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Effect of green tea (*Camellia sinensis*) extract feeding on serum biochemical indices (Cholesterol, HDL, Lipid peroxide and LDL) of hamster under different dietary treatments

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Abstract
Seventy two weaned Syrian hamsters of either sex were randomly assigned to six treatments in single cages (290mm X 220mm X 140mm) in a closed room where the temperature and relative humidity was maintained 24±50C and 60±15%, respectively during the trial of 56 days. The experiment consisted of six treatments. The concentrate mixture of all the groups was formulated using wheat, fish meal, ground nut cake, mineral mixture and common salt. Feed provided to each treatment was same. Treatment T1, T3 and T5were provided with pure water only. While treatment group T2, T4 and T6were provided with green tea extract only. Green tea extract was prepared by dipping green tea bag (1.3g) in 130 ml boiling water so that effectively it formed 1 percent w/v solution. Hemato-biochemical parameters in terms of Cholesterol, HDL, LDL, Lipid Peroxide improved significantly in the treatment groups in which GTE was provided (T2, T4 and T6). It is inferred from the results that T2, T4 and T6 treatment groups (in which green tea was provided) showed better growth profile in comparison to the rest of the treatment groups (T1, T3 and T5). It is also inferred that rice Huskas a bedding material proved to be the best as compared to saw dust and sand.

Keywords: Hamster, green tea extract, rice husk, saw dust, sand, cholesterol, HDL, lipid peroxide and LDL

Introduction
Relatively few animal welfare studies have been conducted on Syrian hamsters (*Mesocricetus auratus*), despite the fact that considerable use is made of these animals in biochemical and behavioural researches. Among the aspects of hamster welfare that have been studied so far are social housing (Arnold and Estep, 1990) [4], cage floor preference (Arnold and Estep, 1994) [5], cage dimensions (Fischer *et al*., 2007) [6], environmental enrichment (Reebs and Maillet, 2003) [7], running wheels (Gebhardt *et al*., 2005; Reebs and St-Onge, 2005) [9, 8] and bedding material requirements for hamsters. Captivity conditions must satisfy the basic needs of laboratory animals and ensure their physical, physiological and psychological welfare.
Tea, prepared from the leaves of *Camellia sinensis*, is the most popular beverage in the world except water. Green tea, made from the mild oxidation of green tea leaves, amounts to 80% of world tea production (Graham, 1992) [10]. Flavonoids are a group of polyphenols present in vegetables, fruits and beverages such as tea and wine and green tea is a major source of dietary flavonoids. Flavonoid intake is inversely associated with mortality from coronary artery disease in a cross-cultural seven country epidemiological study (Hertog *et al*., 1995) [11]. A study found green tea consumption was associated with decreased cholesterol and triglyceride and an increased proportion of HDL (Imai and Nakachi, 1995) [12]. Green tea and black tea are both high in catechins. These compounds are powerful antioxidants, capable of rapid reduction of superoxide radical and alkyl peroxy radicals. Catechins may also repair vitamin E radicals (Jovanovic *et al*., 1996) [13]. Such potent antioxidant ability may be important in inhibiting the *in vivo* oxidation of LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) and the subsequent atherogenesis.
In this backdrop, this experimental study was undertaken to find out the effect of green tea (*Camellia sinensis*) extract on blood biochemical indices of hamsters.

Materials and Methods
The experimental trial was carried out at Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar to evaluate the effect of green tea extract feeding and bedding

material on growth performance and biochemical indices in hamsters. The hamsters were raised at Disease Free Small Animal House (DFSAH), LUVAS, Hisar. The biochemical parameters of blood were analyzed at Department of Veterinary Physiology and Biochemistry Department (VPB).

Selection of animals

Seventy two Syrian hamsters of either sex at 21 days of age having average body weights of 39.26 g were selected from Disease Free Small Animal House, LUVAS, Hisar and randomly divided into six treatment groups of twelve hamster each.

