

## EFFECTS OF POLARITY-BASED EXTRACTS OF PARTIALLY OXIDIZED CAMELLIA SINENSIS L. LEAVES ON LIPID PROFILE OF STREPTOZOTOCIN INDUCED HYPERLIPIDEMIC ALBINO WISTAR RATS

### FARAH NAZ TAHIR

Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road, Lahore, Pakistan.

### ASMA AHMED\*

Associate Professor (Biochemistry), Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road, Lahore, Pakistan.

Corresponding Author Email: asma.ahmad.aridian@gmail.com, asma.ahmed@imbb.uol.edu.pk.

### AISHA RIAZ

Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road, Lahore, Pakistan.

### SAMRA HAFEEZ

Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road, Lahore, Pakistan.

### SHAISTA JAVAID

Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road, Lahore, Pakistan.

### Abstract

*The effects of C. sinensis L. (Camellia sinensis L.) and its extracts on lipid profile are controversial. Plant and their products have been extensively used in the management of dyslipidemia and many plant-based anti-lipidemic products have least toxicity and can be used safely. The aim of current study is to evaluate lipid protective effects of interperitoneally induced n-Hexane, ethyl acetate, methanol, and water extracts of partially-processed leaves of C. sinensis L. (25mg/ml/kg b.w.) against streptozotocin-induced (55mg/ml/kg b.w.) hyperlipidemic albino rats of both genders (300- 400 g), by keeping negative control groups as STZ and high fed diet induced hyperlipidemic, while metformin (250 mg/ml/kg b.w.) treated group was kept as a positive control, for the estimation of total cholesterol (TC), HDL, LDL and VLDL cholesterol, and triglycerides by kit methods. Statistically analyzed results (at  $p \leq 0.05$ ) showed that C. sinensis leaves extracts have protective effect on lipid profile as total cholesterol ( $54.50 \pm 9.40$  mg/dL), triglycerides ( $53.00 \pm 8.04$  mg/dL), HDL ( $59.00 \pm 20.58$  mg/dL), LDL ( $23.33 \pm 4.72$  mg/dL), VLDL ( $14.50 \pm 1.29$  mg/dL) were significantly less than negative control {total cholesterol ( $95.25 \pm 10.47$  mg/dL), triglycerides ( $87.00 \pm 5.72$  mg/dL), HDL ( $57.00 \pm 10.36$  mg/dL), LDL ( $28.75 \pm 5.56$  mg/dL and VLDL ( $13.25 \pm 1.71$  mg/dl)} and high fed diet treated group {total cholesterol ( $310.00 \pm 30.54$  mg/dL), triglycerides ( $250.25 \pm 57.71$  mg/dL), HDL ( $56.75 \pm 5.38$  mg/dL), LDL ( $95.25 \pm 19.92$ mg/dL) and VLDL ( $40.25 \pm 1.71$  mg/dL)} while results of plant extracts were near to positive control group {total cholesterol ( $65.50 \pm 19.94$  mg/dL), triglycerides ( $97.50 \pm 32.65$  mg/dL), HDL ( $46.50 \pm 11.96$  mg/dL), LDL ( $40.25 \pm 26.81$ mg/dL), and VLDL ( $19.25 \pm 9.32$  mg/dL). Active compound from*

active extract can be isolated for the synthesis of its analogue or for the synthesis of new drugs to cure hyperlipidemic disorders.

**Keywords:** *Camellia Sinensis L. Leaves, Hypolipidemic, Streptozotocin*

## 1.0 INTRODUCTION

In the past three decades, the incidence of hyperlipidemia has dramatically increased and now become one of the major causes of death due to its cardiovascular complication (Zheng et Al. 2018).

Asia become a major contributor to a rapid increase in cases of hyperlipidemia. Hyperlipidemia is a condition characterized by an increased level of cholesterol, LDL, triglycerides, VLDL, and decreased level of HDL. There is a correlation between LDL and free radical formation which is the main mechanism involved in the information of foam cells that ultimately leads to cardiovascular events (Rjeibi et al.2019) clotting which occurs due to hyperlipidemia causes blockage of renal, heart, and peripheral vessel (De Sanctis et al., 2018), and this blockage cause failure of organs (Oladiji et al., 2020).

