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INVESTIGATION INTO ANTIOBESITY OF GREEN TEA LEAVE EXTRACT ON EXPERIMENTAL RATS BY DIET INDUCED OBESITY MODEL

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Abstract

Obesity is one of the most common disorders encountered in clinical practice. It is a major risk factor for many chronic diseases. Green tea is reported to contain thousands of bioactive ingredients which are almost contributed by polyphenols which play a key role in prevention and treatment of many diseases including obesity. Our investigation aimed to study the effect of oral administration of aqueous extracts of green tea on obesity by using experimental rats. Thirty adult male albino rats (160-200gm) were divided into five experimental groups: The first considered as control group and fed on normal diet, while other four groups fed on high fat diet for three weeks to induce obesity. Obese rats were divided into three equal groups (n= 4 rats). Second group contain obese rats. Third, fourth and five group fed on 10% and 20% of green tea extract respectively. At the end the experimental period (28 days), the body weight gain, food intake, feed efficiency ratio, blood sugar, liver enzymes (ALT, AST, ALP), and lipid profile were evaluated. Our results revealed that the consumption of green tea extract produced a significant reduction in body weight in obese rats and enhances liver functions.

Keywords: Obesity, green tea, experimental rats

1. INTRODUCTION

Obesity has reached pandemic proportions around the world and now poses one of the greatest public health challenges for the 21st century. One billion of the approximately 6.5 billion people in the world are estimated to be overweight and of these, at least 300million are obese. These numbers are predicted to more than double to 2.3 billion overweight and 700 million obese by 2015.[1,2] Obesity is chronic disease prevalent in both developed and developing countries, it is a complex multi-factorial chronic disease that develops from an

interaction of genotype and environment Also, related to decreased physical activity and increased energy intake. Obesity has implications for both the individual and society in general. For the individual, obesity is associated with an increased risk of mortality, shortening life.[3,4] Obesity is also related to increased morbidity and is a major factor in a number of diseases, including coronary heart diseases, hypertension, non—insulin-dependent diabetes, pulmonary dysfunction, osteoarthritis, and certain types of cancer.[5,6]

A rapidly growing therapeutic area is the use of natural herbal supplements. One of these agents is a green tea-caffeine mixture, whose claimed anti-obesity properties have been ascribed to increased thermogenesis and fat oxidation.[7,8]

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or oolong tea. Green tea is favoured in Japan and China, and initial research on the benefits of green tea was carried out in these countries because of local customs. Green tea has attracted significant attention recently, both in the scientific and in consumer communities for its health benefits for a variety of disorders, ranging from cancer to weight loss.[9,10]

Several epidemiological studies have shown beneficial effects of green tea in cancer, cardiovascular, and neurological diseases. The health benefits associated with green tea consumption have also been corroborated in animal studies of cancer chemoprevention, hypercholesterolemia, atherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging related disorders. The beneficial effects of green tea are attributed to the polyphenolic compounds.[11,12]

The anti-obesity effects of green tea are being increasingly investigated in cell, animal, and human studies. Recent data from human studies indicate that the consumption of green tea and green tea extracts may help reduce body weight, mainly body fat, by increasing postprandial thermogenesis and fat oxidation. Reduce adipocyte differentiation and proliferation, lipogenesis, fat mass, body weight, fat absorption, plasma levels of triglycerides, free fatty acids, cholesterol, glucose, insulin and leptin. Therefore, this investigation aimed to study the possible therapy and protection effect of oral doses of green tea water extracts on obesity and liver status.[13,14]

2. MATERIALS AND METHODS

2.1 Extraction of the green tea leaves: The leaves were shade-dried at room temperature and powdered. The dried powder (500 gm) was successively extracted using methanol, ethanol and water by using a Soxhlet apparatus. After the completion of extraction, the supernatants were filtered through Whatman No.1 filter paper.