Experimental design, housing and feeding

Seventy two weaned Syrian hamsters of either sex were randomly assigned to six treatments in single cages (290mm X 220mm X 140 mm) in a closed room where the temperature and relative humidity was maintained at 24±5 ^{0}C and 60±15%, respectively during the trial of 56 days. The room had an exhaust fitting for ventilation and glass fitted windows and 6 CFL's (Chloro-Fluoro lamps) to maintain a light/dark cycle (approx. 14/10). The selected weaned hamsters were shifted to their allotted cages and allowed an adaptation period of 4 days. The concentrate mixture of all the groups was formulated using wheat, fish meal, ground nut cake, mineral mixture and common salt. Feed provided to each treatment was same. Treatment T_1, T_3 and T_5 were provided with pure water only. While treatment group T_2, T_4 and T_6 were provided with green tea extract only. Green tea extract was prepared by dipping green tea bag (1.3g) in boiling water of volume 130 ml so that effectively it formed 1 percent w/v solution. Feed ingredients used in the concentrate mixture formulation were analysed for proximate composition (AOAC, 2005), presented in (Table 1). The hamsters under different treatments were fed concentrate mixture having minimum 18.92 percent protein and not less than 3.27 percent ether extract. The feed and water bottle in T_1, T_3 and T_5 were provided separately to each hamster. Similarly in case of T_2, T_4 and T_6 feed and GTE (green tea extract) is provided separately.Water bottles were hanged in inverted position over the top of cages as they work on negative pressure near to the feeders and they were regularly cleaned to prevent the chance of any contamination. The study was carried out for a period of 56 days on selected hamsters. Feed and water were supplied *ad libitum* throughout the experiment. Bedding material used for hamsters under treatment groups T_1 and T_2 was rice husk, for T_3 and T_4 was saw dust and for T_5 and T_6 was sand. Old bedding material was changed biweekly with fresh bedding material.

The hamsters were weighed and different body measurements were recorded at the beginning of the experiment after providing an adaptation period of 4 days.

In a completely randomized design (CRD), 72 Syrian hamsters were assigned to 6 treatment groups: T_1, T_2, T_3,T_4, T_5 and T_6 with each treatment group having 12 hamsters.

- **T_1:** Rice husk as bedding material + Standard feeding
- **T_2:** Rice husk as bedding material + Standard feeding + 1% Green tea extract
- **T_3:** Saw dust as bedding material + Standard feeding
- **T_4:** Saw dust as bedding material + Standard feeding + 1% Green tea extract
- **T_5:** Sand as bedding material + Standard feeding
- **T_6:** Sand as bedding material + Standard feeding + 1% Green tea extract

Table 1: Proximate composition (% DM basis) of the feed ingredients

Sr. No	Name of Ingredient	Dry matter (%)	Total ash (%)	Ether Extract (%)	Crude Protein (%)	Crude Fiber (%)
1	Wheat	91.01	2.45	2.67	10.40	2.34
2	Fish meal	90.10	9.09	7.78	41.65	3.32
3	GNC	89.89	7.58	4.60	46.32	6.01

Table 2: Ingredient composition of concentrate mixtures prepared for feeding different treatment groups of Hamsters (g/kg)

Ingredients	Amount
Wheat	740
Ground nut cake	200
Fish meal	50
Common salt	5
Mineral mixture*	5

*Mineral mixture (salt free), Ca (32%), Cu (100 ppm), Zn (0.26%), Iodine (0.01%), P (6%), Mn (0.27%), Fe (1000 ppm) and Co (50 ppm).

Statistical analysis Statistical analyses were performed using the IBM SPSS statistics 20 software package for windows. The results were analyzed using the One-way analysis of variance and it was employed to determine the means along with standard error. Significant differences among the treatments means were determined using Duncan's test. Level of significance was considered at $P< 0.05$.

Result and Discussion

The initial corresponding mean values for cholesterol in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 110.83, 110.83, 110.67, 110.83, 110.83 and 110.37(mg/dl) respectively. While final mean values in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 112.17, 83.67, 112.17, 83.33, 112.00 and 83.17(mg/dl) respectively (Table 12). This indicates that cholesterol level in treatment group T_2, T_4 and T_6 are significantly lower than treatment group T_1, T_3 and T_5. In the case of high density lipoprotein (HDL), initial mean values in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 56.50, 56.33, 56.67, 56.00, 57.33 and 56.33(mg/dl) respectively. While final mean values in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were58.50, 64.33, 58.17, 64.17, 58.50 and 64.67(mg/dl) respectively (Table 12). This indicates that HDL level in treatment group T_2, T_4 and T_6 are significantly higher than treatment group T_1, T_3 and T_5. The results indicate that green tea intake had reduced cholesterol and increased HDL values. That means green tea has improved blood profile in terms of cholesterol and HDL. The changes in Cholsterol and HDL level are depicted in Fig. 13 and Fig. 14 respectively.