Postprandial hyperglycemia and hyperlipidemia are the most important risk factors for the development of cardiovascular diseases (Bore'n et al 2014). Hyperlipidemia not only affects the cardiovascular system but drastically affects other organs like the liver. Clinical data showed an abnormal increase in lipid profile in bile acid metabolism disorders (Sagar et al., 2016). The foremost therapy for the management of dyslipidemia is to rectify the level of cholesterol, LDL, triglycerides, and VLDL and increase the level of HDL (Iyer et al., 2019).

The use of lipid-lowering drugs in the management of dyslipidemia has been restricted due to their many side effects (Chien et al., 2019). The traditional use of plants as a medicine in developing countries is due to its less cost and easy availability (Jamshidi-Kia et al., 2018). However traditional plants are usually given as a drug without dosage monitoring therefore it is very important to monitor its proper dosage and its toxic side effects (Josiah et al., 2019).

Herbal medicine was commonly used before the development of other medicine and there is an increase in the scope of herbal medicine in the coming years (Behrouj et al 2015). Herbal-based medicine has many curative effects on health (Tuhinet al.2017). Therefore this study aimed to evaluate the possible therapeutic effects of polarity base *C. sinensis L.* extracts on hyperlipidemia induced by STZ by studying biochemical markers of lipid profile. Current work has been designed to *evaluate* effects N-hexane, ethyl acetate, methanolic, and distilled water extracts of partially oxidized leaves of *C.sinensis L.* (*C. sinensis L.*) on total cholesterol (TC), HDL, LDL, VLDL and triglycerides in STZ-induced hyperlipidemic rats.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection, identification, and preparation of plant

Fresh leaves of *C. sinensis L.* have been collected from National Tea Research Institute, Shinkiyari, Khyber Pakhtunkhwa, Pakistan, identified and deposited at the university herbarium with the botanical number of GC.Herb.Bot.3779 by a renowned taxonomist, Prof. Dr.Zaheer-ud-Din, at the Department of Botany, Government College University Lahore, Lahore, Punjab, Pakistan. Than fresh leaves were shade dried at room temperature grind, followed by grinding of dried leaves into powdered form (80 mesh) by mechanical means, added n-hexane (1:10 ratio) and kept for shaking for 24 hours in a shaker incubator (K-J-201BD), followed by the centrifugation for 15 minutes at 5000 rpm

(SIGMA 203,43191) and filtration through Whatman filter paper 1.0. Filtrate had been shade dried at room temperature while next solvent (ethyl acetate, methanol and dist. water respectively) has been added in residue with repetition of previous procedure. Dried filtrate has been re-dissolved in 15 % DMSO to prepare stock solution (1.0 mg/ ml) (Asma et al.2016).

## 2.2 Groups of animals for *in vivo* experiment

Healthy albino Wistar rats ((300- 400 g, half male and half female) were kept in the animal house of Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Punjab, Pakistan at 20-26 °C, humidity of 30%–70% and control diet having a mixture of crude protein (23 %), crude fat (3.0%), crude fiber (7.0%), acid insoluble ash (8%) , calcium (1-2.5 %), phosphorus (0.9%), sodium (0.5-1%) and moisture (12%) during whole experiment period. Animals were divided into control groups (Vehicle having only normal saline, negative control group-I having high fed diet, negative hyperlipidemic control having only 55mg/ml/kg b.w. streptozotocin and positive control group having 55mg/ml/kg b.w. streptozotocin and 250 mg/ml/kg b.w. metformin) and experimental groups (treated with 25 mg/ml/kg b.w. of n-hexane, Ethyl acetate, Methanolic and Distilled water extracts of *C. sinensis* L. leaves).

## 2.3 Induction of diabetic hyperlipidemia, plant extracts and metformin

Oral Glucose Tolerance Test (OGTT) was performed to check which animal can become diabetic rapidly before giving STZ in 8-10 hour fasted rats. For this, after the administration of 10% glucose solution, blood glucose level were measure after 0, 30, 60, 90, and 120 minutes by using a pre-calibrated glucometer (Asma et al., 2016) and rats with blood glucose level of more than 200 mg/dL was considered as diabetic rats (Table 1). Than rats were made hyperlipidemic by intraperitoneal injection of STZ (55mg/kg body weight). 10%glucose solution were given for 2 days after STZ induction to prevent the severe hypoglycemic effect of STZ along with normal diet, followed by the intraperitoneal induction of 25mg/ml/kg b.w. of n-hexane extract, ethyl acetate, methanolic and distilled water extract of *C. sinensis* L leaves and 25mg/ml/kg b.w. of metformin.

