

EFFECT OF CAFFEIC ACID LOADED BIOPOLYMERS ON CORTISOL AND KIDNEY AGAINST HIGH FAT DIET INDUCED METABOLIC SYNDROME IN ALBINO MICE

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Abstract

The current study aimed to assess the impact of biopolymers loaded with caffeic acid on cortisol of albino mouse models of experimentally induced metabolic syndrome (MetS). 48 healthy adult albino mice were randomly assigned to 8 equal groups. For 4 weeks, the "Control group," Group 1, was fed a typical chow diet. For 35 days, caffeic acid-loaded biopolymers (300 mg/kg) were administered to Groups 3, 4, and 5 (the control group). MetS was initially induced in Group 2 (MetS), Groups 6, 7, and 8 (MetS + Caffeic Acid Loaded Biopolymers) by an oral dosage of 30% fructose and a high-fat diet for 4-6 weeks. After that, Group 2 continued to consume a high-fat, high-fructose diet for 35 days, whereas Groups 6, 7, and 8 got biopolymers enriched with caffeic acid for the same amount of time. Blood samples from each animal were taken on days 15th and 35th. The centrifuged samples were then stored at -40°C to estimate biochemical and hormonal parameters. According to findings, MetS highly significantly raised levels of triglycerides, cholesterol, LDL, creatinine, urea, and cortisol while non significantly increased albumin, highly significantly lowered levels of total protein and HDL, and non-significantly decreased levels of globulins. Contrarily, delivery of caffeic acid ladened with biopolymers led to the maintenance of all parameters' normal levels. After caffeic acid loaded biopolymers were administered to the MetS group, there was a decrease in the levels of triglycerides, albumin, cholesterol, LDL, creatinine, urea, and cortisol and an increase in the levels of total protein, HDL, and globulins. Accordingly, it can be said that caffeic acid loaded biopolymers are a promising drug with the potential to ameliorate MetS morbidities on several biochemical parameters based on the findings of the current investigation.

1.1 Metabolic Syndrome

The unified parameters, which continue to be used to describe MetS (Metabolic Syndrome), specify that aberrant results of three out of the four risk factors—increased blood pressure, dyslipidemia (raised triglycerides (TG) and reduced high-density lipoprotein cholesterol),

higher fasting glucose, and central obesity—would designate a person as having MetS. (Alberti et al., 2009; Eckel et al., 2010). Individually, these elements can cause cardiac dysfunction, but when combined, they can increase morbidity and mortality (Li et al., 2021). The clinical community has yet to agree

on a common nomenclature and diagnostic criteria for MetS, despite its widespread recognition as a serious illness. This is mostly due to the genetic predisposition, dietary history, and physical, geographical, and endocrine variables that all contribute to the development of this complicated disease (Etchegoyen et al., 2018). MetS is caused by increased oxidative stress and chronic inflammation. In a number of instances, it has been proven that an antioxidant deficiency may play a role in its development, where there is an overproduction of reactive oxygen and nitrogen species (ROS and RNS) that may cause oxidative damage to almost all biomolecules (Li et al., 2015; Maritim et al., 2003). Similarly, human studies have shown that MetS is associated with oxidative stress and a proinflammatory state in peripheral blood mononuclear cells, which are believed to originate from a preactivated state of human cells (Montserrat-Mesquida et al., 2020).

1.2 Cortisol

The hypothalamus-pituitary-adrenal axis (HPA) and obesity have a convoluted connection. Because 11-hydroxysteroid dehydrogenase type 1 (11-HSD1) in the liver reactivates cortisol in obese individuals, there is a greater metabolic clearance of cortisol in these individuals (Andrew et al., 1998; Stewart et al., 1999; Rask et al., 2002). Even though cortisol release is considerably higher in obese people, their cortisol concentrations are normally normal or low. Some study, however, suggests that a subset of individuals who maintain high plasma cortisol levels while being obese have a high risk of metabolic syndrome. When assessing the risk of glucose intolerance, high blood pressure, and the metabolic syndrome, obesity has an impact because of increased plasma cortisol levels or HPA reactivity in these individuals (Phillips et al., 2000; Walker et al., 2000; Reynolds et al., 2001).

Stress enhances the activity of the hypothalamus-pituitary-adrenal (HPA) axis and leads the adrenal cortex to release more corticosteroids. Therefore, cortisol and corticosterone are often used as biomarkers for anxiety and depressive disorders. Corticosterone is believed to be the major

glucocorticoid involved in regulating stress responses in mice. However, researchers typically test cortisol for stress indicators since it is more practical and kits are easily accessible. Cortisol is the primary glucocorticoid in humans, while modest 17 α -hydroxylase activity in the adrenal glands of rats and mice suggests that corticosterone is the primary glucocorticoid in these species (Keeney et al., 1995). However, this does not indicate that these animals do not produce cortisol, particularly under stressful situations. Although this enzyme is not visible in the adrenal cortex of adult mice or rats, it is expressed in the gonads (Goascogne et al., 1993) and may be visible in the adrenal glands of mouse embryos (Keeney et al., 1995). In fact, many kinds of stress have been shown to increase cortisol levels in the plasma and adrenal glands of mice (Nakamura et al., 1990). (Won and Lin, 1995). The antibodies used for the detection of corticosterone may have some, albeit modest, cross-reactivity with other glucocorticoids or steroids, despite the fact that this is not explicitly stated in the majority of published studies.