For lipid peroxide, the corresponding initial mean values in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 0.82(µM) with non-significant difference. While the final mean values in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 0.83, 0.37, 0.83, 0.36, 0.83 and 0.37(µM) (Table 12). This indicate that lipid peroxide level in treatment group T_2, T_4 and T_6 are significantly lower than treatment group T_1, T_3 and T_5 The corresponding initial mean value for low density lipoprotein (LDL) in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 were 61.0, 61.17, 61.67, 61.17, 61.67 and 61.17(mg/dl) respectively. The

final mean values were 61.67, 55.50, 61.67, 56.50, 61.83 and 57.00(mg/dl) (Table 12) respectively. This indicates that low density lipoprotein level in treatment group T_2, T_4 and T_6 are significantly lower than treatment group T_1, T_3 and T_5.These results clearly manifest the beneficial impact of green tea intake on health profile of hamsters. HDL/LDL ratio is depicted in table 11. The changes in lipid peroxide and LDL level are shown in Fig. 15 and Fig. 16 respectively.

Serum biochemical parametersof all the experimental groups (T_1, T_2, T_3, T_4, $T_5$and T_6) at the start, bi-weekly and at the end of experiment are presented in (Table12). There were no significant changes in the hemato-biochemical parameters (cholesterol, HDL, LDL, lipid peroxide, total bilirubin, SGOT, SGPT, alkaline phosphates and BUN) of the groups at the start of the experiment. But there were significant differences (P<0.05) in the hemato-biochemical parameterson the subsequent observations as shown in (Table 12). All the values of hemato-biochemical parameters were declined significantly in T_2, T_4 and T_6 (in which GTE was provided) except HDL values which increased. This indicates that GTE had improved blood profile of hamsters significantly. Similarly in a study conducted by Vinson and Dabbagh (1998) found that cholesterol and lipid peroxide values dropped significantly in the hamsters provided with green tea. At the same time HDL values spiked in the hamsters provided with green tea.

Similar to our study in a experiment conducted on Syrian hamsters by Chan *et al.* (1999) [3], it was reported that cholesterol and triglyceride values decreased in the hamsters provided with GTE. The cholesterol-lowering effects of green tea could be due to the inhibition of cholesterol absorption. They further explained that catechins with gallate esters were shown to interfere with the biliary micelle system in the lumen of the intestine by forming insoluble co-precipitates of cholesterol and increasing the fecal excretion of cholesterol. Bursill and Roach (2007) [1] explained this apparent decrease in cholesterol absorption and reduction in liver cholesterol concentrations lead to an increase of LDL-receptor expression and activity. This cell-surface protein is present on the outer surface of most cells, but in particular liver cells, it can remove cholesterol-carrying LDL from the circulation. Studies in animals have provided evidence that green tea extracts and their catechin constituents can reduce plasma, liver, and thoracic aorta cholesterol and up-regulate hepatic LDL receptors. Bursill and Roach (2007) [1] have concluded that the administration of green tea extract was able to significantly increase both the LDL-receptor binding activity and relative amounts of LDL-receptor protein. In addition, there is another possible major mechanism by which green tea lowers cholesterol: catechins have direct inhibitory effects on cholesterol synthesis. A recent *in vitro* study has revealed that green tea catechins were potent and selective inhibitors of squalene epoxidase, which is likely a rate-limiting enzyme of cholesterol biosynthesis (Abe *et al.*, 2000) [2].

Table 3: Serum biochemical indices (Cholesterol, HDL, lipid peroxide and LDL) of hamster (bi-weekly)