**Table 1: OGTT of rats**

Groups	Extract or drug/ Time (Minutes)	Zero	Thirty	Sixty	Ninety	One hundred and Twenty
Vehicle	Normal saline	78±5.33	124.6±8	207.3±12	207.3±8.2	71.33±17.5
Negative control group	STZ	145.3±2.9	172±14	299.33±5	201.6±20	89.6±37.11
Positive control group	STZ+ metformin	100.67±2.4	159.6±31	221.67±31.3	233±20	115.3±51.7
High fed diet treated group	HFD	73±.67	151±31	289±6	290.33±23	68±30
Experimental group	n-hexane	115.6±8.2	124.3±67	201±2	233±2.5	115±3.5
Experimental group	Ethyl acetate	124±53	98±22	221±2.4	233±31	67±32
Experimental group	Methanol	99±7.2	105±	266±29	202±9.1	91±22
Experimental group	Distill water	78±5.33	107.3±12	259.6±31	207.3±8.2	71.33±17.5

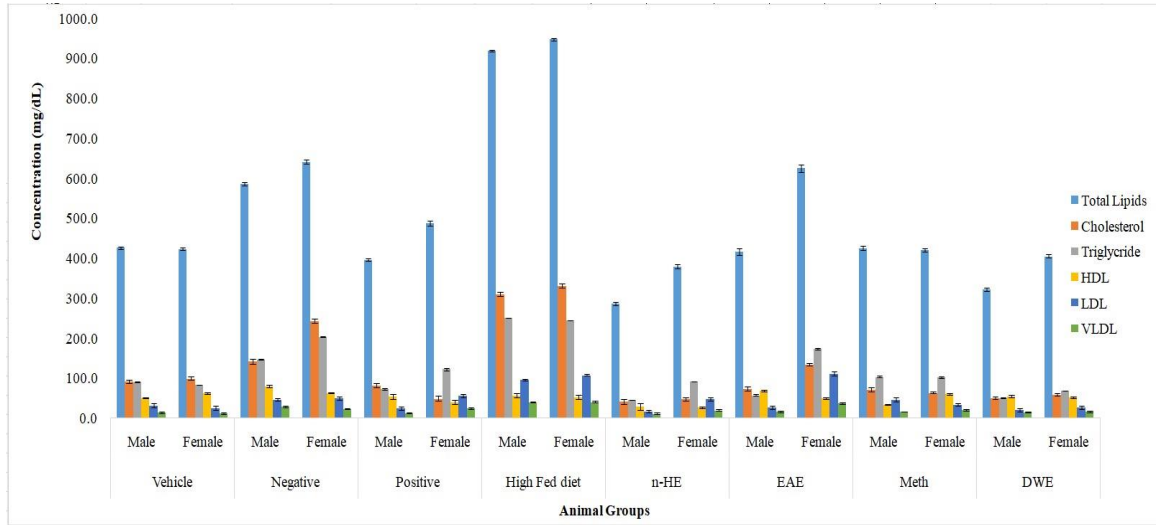
## 2.4 Determination of Lipid Profile

The rats were anesthetized by giving inhalant anesthesia (chloroform, halogenated ether) and placed in closed container for 2 to 3 minutes. After sedation, rats were removed from container in placed on a slap and cleaned it with spirit swab to avoid any contamination of skin and then made its dissection by open chest method (Greenfield EA et al.2019) for the collection of whole blood in EDTA and non-EDTA vacutainers, followed by centrifugation at 2000 rpm for 5 minutes to separate the serum and RBC for the estimation of total cholesterol (TC), triglycerides, HDL, LDL and VLDL cholesterol, by using UV- spectrophotometer and cholesterol liquid enzyme kits. Results has been analyzed statistically by using SPSS2020 to calculate mean, standard deviation and P value. Whole experiment has been done in triplicate for the authenticity of experiment.