2.2 Induction of obesity:

Lard or saturated oil added to diet takes 8 weeks to develop obesity most Commonly Used model HFD contains (32.6% Protein, 33% Fat, 30% Carbohydrate, Normal chow, Lard, Casein, cholesterol, Vitamins, minerals, Yeast powder, Methionine, Nacl).

2.3 Experimental Animals:

Albino rats of either sex weighing 160-200 g were used. Animals used in the study were procured from Daksh Institute of Pharmaceutical Science, Chhatarpur (MP). Animals were acclimatized to the experimental condition for one week prior to the experiment under controlled conditions of temperature (27 ± 2^0C) and were housed in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka.1582/PO/E/S/11/CPCSPA.

2.4 Experimental design

Thirty albino rats weighing 140-180 gm were randomly divided into five groups of six each and kept in their cages for 1-week prior dosing to allow for acclimatization to the laboratory conditions. The obesity was produced in rats by the hight fat diet.

Group	Treatment	Induction of obesity
Control group	Normal saline	Normal diet
Negative group	High fat diet	High fat diet
Lipton tea group	300mg/kg	High fat diet
Himalaya tea group	300mg/kg	High fat diet
Local tea group	250mg/kg	High fat diet
Local tea group	500mg/kg	High fat diet

Table 1. Experimental design

2.5 Pharmacological Studies:

1. Body weight: Body weight was measured on every alternative day for 10 weeks.

2. Estimation of Serum Cholesterol: To 0.1ml of serum 10 ml of ferric chloride reagent was added in a test tube. Mixed well and kept for 10 min at room temperature. It was then centrifuged at 3000 rpm for 30 min. 5 ml of the supernatant was pipette out into a test tube and 3 ml of concentrated sulphuric acid was added and mixed well. To prepare standard, 10 ml of working standard (200 mg of cholesterol dissolved in 10 ml of ferric chloride reagent and made up to 100 ml with glacial acetic acid) was mixed with 0.1 ml of sodium chloride and kept for 10 min and centrifuged. 5 ml of supernatant was taken and to this 3 ml of concentrated sulphuric acid was added. Both the tubes were kept for 30 min at room temperature. To prepare the blank, 5 ml of ferric chloride solution was mixed with 3 ml of concentrated sulphuric acid. This was kept for 30 min. Read the test samples and standard against blank at 560 nm.

3. Estimation of Triglycerol (TGL): 0.1 ml of serum was added to 4 ml of isopropanol, mixed well and to this 0.4 g of alumina was added and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2 ml of the supernatant was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65° C for 15 min for saponification after adding 0.6 ml of the saponification reagent (0.5g of potassium hydroxide dissolved in 6 ml of distilled water and then 44 ml of isopropanol was added to it). After cooling down to room temperature 1 ml of sodium metaperiodate regent followed by 0.5 ml of acetyl acetone reagent

were added. After mixing, the tubes were incubated in a water bath at 65°C for 30 min. The contents were cooled and read at 430 nm. Against appropriate blank. Tripalmitin (10 mg of tripalmitin was dissolved in 100 ml of isopropanol) was used as standard.

4. Estimation of HDL Cholesterol: To 1.0 ml of lipid extract, 0.18 ml of heparin- manganese chloride reagent (3.167g of manganese chloride was added to 1 ml solution of heparin containing 20,000 units. The mixture was made up to 8 ml with distilled water) was added and mixed. This was allowed to stand in an ice bath for 30 min and then centrifuged in a refrigerated centrifuge at 2500 rpm for 30 min. The supernatant contained HDL fraction. Aliquots of the supernatant were estimated for LDL and HDL cholesterol and phospholipids.

5. Blood Glucose: Fasting blood glucose was estimated by using a commercial glucometer and test strips Accucheck Sensor test meter.