1.3 Caffeic Acid

Coffee, honey, potatoes, berries, herbs, and vegetables such as olives, Swiss chard, and carrots contain CA (Armutcu et al., 2015). In vitro and in vivo studies have demonstrated that CA has antibacterial (Kim et al., 2014), neuroprotective, anti-anxiolytic (Koga et al., 2019), antiproliferative, and anti-inflammatory properties, as well as the ability to alleviate inflammation and oxidative stress in chronic metabolic diseases. This strengthens the argument for using CA as a feasible oral delivery method for phytonutrients. Aside from pure CA, studies have indicated that the anti-MetS effects of its derivatives caffeic acid phenyl ester (CAPE) and caffeic acid phenylethyl amide (CAPA) have also been shown. In contrast, CAPA and CAPE are less stable in their form than CA (Weng et al., 2012). CAPE is a propolis component with anti-inflammatory, anti-oxidant, and anti cancer effects (Shin et al., 2019). CA (Caffeic Acid) has been shown to increase insulin sensitivity under hyperglycemic conditions by decreasing

proinflammatory cytokines and increasing adiponectins (Vanella et al., 2016).

1.4 Biopolymers

1.4.1 Guar gum

It is a natural water-soluble dietary fiber called partially hydrolyzed guar gum (PHGG) that is created by the controlled partial enzymatic hydrolysis of guar gum (GG). PHGG is flavorless and may be readily included into any diet (Yoon et al., 2008). In individuals with type 2 diabetes and the MetS, the potential effects of GG on MetS components are yet unclear.

1.4.2 Sodium alginate

The sodium alginate in brown seaweeds, which functions as a matrix polysaccharide in the cell wall and accounts for over 40% of their dry weight, is extracted (Hay et al., 2013). Sodium alginate is an extensively used food ingredient in the food processing industry. And earlier studies suggested that sodium alginate might decrease postprandial blood glucose levels, increase fat excretion, decrease calorie intake, and reduce inflammation related to obesity (Wilcox et al., 2014). However, further research is required in the lower intestine canal to discover if sodium alginate is employed as a satiety agent in a pleasant food vehicle consumed on a regular basis. Regarding dietary fiber, sodium alginate has the capacity to modify gut flora and regulate body metabolism (Strugala et al., 2005). It is not surprising that sodium alginate may significantly enhance intestinal function and protect against obesity and other chronic metabolic illnesses (Liu et al., 2018).

1.4.3 Tween -80 (polysorbate 80)

Dietary polysorbate 80 (PS80) is often used as an emulsifier in a range of processed meals. It has been discovered that a continual low dose of PS80 reduces the intestinal permeability of mice (Chassaing et al., 2015). In addition, PS80 treatment leads to the development of obesity and metabolic dysfunction, which are associated with dysbiosis of the gut microbiota and low-grade inflammation (Chassaing et al., 2017). Intestinal permeability induced by PS80 may rise to the point that the whole body's metabolism is compromised, perhaps due to

leakage agents such as bacteria and endotoxins. It may also serve as a model for diet-induced intestinal permeability. More than 70% of blood glucose is used by skeletal muscle, making it a large nutritional consumer whose optimum metabolic function is crucial for avoiding metabolic diseases (Egan and Zierath, 2013). It is well accepted that noncommunicable diseases such as type 2 diabetes and dyslipidemia are caused by dysfunctional muscle metabolism (Lavie et al., 2015; Milton et al., 2014). In addition, metabolic mechanisms involving glucose and lipids are essential for maintaining muscle contraction (Hargreaves and Spriet, 2015). Consequently, muscular metabolic capacity influences endurance. Based on these findings, we hypothesized that an increase in intestinal permeability would impair the glucose tolerance and endurance associated with atrophic skeletal muscle glucose metabolism. In the PS80-induced leaky gut model, we see systemic glucose intolerance accompanied with inactive skeletal muscle metabolism.

MATERIAL AND METHODS

2.1 Chemicals and diets

Mice were fed with Chow diet (Almeida-Suhett et al., 2019) from Tollinton Market, Cheese (Kim et al., 2021) of Adam brand, Sunflower oil (Masi et al., 2012) from Ichra, Galaxose -D (Moreno et al., 2018) of Unilever brand, Sunflower seeds (Pritchett-Corning et al., 2013) from Tollinton Market and Ad Libitum drinking water, Caffeic acid of Unicamp brand, Guar gum, Sodium alginate and Tween-80 of Sigma Aldrich Germany.

2.2 Synthesis of biopolymers of Caffeic acid

Different amounts of each polymer, namely guar gum and sodium alginate (as described below), were taken separately in a beaker and mixed continuously with hot plate to dissolve in deionized water. Both polymeric solutions' temperatures were held constant at 45 °C. Tween 80 was gently added with a dropper after both solutions had completely dissolved. Following that, for 5 hours, the blended solution was continuously stirred on a hot plate (temperature 45 °C). Finally, a homogenized formulation was produced.

2.3 Loading of Caffeic acid

The combined formulation was prepared for the loading test. Weighed and dissolved in solvent was 300 mg of CA (water: ethanol; 20:

80). To create miscible formulations, all samples were combined with CA and rapidly agitated for an hour at room temperature. The sample was taken off the hot plate and placed in a low temperature storage container for future use.

Chemical No.	Polymer (A)- Alginate (g)	Polymer (B)- Gaur gum (g)	Plasticizer (g)	CA (mg)
3.	0.8	----	0.2	300
4.	----	0.8	0.2	300
5.	0.4	0.4	0.2	300

- 300 mg of caffeine acid are present in each of the samples above.
- Every sample is made using deionized water.

2.4. Purchasing and Housing of animals

48 healthy mice, 4 to 6 week old, weighing 25 to 40 grams each were acquired from the University of Veterinary Sciences in Lahore for this study and housed in the laboratory's animal housing area at Lahore College for Women University. Prior to the commencement of the experimental treatment, animals underwent a two-week acclimatization period in a regular laboratory setting. Every day, a kitchen scale was used to weigh each mouse.