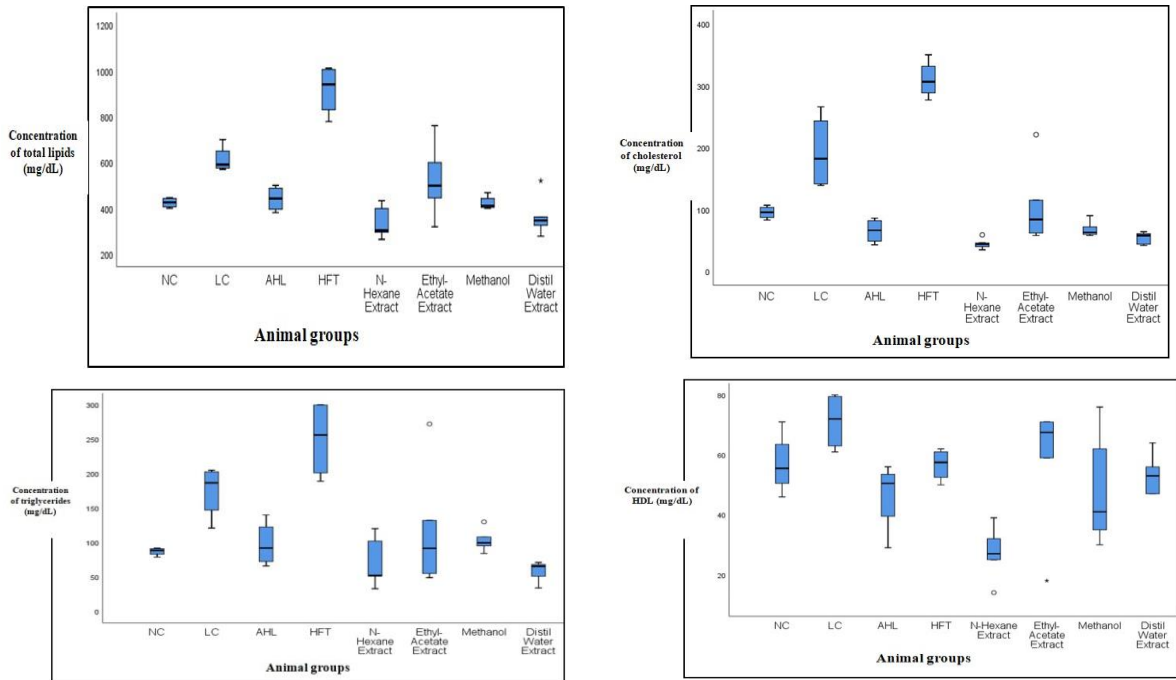
## 3.0 RESULTS

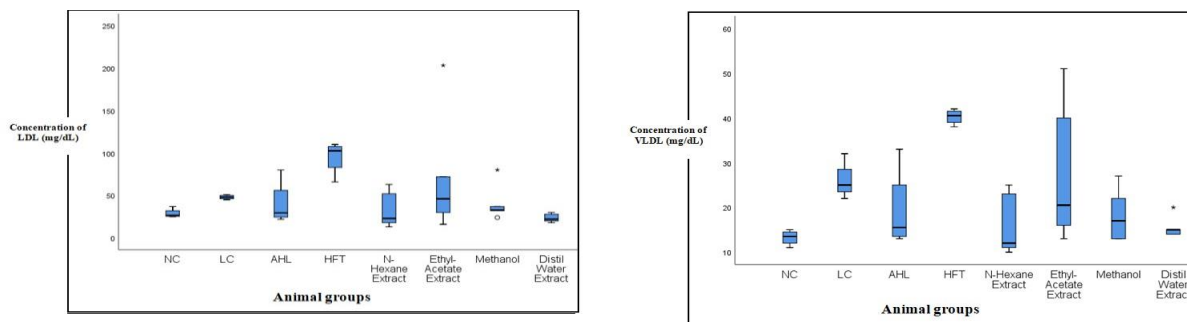
### 3.1 Analysis of Lipid Profile

Among the experimental groups, n-hexane extract treated male and female rats showed least significant effects ( $p \leq 0.05$ ) on the reduction of cholesterol ( $41.30 \pm 3.75$  mg/dL and  $48.00 \pm 2.75$  mg/dL), triglycerides ( $45.3 \pm 2.90$  mg/dL and  $91.3 \pm 4.45$  mg/dL) and HDL ( $28.30 \pm 2.58$  mg/dL and  $26.30 \pm 2.58$  mg/dL) and improvement in LDL ( $16.7 \pm 3.83$  mg/dL and  $47.3 \pm 2.37$  mg/dL) and VLDL ( $40.3 \pm 5.17$  mg/dL and  $40.5 \pm 4.7$  mg/dL), followed by male and female rats treated with ethyl acetate {cholesterol ( $73.7 \pm 4.01$  mg/dL and  $134.3 \pm 6.9$  mg/dL), triglycerides ( $57 \pm 5.51$  mg/dL and  $173.3 \pm 15.5$  mg/dL), HDL ( $68.7 \pm 4.82$  mg/dL and  $49.3 \pm 3.86$  mg/dL), LDL ( $26.3 \pm 2.13$  mg/dL and  $111.3 \pm 12.3$  mg/dL) and VLDL ( $16.00 \pm 5.49$  mg/dL and  $37.7 \pm 4.9$  mg/dL) and distilled water extracts {cholesterol ( $50.00 \pm 3.08$  mg/dL and  $59.00 \pm 3.5$  mg/dL), triglycerides ( $50.00 \pm 8.04$  mg/dL and  $68.3 \pm 4.9$  mg/dL), HDL ( $55.30 \pm 5.29$  mg/dL and  $51.30 \pm 6.9$  mg/dL), LDL ( $20.3 \pm 6.95$  mg/dL and  $26.3 \pm 4.25$  mg/dL), VLDL ( $14.7 \pm 1.29$  mg/dL and  $16.3 \pm 3.19$  mg/dL). Rats treated with methanolic extract showed more significant results as compared to other experimental group in the reduction of cholesterol ( $71.70 \pm 9.40$  mg/dL and  $63.70 \pm 5.8$  mg/dL), triglycerides ( $103 \pm 14.13$  mg/dL and  $102.3 \pm 9.3$  mg/dL), HDL ( $34.3 \pm 6.47$  mg/dL and  $60.7 \pm 5.7$  mg/dL), LDL ( $45.7 \pm 6.95$  mg/dL and  $34.00 \pm 6.5$  mg/dL), VLDL ( $16.00 \pm 1.29$  mg/dL and  $20.00 \pm 3.2$  mg/dL). Results of experimental groups are