3. RESULT AND DISCUSSION

3.1 PHARMACOLOGICAL STUDY

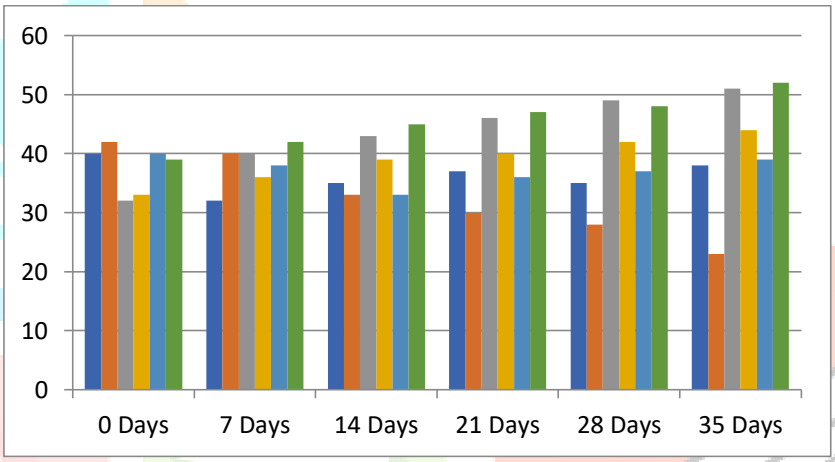

Figure 1. Effect of Ethanolic extract of Local Green Tea on Total Cholesterol level

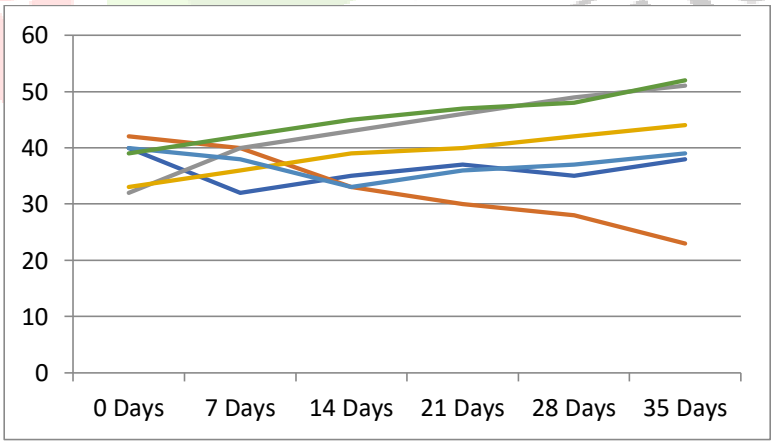

Figure 2. Effect of Ethanolic extract of Local Green Tea on Total Cholesterol level