2.5. Dosage Protocol

Control group was served normal diet. Met S group were served with 25%Galaxose-D (Moreno et al., 2018) and high fat diet containing 2.2g Cheese (Kim et al., 2021), 2g/kg Sunflower oil (Masi et al., 2012) 4-6 weeks. Control Caffeic acid group received normal diet with caffeic acid (300mg/kg b.wt) along with loaded polymers for 35days. Albino mice (n=6) in Met S+ Caffeic acid group received caffeic acid (300mg/kg b. wt) along with loaded polymers (Koga et al., 2019) orally daily for 35 days after the induction of metabolic syndrome.

2.6 Experimental Design

48 albino mice were used in the trial, which lasted between 10 to 14 weeks. Mice were

divided into 8 groups (Control group, MetS group, Caffeic acid concentration 1 group (CA 1),Caffeic acid concentration 2 group (CA 2),Caffeic acid concentration 3 group (CA3),Caffeic acid concentration 1 group + MetS (MetS+CA1),Caffeic acid concentration 2 group +MetS (MetS+CA2),Caffeic acid concentration 3 group +MetS (MetS+CA3).

Grouping (n=48)

MetS induced mice were further divided into two random groups. Each group was treated orally with doses.

Control group (n=6): It was provided with normal food

Met S (n=6): It was provided with 30% fructose, HFD (2.2g Cheese, 2g/kg Sunflower oil and oily chapatis) for 4-6 weeks.

Caffeic acid concentration 1 group (n=6): It was provided with normal diet along caffeic acid (300mg/kg b. wt.).

Caffeic acid concentration 2 group (n=6): It was provided with normal diet along caffeic acid(300mg/kg b.wt.) concentration 2 for 35 days.

Caffeic acid concentration 2 group (n=8): It was provided with normal diet along caffeic acid (300mg/kg b.wt.) concentration 3 for 35 days.

Met S Caffeic acid (n=6): It was provided with same diet as Met S group for 35 days and then caffeic acid (300mg/kg b.wt) was given orally for 35 days along HFD.

2.7. Sampling protocol

3 Animals from Control group were sacrificed fortnightly through euthenization. 2nd sampling from every group was done at 35th day. Their Kidneys and testes were collected, washed twice in ringer solution and weighed on electronic balance (Sartorius TE 313S-DS) for the calculation of renal somatic index by using the formula. After rinsing in 10% formalin 2-3 times, the organs were stored in 10 % formalin in glass vials for histology.

2.8. Blood Collection and Centrifugation

A substantial amount of serum was sampled using the cardiac puncture technique from albino mice that had been put to sleep or albino mice that were under profound terminal anesthesia. Depending on the size of the mouse while the heart is pumping, the maximum amount of blood was collected. Blood samples were obtained from the heart, ideally the ventricle, which may be reached from the top of the sternum, the left side of the chest, or the diaphragm. Blood was gently dragged out. Blood was transferred to an eppendorf coated with heparin. The Eppendorf containing obtained blood sample was centrifuged at 3000rpm for 15 min by using microcentrifuge machine (WiseSpin ® CF-10) for plasma separation which was then stored at - 20°C, until it was used for estimation of biochemical and hormonal testing.

2.9. Biochemical assays

Plasma Triglyceride, Plasma Total Protein, Albumin, Globulin, Plasma Urea, Plasma Creatinine was determined to study the effect of Caffeic acid loaded biopolymer by using biochemical assay kits/Chemistry analyzer.

2.10. Hormonal assay

Plasma cortisol level was determined to study the effect of caffeic acid loaded biopolymers.

2.11. Histology procedure

Histological procedures were performed to make slides of the tissues for histopathological examination of kidney tissues due to metabolic syndrome and protective effect of caffeic acid after treatment.

2.12. Microscopic study

The prepared slides containing the tissue slices of kidney were examined, and images of the slides were taken using a camera attached to a microscope (Optika B-15). Under a 10X magnification, tissues were examined. All control and treatment group slides were taken photos of and compared under a microscope (Optika B-15).

2.13. Statistical analysis

Tables and graphs were used to show the statistically examined data. The statistical analysis was conducted using IBM SPSS statistics V21*86. To determine the differences between several groups of various hormonal and biochemical parameters, mean and SEM of various biochemical and hormonal parameters were measured and graphed. One-way ANOVA was then used to examine significance.

3. RESULTS AND DISCUSSION

This experimental study's goal was to compare the metabolic syndrome induced by the HF diet to caffeic acid's ameliorative effect on cortisol levels in male albino mice. The term "metabolic syndrome" refers to a group of metabolic abnormalities, including central obesity, decreased HDLC, elevated triglycerides, hypertension, and hyperglycemia. After a month of research, the implications of a high-fat diet's role in metabolic disease became clear. Caffeic acid's ability to reduce inflammation and fight free radicals was evaluated using a variety of metrics, including biochemical parameters assessment and cortisol, a hormone, which was measured by changes in body weight, fasting blood sugar, and other biochemical metrics like tests for globulin, total protein, bilirubin, triglycerides, total cholesterol, HDL, and LDL. In order to assess similarities and differences, a comparison with prior research was conducted to determine the effectiveness of

caffeic acid-loaded biopolymers on the aforementioned parameters.