compatible to that of vehicle in which total cholesterol ( $91.5 \pm 10.47$  mg/dL and  $99.00 \pm 9.7$  mg/dL), triglycerides ( $91.00 \pm 6.5$  mg/dL and  $83.00 \pm 5.72$  mg/dL), HDL ( $51.00 \pm 6.36$  mg/dL and  $63.00 \pm 5.72$  mg/dL), LDL ( $32.00 \pm 5.56$  mg/dL and  $25.50 \pm 5.72$  mg/dL) and VLDL ( $14.5 \pm 1.71$  mg/dL and  $12.00 \pm 5.72$  mg/dL) were normal as compared to negative control group in which total cholesterol ( $141.5 \pm 21.00$  mg/dL and  $243.00 \pm 10.47$  mg/dL), triglycerides ( $147.00 \pm 5.72$  mg/dL and  $202.5 \pm 10.47$  mg/dL) and LDL ( $79.5 \pm 5.56$  mg/dL and  $63 \pm 10.47$  mg/dL) had been increased while HDL ( $46.00 \pm 5.36$  mg/dL and  $50.00 \pm 5.47$  mg/dL) and VLDL ( $28.5 \pm 7.71$  mg/dL and  $23.5 \pm 8.47$  mg/dL) didn't varied significantly. Total cholesterol ( $310.00 \pm 30.54$  mg/dL and  $331.5 \pm 10.47$  mg/dL), triglycerides ( $250.3 \pm 27.71$  mg/dL and  $244.5 \pm 10.47$  mg/dL), HDL ( $56.8 \pm 5.38$  mg/dL and  $52.5 \pm 8.47$  mg/dL), LDL ( $95.3 \pm 19.92$  mg/dL and  $107.5 \pm 10.47$  mg/dL) and VLDL ( $40.3 \pm 8.71$  mg/dL and  $40.5 \pm 6.47$  mg/dL) was higher in rats treated with high fed diet as compared to animals in positive control group where there was significant decrease in total cholesterol ( $82.00 \pm 9.94$  mg/dL and  $49.00 \pm 6.47$  mg/dL), triglycerides ( $72.50 \pm 12.65$  mg/dL and  $122.5 \pm 10.47$  mg/dL), and HDL ( $53.5 \pm 6.81$  mg/dL and  $39.5 \pm 5.17$  mg/dL) while LDL ( $24.50 \pm 4.96$  mg/dL and  $56.00 \pm 5.47$  mg/dL), and VLDL ( $13.5 \pm 2.32$  mg/dL and  $25.00 \pm 4.47$  mg/dL) were significantly improved as compared to negative control and high fed diet group. There was more improvement in the lipid profile of male rats as compared to female rats (Figure 1 and Figure 2).