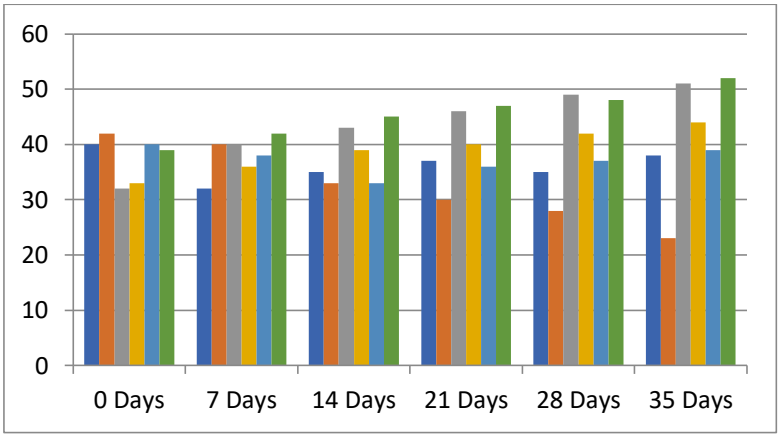

Figure 3. Effect of Ethanolic extract of Local Green Tea on HDL level

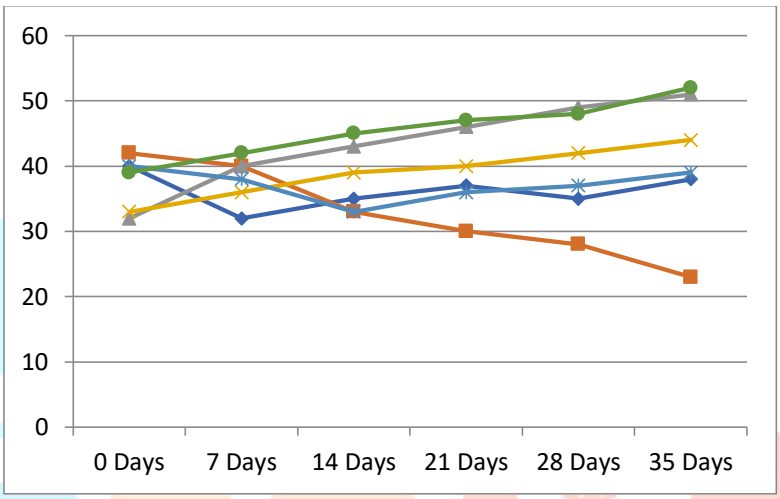

Figure 4. Effect of Ethanolic extract of Local Green Tea on HDL level

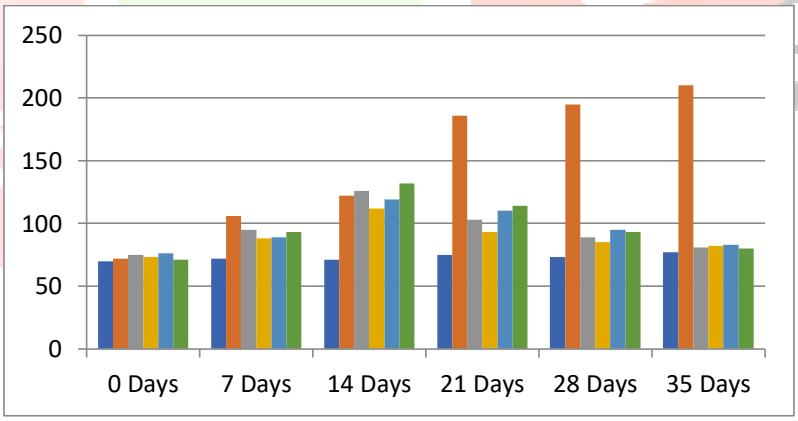

Figure 5. Effect of Ethanolic extract of Local Green Tea on Triglyceride level

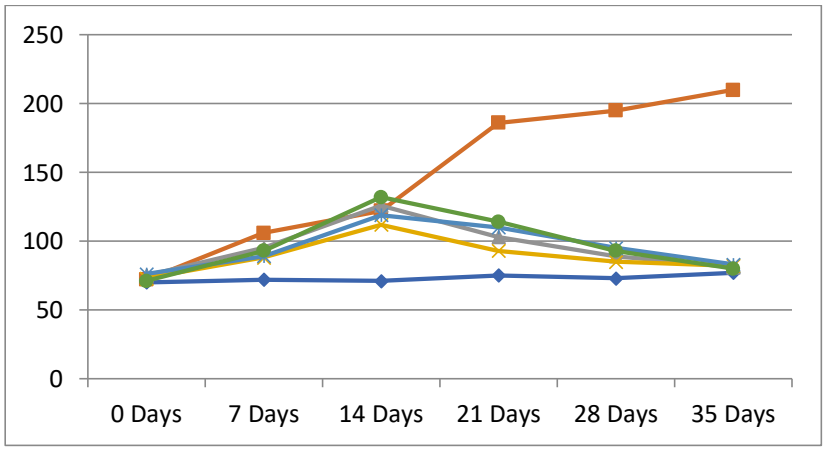

Figure 6. Effect of Ethanolic extract of Local Green Tea on Triglyceride level