An earlier study discovered that caffeic acid reversed changes brought on by a high-fat diet (Ibitoye and Ajiboye, 2018). When body weight was compared to the 1st sampling of the normal control group, the current investigation revealed a highly- significant rise in the 2nd sampling of MetS. In a prior research by Narcisso et al., (2022), it was also demonstrated that both male and female mice put on weight while taking the HCD, however males did so more quickly than females over the course of the 4-month experiment. A prior research by Xu et al., (2020) reported that HFD mice's body weight grew considerably. According to Liao et al., (2013), HFD mice's body weight dramatically increased. The sedentary and high-calorie lifestyle that prevented them from exertion may have contributed to their weight gain. Rapid onset of fat deposition was brought on by a high-calorie diet. Therefore, this weight increase is the result of an imbalance between energy intake and expenditure, which causes fat to deposit in the white adipose tissues of the belly. Body weight decreased after caffeic acid administration in the MetS group. When the CA 1+ MetS and CA 3 + MetS groups of the 2nd sampling were compared to the MetS control of the 2nd sampling, there was a highly significant decrease, but there was a significant decrease when the CA 2 + MetS group of the second sampling was compared to the MetS control of the second sampling. A prior study by Xu et al., (2020) demonstrated that caffeic acid therapy resulted in a substantial reduction in body weight in HFD mice. According to a previous study by Liao et al., (2013), caffeic acid dramatically reduced body weight in HFD mice. The reason of decrease of body weight after treatment with caffeic acid might be due to the loaded biopolymers such as sodium alginate and guar gum which treat obesity and diabetes caused by HFD.

In MetS, glucose is the main cause of diabetes. Thus, it is crucial that blood glucose levels stay within a safe range. Estimates of fasting blood glucose levels were made. Mice blood was collected on strips linked to a glucometer after a number of hours of fasting to determine the fasting blood glucose level. Comparing the

MetS control of the 1st sampling to the normal control of the 1st sampling, the current investigation revealed a highly significant rise. There was substantial increase in fasting blood glucose levels in the mice fed a high-fat diet in the earlier work by Goodarzi et al., (2019). The elevated fasting blood sugar level might be the result of sedentary lifestyle and HFD-induced insulin resistance. When comparing the caffeic acid treated groups in the 1st and 2nd sampling to the MetS control group in the 2nd sampling, it was discovered that there had been a highly significant drop. According to research by Xu et al., (2020), caffeic acid administration significantly lowered fasting blood glucose levels in diabetic rats. According to the findings of Orsolic et al., (2021), caffeic acid is a robust and safe anti-diabetic medication that also works as a powerful antioxidant to lower blood glucose levels, extending the longevity of mice. According to the Kim et al., (2018) study, caffeic acid-treated HFD mice substantially decreased fasting blood glucose. The treatment with caffeic acid-loaded biopolymers, such as guar gum and sodium alginate, which delay the absorption of sugar in the blood and decreased the fasting blood sugar level, might be the cause of the reduced fasting blood glucose.

The current investigation shown that the triglyceride level in the MetS control 2nd sampling increased highly- significantly when compared to the 2nd sampling's normal control. Research by Moreno-Fernandez et al., (2018) revealed that mice fed a high-fat diet had their plasma triglyceride levels significantly increase. Liao et al., (2013) reported that the triglyceride level in the HFD mice dramatically rose. According to a study by Bezerra et al., (2012) the HFD group's blood triglyceride levels considerably increased. A study by Cho et al., (2010) revealed a significant rise in triglyceride levels in mice fed a high-fat diet. Triglyceride levels in rats fed a high-fat diet significantly increased, according to a prior Yang et al., (2006) study. An increase in plasma TG in MetS mice might be caused by insulin resistance or insulin deprivation, both of which were linked to an increase in lipolysis. The metabolically active intra-abdominal fat released free fatty acids (FFA) into the portal circulation, and the liver then converted these FFA into

triglycerides, resulting in a high plasma TG level. When compared to the MetS control of the second sampling, the caffeic acid treated groups of the first and second sampling exhibited a highly- significant reduction. In a research by Liao et al.,(2013) caffeic acid dramatically reduced the level of triglycerides. Similar findings were obtained by Chao et al., (2009), who found that caffeic acid reduced the amount of triglycerides in cardiac tissue and plasma. According to a Kim et al., (2018) study, caffeic acid treatment caused HFD mice's triglyceride levels to drastically fall. The decrease in plasma triglyceride level might be due to caffeic acid as caffeic acid reduce insulin resistance and due to which there was not abnormal level of inulin which lessened the adipose tissue and as a result it did not rendered the conversion of free fatty acid from adipose tissue to triglyceride.

When compared to the first sampling's normal control group, the MetS group's HDL levels decreased in the current study in a highly-significant manner. Jia et al., (2013) study revealed a substantial reduction in HDL levels in rats receiving HFD. The HDL levels of obese mice on a high-fat diet significantly decreased, according to a research by Cho et al., (2010). In a prior research by Yang et al., (2006), it was shown that rats fed a high-fat diet had a substantial drop in HDL levels. A high-fat diet might lower HDL because it reduces the transporters that carry apo-lipoproteins, which lowers HDL levels. When compared to the MetS control group from the first sampling, the CA 3+ MetS group after treatment showed a highly-significant rise, the CA 1+ MetS group showed a significant increase, and the CA 2 + MetS group showed a non-significant increase. Xu et al., (2020) showed that there was no appreciable rise in HFD mice in a research. A research by Cho et al., (2010) revealed that caffeic acid therapy against HFD- in obese mice resulted in a statistically significant rise in HDL levels. A prior work by Mu et al., (2021) shown a considerable rise in HDL levels in mice fed a high fat diet who had been given caffeic acid treatment. The increase in HDL level might be brought on by loaded polymer guar gum as it is dietary fibre and increases HDL level, Comparing the MetS control of the first