**Figure 1: Comparison of Lipid Profile in Control and experimental groups in both genders of rats**  
 n-HE= n- Hexane extract, EAE= Ethyl acetate extract, Meth= Methanolic extract, DWE= Distilled water extract





**Figure 2: Boxplot showing comparative effects of various extracts on lipid profile of experimental groups**

**NC= Vehicle, LC= Lipidemic control/ negative control, AHL= Anti-hyperlipidemic control/ positive control, HFT= High fed-diet treatment**

#### 4.0 DISCUSSION

As a traditional functional tea, *C. sinensis L.* is most commonly used in the treatment of weight loss and other obesity-related disorders due to its remarkable activity. Additionally, *C. sinensis L.* is used as a health tea to protect different organs of the body like the liver and to control blood lipid and sugar levels (Zheng, et al., 2019). Previous data and research supported the traditional use of *C. sinensis L.* as medicine and found that *C. sinensis L.* can effectively control insulin resistance and metabolic disorders caused by long-term intake of a high-fed diet. The current study clarified the mechanism of *C. sinensis L.* to control lipid metabolism disorder in STZ-induced diabetic hyperlipidemic rats (Le et al., 2016).

*Phytochemical screening of C. sinensis L. and HPTLC confirmed tannin and EGCG in C. sinensis L. extracts. Ex vivo studies also confirmed the permeability of these contents across the gut in rats and in vivo studies showed no toxic side effects at the dose of 100 mg/kg (Pandit et al., 2019). The main mechanism which is involved in lipid metabolism regulation is EGCG which emulsifies and hydrolyze the lipids in the gut and increases the uptake of cellular remnants of lipids and secretion of chylomicron. EGCG and curcumin both are mainly involved to reduce hyperlipidemia (Pandit et al., 2019).*

In current study group rats developed dyslipidemia which agrees with previous studies (Kleiner et al., 2018 Zhao et al., 2018). In another study (Ma et al., 2019) tea polyphenols that are present in *C. sinensis L.* reduce hyperlipidemia by improving the expression levels of a gene which are involved in lipid metabolism and also modulates gut microbiota (Tian, C et al., 2013). Gene that is mainly involved in hyperlipidemia is SREBP-1c which is up-regulated and codes for major enzymes that cause increase fatty acid synthesis and also increase cholesterol uptake. PPAR- $\alpha$  is another gene that is involved in beta-oxidation and therefore decreases lipid accumulation (Petta et al., 2009). TLR4 can activate NF $\kappa$ B through MyD88- dependent and -independent pathways it causes cytokines expression (Li et al., 2017). In this study *C. sinensis L.* cause reverse changes in the hepatic mRNA expression levels of PPAR $\alpha$ , SREBP-1c, SREBP-2, and TLR4 and therefore decrease fatty liver (Borrelliet al2018).

Another study showed the anti-obesity effect of ethanolic extracts from *C. sinensis L.* along with glucose intolerance. The GallocatechinGallate-Containing (GCG) component of *C. sinensis L.* plays a major role in controlling cholesterol and triglyceride level in this study (Seo et al., 2015). (GCG) control lipid metabolism through LDL receptor induction. In this study, it also increases the mRNA expression

in adipocytes and monocyte and controls lipid synthesis, storage, and transport (Lee et al 2015). it has been reported that the effect of *C. sinensis L.* increased in the presence of caffeine in controlling weight gain and reducing body fats.