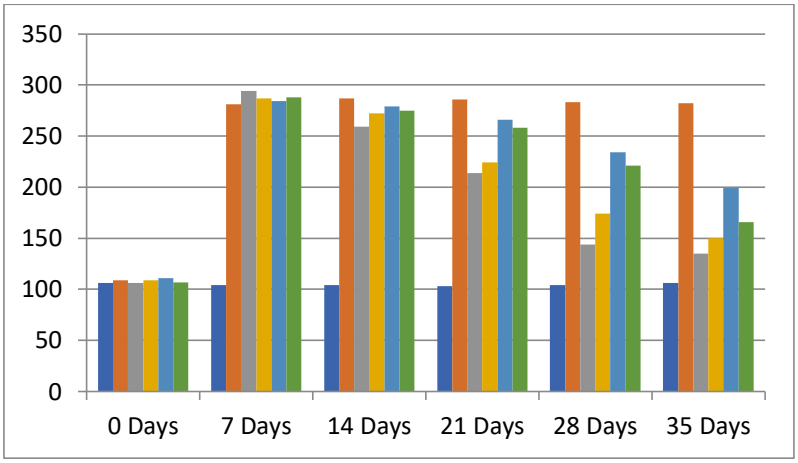

Figure 7. Effect of Ethanolic extract of Local Green Tea on Blood Glucose level

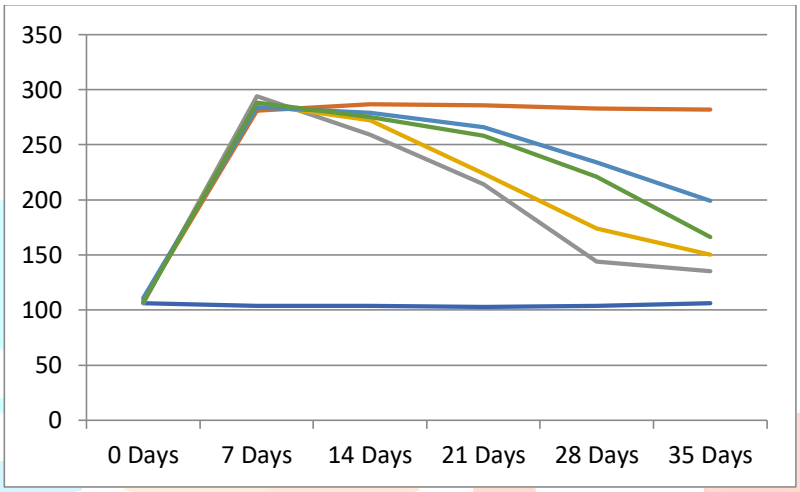

Figure 8. Effect of Ethanolic extract of Local Green Tea on Blood Glucose level

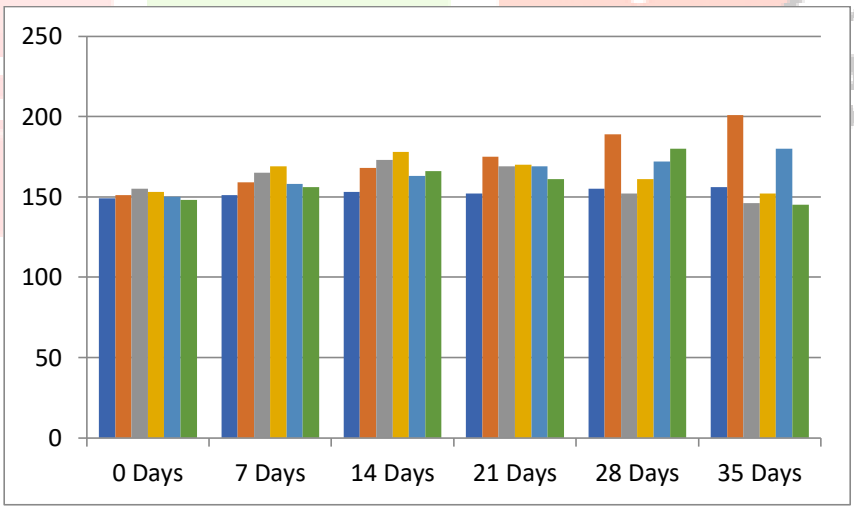

Figure 9. Effect of Ethanolic extract of Local Green Tea on Blood Glucose level

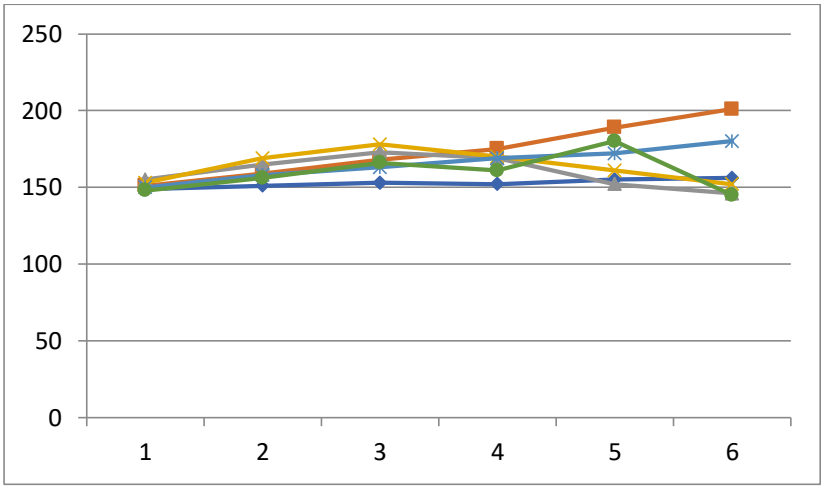

Figure 10. Effect of Ethanolic extract of Local Green Tea on Blood Glucose level