sampling to the Normal control of the first sampling, the current investigation revealed a highly-significant rise in LDL levels. A prior Buren et al. research from 2021 demonstrated a significantly substantial rise in LDL levels as a result of the HFD-diet. A prior research by Yu et al.,(2010) shown a considerable rise in LDL levels as a result of a high-fat diet. A prior research by Liang et al. in 2020 shown that mice on high-fat diets had considerably increased levels of LDL. An earlier study by Yang et al., (2006) revealed a substantial rise in LDL levels in rats on a high-fat diet. Since saturated fats raise LDL levels, a high-fat diet might be to blame for the rise in LDL. After treatment, all groups of the Caffeic acid + MetS treated group of the first and second sampling exhibited a highly-significant reduction when compared to the MetS control of the second sampling. In a prior research, obese mice fed with caffeic acid demonstrated a non-significant reduction in LDL levels (Xu et al., 2020). A prior work by Mu et al., (2021) shown a substantial reduction in LDL levels in mice fed a high-fat diet after caffeic acid treatment. In a prior research by Matboli et al., (2017), high-fat diet-induced diabetic rats in the caffeic acid-treated group exhibited a significantly lower LDL level. The decrease in LDL level might be due to guar gum as guar accelerates the oxidation of LDL in bile acids which reduce plasma level of LDL. When compared to sampling 1's normal control, the current investigation demonstrated a highly significant rise in sampling 1's MetS creatinine level. A study by Matboli et al.,(2017) established that HFD mice had a considerable rise in blood creatinine levels. In a prior study, Chang et al., (2015) found that HFD rats' creatinine levels significantly increased. In a prior study, Amin et al., (2011) found that HFD-induced MetS significantly raised the blood creatinine level. In a prior investigation, Lacerda et al., (2018) found that HFD-induced MetS led to a non-significant rise in blood creatinine levels. In a prior research, Yu et al., (2022), it was shown that mice given a high-fat diet had higher plasma creatinine levels. The rise in plasma creatinine level might be the result of renal damage brought on by a high-fat diet. When compared to Sampling 1 MetS, Sampling 1

MetS+CA3 after treatment revealed a highly-significant drop, Sampling 1 MetS+CA1 a significant decrease, and Sampling 1 MetS+CA2 a non significant decrease. A prior work by Xu et al., (2020) found that caffeic acid-treated HFD mice had a non-significant reduction in creatinine content. A prior work by Matboli et al., (2017), shown that caffeic acid-treated HFD mice had a substantial reduction in blood creatinine levels. Caffeic acid might be the reason for the drop in plasma creatinine levels because it attenuates kidney damage by inducing autophagy, which lowers plasma creatinine levels. When compared to the normal control of sampling 1, the current study demonstrated a highly- significant increase in sampling 1 MetS of urea. A prior research by Amin et al. from 2011 shown a substantial rise in serum urea levels due to MetS caused by HFD. An earlier work by Matboli et al., (2017), shown that HFD mice had a considerable rise in blood urea level. In contrast, a study by Lacerda et al., (2018) revealed a substantial reduction in blood urea level caused by MetS caused by HFD. The rise in plasma urea might be the result of renal damage brought on by a high-fat diet. MetS+CA1, MetS+CA2, and MetS+CA3 highly-significantly decreased following treatment. The blood urea content in caffeic acid-treated HFD mice significantly decreased, according to a prior research by Xu et al., (2020). A prior work by Matboli et al., (2017), shown that caffeic acid-treated HFD mice had a substantial drop in blood urea levels. Caffeic acid might be the reason for the drop in plasma urea levels because it attenuates kidney damage by inducing autophagy, which lowers plasma urea levels. Although corticosterone is thought to be the primary glucocorticoid involved in

controlling stress responses in mice, cortisol is frequently used by researchers to identify stress markers. In reality, several studies have employed cortisol as the measure for stress activation in both mice and rats and have found elevated cortisol in the plasma and adrenal gland of stressed animals. Additionally, research has demonstrated that cortisol has a substantially higher glucocorticoid potency than corticosterone (Gong et al., 2015). When compared to sample 1's normal control, the current investigation demonstrated a highly significant rise in cortisol levels in Sampling 1 MetS. A prior study conducted by Nakamura et al., (1990) found that the stress of food restriction raised the cortisol level in mice. In a research conducted by Ayada et al., (2002), it was discovered that caged mice exhibit greater chewing activity, and their cortisol levels considerably rise after just one hour of confinement. The confinement of mice in a cage during MetS induction, the impact of temperature, and insulin resistance might all be contributing factors to the rise in cortisol levels. When compared to Sampling 1 MetS, Sampling 2 MetS+CA1 and Sampling 2 MetS+CA2 after treatment with caffeic acid exhibited a non-significant drop and a significant decrease in Sampling 2 MetS+CA3, respectively. Cortisol levels in insulin resistance stressed-induced mice treated with caffeic acid were shown to be considerably lower in an experimental investigation by Choudhary et al., (2016). According to a prior study by Gul and Gunay (2020), caffeic acid reduces cortisol levels and treats vascular inflammation brought on by diabetes in rats. Caffeic acid, which treats vascular inflammation brought on by diabetes by reducing cortisol levels, might be the reason for the drop in cortisol levels.

Table 3.1: Comparison of mean $\pm$ SEM of Metabolic Syndrome Parameters before induction, after induction and after treatment.