*C. sinensis L.* contains carbohydrates, minerals, antioxidants, flavonoids, and many other important constituents. There is a strong relationship between *C. sinensis L.* intake and cardiovascular disease. Recent studies have shown that those subjects who drink 2 cups of *C. sinensis L.* had lower plasma cholesterol concentration and their incidence of death from cardiovascular disease is reduced to 22% to 33 % this important role is due to the presence of catechins (the main one being epigallocatechin-3-gallate), which is cardioprotective and inhibit thrombin formation, inhibit oxidation and improve blood lipid concentration by inhibit the main enzyme for its synthesis and decrease intestinal absorption (Stangl V et al., 2007 and Kobayashi et al.,2014). *C. sinensis L.* also contains flavonols ( quercetin, kaempferol, myricetin), phenolic acids ( gallic and chlorogenic acids), proanthocyanidins (prodelphinidin), xanthic bases (caffeine, theophylline), polysaccharides, essential amino acids (glycine, serine, valine, leucine, threonine, and amino acid theanine), also vitamins (B, C, E), minerals and trace elements (calcium, magnesium, manganese, copper, zinc, selenium, potassium) (Sinija et al., 2008). Tea flavin plays an important role in the expression of pancreatic lipase which is the main enzyme involved in obesity and deranged lipid metabolism by regulating the AMPK–FoxO3A– MnSOD pathway in 3 T3-L1 adipocytes (Lijun et al.,2016). In the presence of a high-fed diet nutrient infusion increase in Wistar rats because of increased expression of lipogenic genes. This occurs due to a negative feedback mechanism to prevent excess storage of fats and adipocyte hypertrophy occur due to excess accumulation of fats because of gene expression. *C. sinensis L.* extract not only control hypertrophy but also decrease the intracellular content of lipid. The same mechanism occurs in the liver and skeletal muscle cells (Cho et al.2019).

Another study showed the presence of saponins in the water extract of *C. sinensis L.* showed a hypolipidemic effect in high-fed diet-induced rats. The result showed serum TC, LDL, HDL, and atherogenic index (AI) were reduced after the treatment and these extracts increased the expression of liver receptors SR-AI, SR-BI, and CD36 (Song C et al., 2016). Liver biopsy showed an improvement in steatosis after treatment with extracts in hyperlipidemic rats. The result showed that saponins block steatosis by inhibiting the accumulation of lipid droplets in the liver. It is reported that there is a strong correlation between LDL cholesterol and atherogenic index 4% decrease in LDL cause a 5 to 10 % decrease in the risk of atherosclerosis (Katan et al 2003). Enzymes involved in cholesterol synthesis were inhibited by water extracts of *C. sinensis L.* resulting in decreased cholesterol level (Song et al., 2012). Hepatic LDL-R present on the cell surface plays an important role in controlling lipid metabolism. This study showed the increased expression of LDL-R after extracts treatment indicates the hypolipidemic effect of *C. sinensis L.* water extracts. It is reported that SR-A and CD36 are involved in oxidized LDL metabolism 75% to 90%.and play role in atherosclerosis.

Another study which was conducted on *C. sinensis L.* extracts showed that it increases leptin levels and decreases LDL levels in obese women and showed no effects on other biochemical parameters (Huang et al., 2018). This showed that GTE has anti-diabetic and anti-obesity effects. Due to the presence of EGCG, these extracts also increase fatty acid oxidation and energy expenditure (Chia-Yu Liu et al., 2014). EGCG also inhibits the COMT enzyme that causes epinephrine degradation and increases lipid breakdown (Most et al., 2015). This study showed that the decrease in LDL level after GTE is due to a decrease in lipid and protein absorption in the intestine and activating the pathway which decreases gluconeogenesis and fatty acid synthesis and it proved that the lowering of LDL is due to EGCG (Yang et al., 2016). Li et al suggested that the effect of *C. sinensis L.* occurs after 8 weeks

of usage. Many studies conducted on *C. sinensis L.* and its extracts in humans and animals showed that it markedly decreased LDL levels and increased HDL levels. It also reduced total cholesterol levels but not in all (Li et al., 2016). Another study conducted in triton-induced hyperlipidemic rats showed that *C. sinensis L.* extracts exhibit its hypolipidemic effects by inhibiting the pancreatic lipase enzyme and increasing the energy expenditure. It mainly decreases the triglycerides level in this study. It is due to the suppression of lipogenic genes and stimulation of lipid catalytic genes in peripheral tissue. Serotonin secretion and pancreatic lipase inhibition are mainly involved in decreasing the triglycerides level in rats in this study (Seo et al., 2017).

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