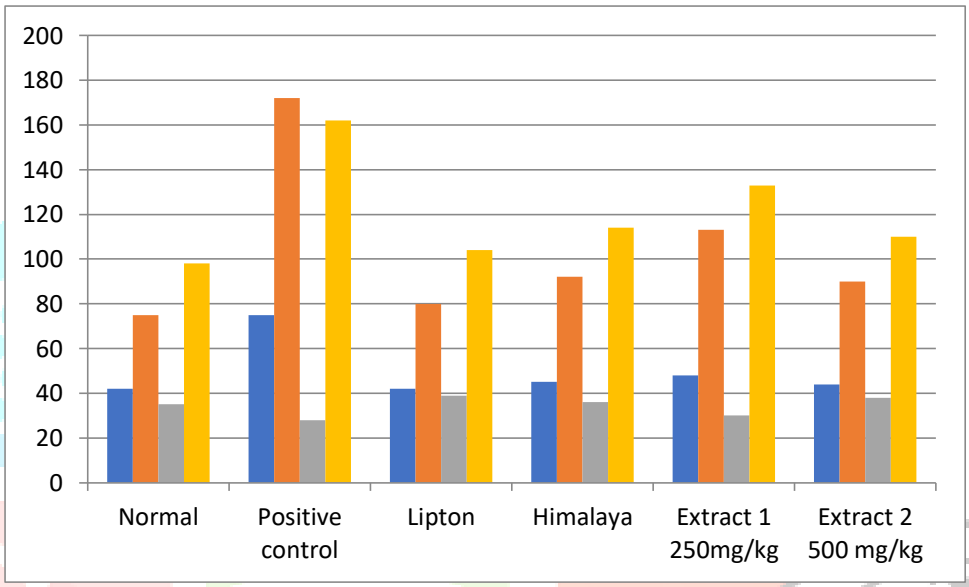

Figure 11. Effect of Ethanolic extract of Local Green Tea on Obesity

4. SUMMARY AND CONCLUSION

Present study was under taken for the screening of anti-obesity property of green tea leaves by using ethanolic extract on diet induced rat animal model. By using various species of green tea i.e., Lipton Green tea, Himalaya green tea, and local green tea we compare anti-obesity property.