Parameters of metabolic syndrome	MetS				
	Before Induction	After induction	After Treatment		
			T1	T2	T3
Weight(g)	35.82 $\pm$ 0.65	42.14 $\pm$ 1.30	35.98 $\pm$ 0.2	37.02 $\pm$ 0.19	34. $\pm$ 2.46
Glucose(mg/dl)	82.61 $\pm$ 0.62	182.51 $\pm$ 4.4	146.56 $\pm$ 0.6	156.38 $\pm$ 0.09	134 $\pm$ 0.5
Triglycerides (mg/dl)	138. $\pm$ 1.23	175 $\pm$ 9.01	120.5 $\pm$ 1.06	129 $\pm$ 1.41	102 $\pm$ 2.8

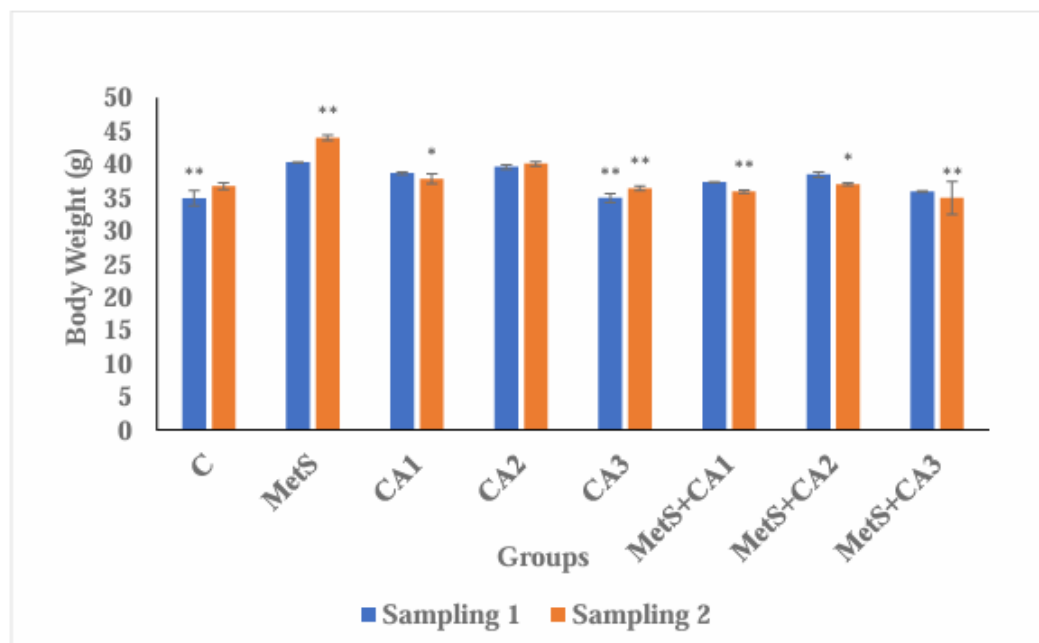


Figure 3.1

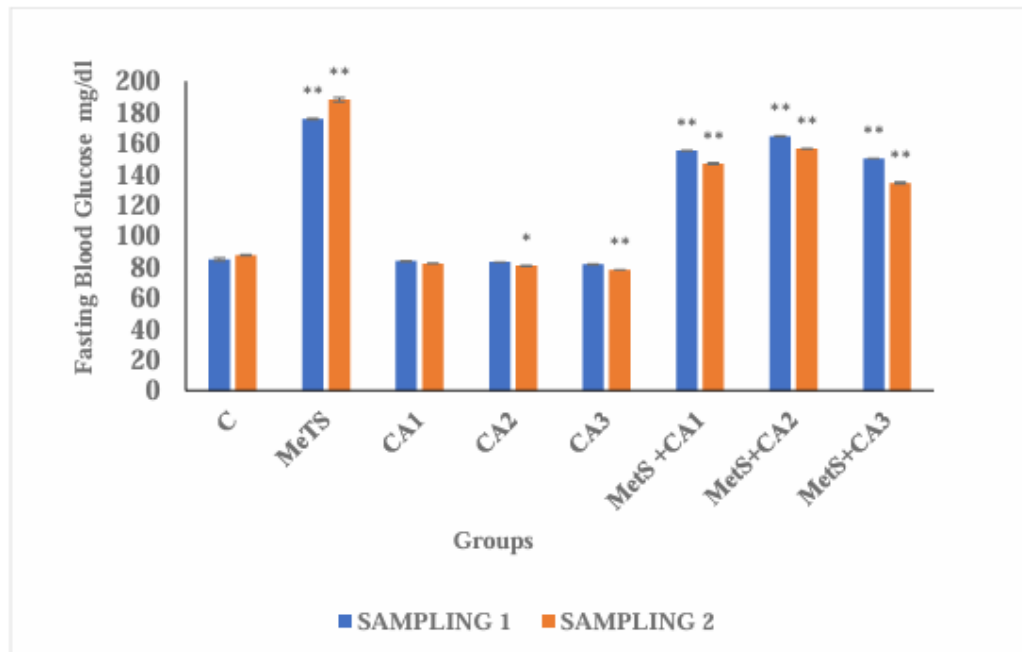


Figure 3.2

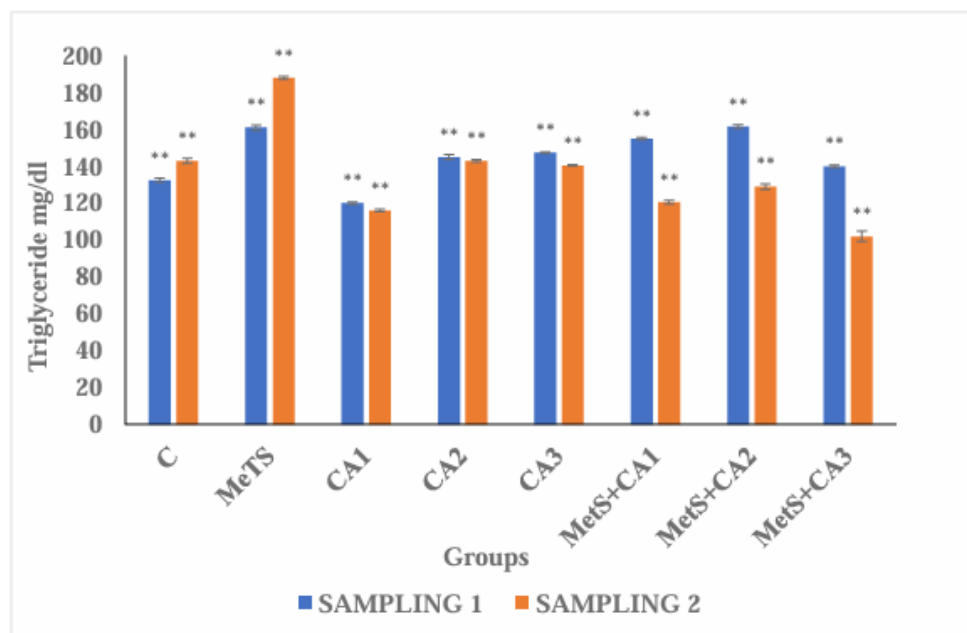


Figure 3.3

Figure 3.1, 3.2. and 3.3. : Comparison of mean $\pm$ SEM of Body Weight ,Fasting Blood Glucose and Triglycerides (mg/dl) group I (C), group II (MetS), group III (Caffeic acid conc. 1), group IV (Caffeic acid conc. 2), group V (Caffeic acid conc. 3), group VI (MetS+Caffeic acid conc. 1), group VII (MetS+Caffeic acid conc. 2) and group VIII (MetS+Caffeic acid conc. 3) after Sampling 1 (15th day) and Sampling 2 (35th day) in albino mice

Table 3.2 showed biochemical and hormonal parameters associated with kidney in control and all experimental groups from sampling 1 to 2.

	Total Proteins	Albumin	Globulin	Urea	Creatinine	Cortisol
C	3.95 ± 0.1** to 4.02 ± 0.08	22.5 ± 0.2** to 19 ± 0.57*	1.3 ± 0.1 to 1.27 ± 0.005	22.5 ± 0.2** to 19 ± 0.57*	0.35 ± 0.02 to 0.45 ± 0.02	124 ± 1.15 to 112.5 ± 0.28**