Days	T_1	T_2	T_3	T_4	T_5	T_6
Cholesterol						
0 days	110.83±0.48	110.83±0.31	110.67±0.49	110.83±0.31	110.83±0.4	111±0.37
14 days	112.33^{b}±0.21	95.67^{a}±1.05	112.50^{b}±0.22	94.83^{a}±1.19	112.00^{b}±0.26	94.50^{a}±1.28
28 days	111.8^{b}3±0.54	90.33^{a}±1.99	112.00^{b}±0.52	89.33^{a}±2.36	112.00^{b}±0.58	89.50^{a}±2.28
42 days	112.83^{b}±0.48	86.67^{a}±1.6	112.17^{b}±0.6	86.67^{a}±1.65	112.00^{b}±0.68	86.50^{a}±1.75
56 days	112.17^{b}±0.31	83.67^{a}±1.31	112.17^{b}±0.31	83.33^{a}±1.41	112.00^{b}±0.26	83.17^{a}±1.3
HDL						
0 day	56.50±0.43	56.33±0.33	56.67±0.33	56.00±0.37	57.33±0.42	56.33±0.33
14 day	56.67^{a}±0.21	58.83^{b}±0.17	56.83^{a}±0.48	59.50^{b}±0.34	57.00^{a}±0.63	59.33^{b}±0.42
28 day	57.67^{a}±0.42	61.67^{b} ±0.33	57.0^{a}±0.43	61.33^{b}±0.42	57.33^{a}±0.49	61.67^{b}±0.33
42 day	57.00^{a}±0.63	62.83^{b}±0.48	57.67^{a}±0.61	62.67^{b}±0.42	57.00^{a}±0.63	62.83^{b}±0.48
56 day	58.0^{a}±0.22	64.33^{b}±0.33	58.17^{a}±0.4	64.17^{b}±0.48	58.50^{a}±0.43	64.67^{b}±0.33
Lipid Peroxide						
0 day	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01
14 day	0.83^{b}±0.01	0.62^{a}±0.03	0.83^{b}±0.01	0.59^{a}±0.04	0.82^{b}±0.01	0.57^{a}±0.03
28 day	0.83^{b}±0.01	0.52^{a}±0.04	0.83^{b}±0.01	0.51^{a}±0.04	0.83^{b}±0.01	0.51^{a}±0.04
42 day	0.83^{b}±0.01	0.41^{a}±0.02	0.82^{b}±0.01	0.41^{a}±0.02	0.83^{b}±0.01	0.41^{a}±0.02
56 day	0.83^{b}±0	0.37^{a}±0.01	0.83^{b}±0.01	0.36^{a}±0.01	0.83^{b}±0.01	0.37^{a}±0.01
LDL						
0 day	61.50±0.43	61.17±0.48	61.67±0.33	61.17±0.48	61.67±0.33	61.17±0.48
14 day	61.67^{b}±0.49	58.67^{a}±0.33	61.50^{b}±0.56	58.83^{a}±0.4	61.67^{b}±0.49	58.83^{a}±0.4
28 day	61.67^{b}±0.21	57.33^{a}±0.21	61.83^{b}±0.17	57.0^{a}±0.22	61.83^{b}±0.17	57.83^{a}±0.31
42 day	61.83^{c}±0.4	56.00^{a}±0.26	61.83^{c}±0.4	56.67^{ab}±0.21	62.00^{c}±0.37	57.17^{b}±0.17
56 day	61.67^{c}±0.49	55.50^{a}±0.22	61.67^{c}±0.42	56.50^{ab}±0.34	61.83^{c}±0.4	57.00^{b}±0.37

Means with different superscripts row wise differ significantly (P< 0.05).

Table 4: HDL/LDL ratio of different treatment groups(bi-weekly)

Mean±SE, n=12

Days	T_1	T_2	T_3	T_4	T_5	T_6
0 day	0.918±0.011	0.92±0.008	0.918±0.007	0.915±0.007	0.928±0.009	0.922±0.008
14 day	0.918^{a}±0.010	1.005^{b}±0.008	0.925^{a} ±0.015	1.012^{b} ±0.007	0.927^{a} ±0.016	1.010^{b} ±0.009
28 day	0.935^{a} ±0.008	1.077^{b} ±0.007	0.930^{a} ±0.007	1.067^{b} ±0.010	0.928^{a} ±0.011	1.067^{b} ±0.010
42 day	0.923^{a} ±0.013	1.123^{b} ±0.010	0.933^{a} ±0.013	1.108^{b} ±0.007	0.922^{a} ±0.012	1.100^{b} ±0.008
56 day	0.950^{a} ±0.009	1.158^{b} ±0.010	0.943^{a} ±0.009	1.137^{b} ±0.013	0.947^{a} ±0.01	1.133^{b} ±0.007

Means with different superscripts row wise differ significantly (P<0.05).

Conclusion

It is inferred from this study that Hemato-biochemical parameters in terms of cholesterol, HDL, LDL, lipid peroxide improved significantly in the groups in which GTE was provided (T_2, T_4 and T_6). However, from economical perspective, green tea and saw dust were found to be more expensive than the other treatments.

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