compounds account for 30–42 % dry mass of the solids in brewed GT (Balentine *et al.*,1997).

5 CONCLUSION

The experiment concluded that increase in concentration of GT extract led to lowering body weight, serum glucose, cholesterol, triglycerides, and activity of ALP and increased the activity of LDH. Whereas, there was no significant change in Na and K, there was a clear reduction in Ca and P.

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