MetS	2.75 ± 0.17** to 2.44 ± 0.22**	37.5 ± 0.8** to 45 ± 1.73**	0.94 ± 0.115 to 0.4 ± 0.2	37.5 ± 0.8** to 45 ± 1.73**	0.75 ± 0.02** to 0.85 ± 0.02**	149.16 ± 1.73** to 259 ± 1.73**
CA1	3.64 ± 0.12** to 3.68 ± 0.17	21 ± 0.5** to 19.5 ± 0.28**	1.29 ± 0.22 to 1.42 ± 0.34	21 ± 0.5** to 19.5 ± 0.28**	0.45 ± 0.02 to 0.35 ± 0.02	129 ± 0.57* to 123 ± 1.15
CA2	3.33 ± 0.06 to 3.41 ± 0.04	23.5 ± 0.2** to 23 ± 0.57**	0.66 ± 0.31 to 0.86 ± 0.1	23.5 ± 0.2** to 23 ± 0.57**	0.55 ± 0.02* to 0.55 ± 0.02*	133.16 ± 1.04** to 130 ± 1.3**
CA3	4.07 ± 0.04** to 3.89 ± 0.02	21 ± 0.57** to 19.5 ± 0.28**	1.8 ± 0.17** to 1.68 ± 0.005**	21 ± 0.57** to 19.5 ± 0.28**	0.35 ± 0.02 to 0.2 ± 0.05	129.16 ± 0.6 to 122 ± 1.32
MetS+CA 1	4.45 ± 0.12** to 4.67 ± 0.13	27.5 ± 0.2** to 25 ± 0.57**	2.06 ± 0.005** to 2.35 ± 0.14**	27.5 ± 0.2** to 25 ± 0.57**	0.55 ± 0.02* to 0.46 ± 0.03	137 ± 1.76** to 133 ± 0.88**
MetS+CA 2	3.995 ± 0.01** to 4.1 ± 0.01	29.5 ± 0.28** to 28.5 ± 0.28**	1.42 ± 0.08* to 1.61 ± 0.19**	29.5 ± 0.28** to 28.5 ± 0.28**	0.7 ± 0.05** to 0.65 ± 0.02**	142 ± 1.76** to 140 ± 0.6**
MetS+CA 3	4.85 ± 0.07* to 5.14 ± 0.06**	27.5 ± 0.28** to 24 ± 0.57**	2.44 ± 0.2** to 2.97 ± 0.11**	27.5 ± 0.28** to 24 ± 0.57**	0.45 ± 0.02 to 0.35 ± 0.02	134 ± 0.57** to 132 ± 0.88**