For this project work Local green tea extracted with 99.99% ethanol and extraction data showed presence of flavonoids alkaloids and glycoside. These secondary metabolites may be interacted with high fat deposition special in abdominal region. And also re-established balance between metabolism.

By using diet induced model we examined various parameter like blood glucose level, triglyceride, total cholesterol level, HDL level. data represents effect of Ethanolic extract of Local Green Tea on Total Cholesterol level of experimental rats by diet induced obesity model. The Data illustrated show the effect of green tea on cholesterol, in obese rats. Apparently from the table that there were significant decreases ($^{*}p\leq0.05$) & ($^{**}p<0.001$) in serum of total cholesterol, after 14 days when treatment was start when compared to normal groups (33±1.95) Obese rats and administered with green tea extract at 250 mg/kg (45±3.11) and 500 mg/kg. (36±3.07) showed significant decrease in all lipid profiles when compared to control positive

group. In table no 8.3 Data illustrated HDL level and it is compared with two branded green tea i.e., Lipton green tea & Himalaya Green tea. Rats administered with Lipton green tea green tea and Himalaya green tea extract at dose 300 mg/kg showed the highest significant increases (51±2.11) & (44±1.50) in the total HDL level.

Regarding high density lipoprotein (HDL) it was showed a high significant decrease in the obese group compared to normal. Rats of obesity and orally administered with green tea extract at dose 250 mg/kg and 500 mg/kg showed significant increase (39±2.54) & (52±1.36) when compared to control positive group.

In table no 8.4 data illustrated total triglyceride content in serum when administered in obese rats and treatment start after 14 days. The data showed significant decreases in total triglyceride level compare than control group which showed (210±3.55*) and local green tea administered in two different doses 250 mg/kg and 500 mg/kg. Which showed (83±3.01) & (80±3.23) result.

GTE intake decreases the absorption of triglycerides and cholesterol, and these findings are in accordance with the fact that fat excretion increases. Nevertheless, the mechanism remains to be determined. Some studies report that green tea catechins decrease plasma total cholesterol and blood triglyceride levels, green tea extract reducing activity of cholesterol and lipid oxidation in rats by significant hypolipidemic action of polyphenols and catechins that will be responsible for the observed effects and reducing cholesterol and TGY, and relative increase HDL.

The data illustrated with rats body weight. rats were feed diet for 35 days and divided into 5 groups and our results revealed that green tea extract decreased body weight gain and had an anti-obesity potential. When compared result with control groups (201±4.42) and for GTE at two different dose is (180±6.54) & (180±6.54). As per previous study green tea powder (GTP) to the diet suppressed fat accumulation and body weight increase. And it indicated that caffeine and theanine were responsible for the suppressive effect of GTP on the body weight increase and fatty accumulation. It has been reported that caffeine ingestion elevated the metabolic rate and fat oxidation in vivo through lipolysis in fat cells and the release of catecholamines. Moreover, caffeine enhanced noradrenalin- or adrenaline induced lipolysis in fat cells. They also obtained results which supported the anti-obesity activities of caffeine. Thus, it seems that the anti-obesity effect of green tea was due to enhancement of thermogenesis and fat metabolism significantly reduced or prevented body weight gain with reduction of food intake in lean and obese rats

In addition to its weight loss effects, there are studies that have suggested that tea consumption may alleviate other metabolic abnormalities related to obesity our result showed that green tea decrease body weight gain by decrease food intake that contract with who found the decrease in body fat accumulation was observed after an even shorter time (35 days) this decrease occurred without a change in food intake but with a significant reduction in food digestion.

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