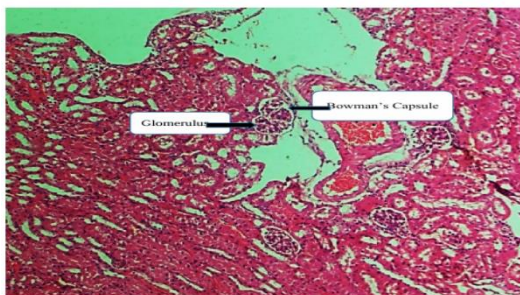


Figure 3.4

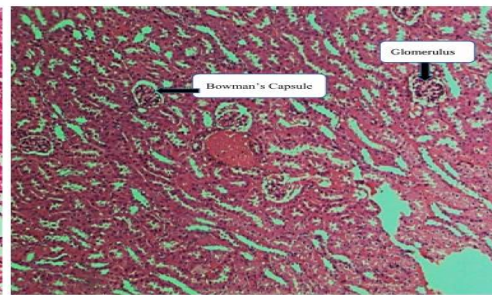


Figure 3.5

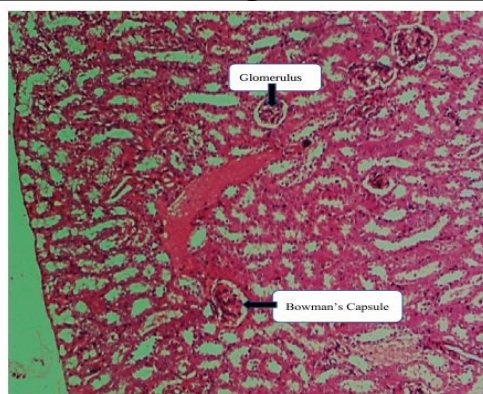


Figure 3.6

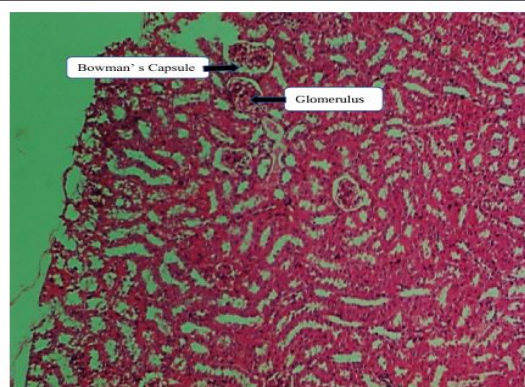


Figure 3.7

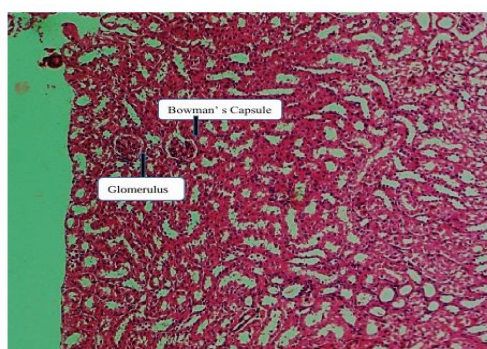


Figure 3.8

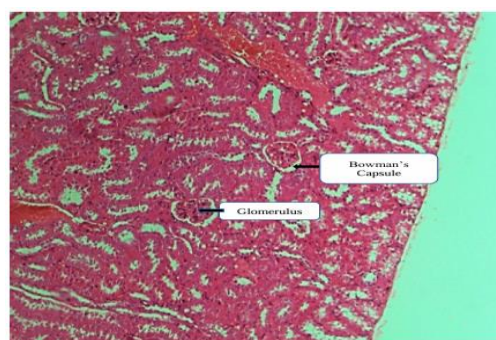


Figure 3.9

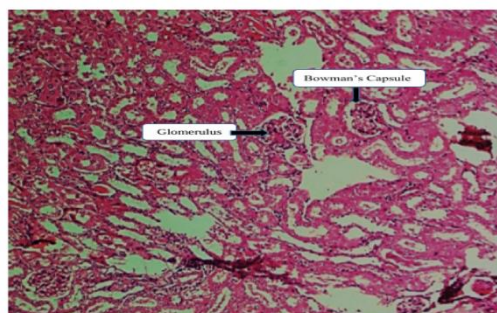


Figure 3.10

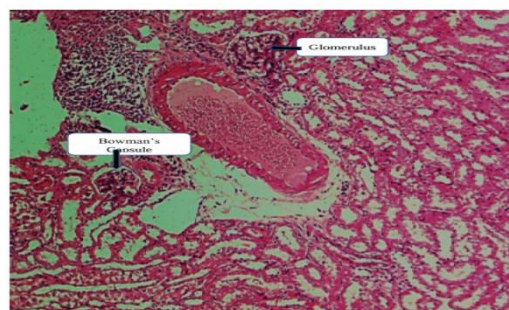


Figure 3.11

Figure. 3.4, 3.5, 3.6 , 3.7, 3.8, 3.9, 3.10, 3.11: Cross Section of Kidney of Male Albino Mice from (3.4) Control, (3.5) MetS , (3.6) Caffeic Acid Concentration 1 , (3.7) Caffeic Acid Concentration 2, (3.8) Caffeic Acid Concentration 3, (3.9) MetS+ Caffeic Acid Concentration 1, (3.10) MetS+ Caffeic Acid Concentration 2, (3.11) MetS+Caffeic Acid Concentration 3 at 10X

Conclusion

The aim of this study was to investigate the effects of biopolymers containing caffeic acid on the biochemical, hormonal, and renal histology in albino mice with metabolic

syndrome brought on by a high-fat diet. The body weight, biochemical, and hormonal parameters altered when a high-fat diet was administered. By increasing the levels of cortisol, urea, and creatinine, MetS induced

inflammation. Light-microscopy examination of kidney sections from the MetS group revealed inflammation. Caffeic acid's anti-inflammatory and antioxidant properties shield the kidney from harm. Based on our work, caffeic acid loaded biopolymers should be seen as a new and promising agent with a lot of potential for treating kidney damage and kidney diseases. The reason for this is because it reduced triglyceride, albumin, cholesterol, and LDL. Creatinine, Urea and Cortisol and raised Total Protein, HDL and Globulins.

Recommendations

A few recommendations have been obtained from this inquiry as follows: To further investigate the protective properties of caffeic acid loaded biopolymers, mice should be given caffeic acid over a prolonged length of time. The molecular characteristics of the therapy of MetS by caffeic acid loaded biopolymers were not examined because this was a brief experiment. As a result, a more thorough examination that focuses particularly on the treatment's molecular components has to be done. It is crucial to look at how caffeic acid biopolymers affect both human bodies and those of other mammalian species.

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