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# Effects of Verbena (*Lippia citriodora*), Tilia (*Tilia argentea)*, Zucchini flowers *(Cucurbita pepo L.*) on Lowering the Blood Glucose of Streptozotocin Injected Male Rats

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**Abstract:**

**Objective:** This investigation aimed to evaluate the effects of Verbena (*Lippia citriodora* **(**LC), Tilia (*Tilia argentea* (TA**)** and Zucchini (*Cucurbita pepo* (CP) flowers powder on lowering blood glucose level of diabetic male rats injected with streptozotocin. **Methods:** A total of thirty adult male Sprague Dawley rats were classified into six equal groups as follows: group (1) negative control rats that were fed of a basal diet; group (2) diabetic rats injected with streptozotocin and kept as a positive control (C+), Groups (3), (4), and (5), were fed of basal diet fortified with 5% of LC, TA, and CP, powder, respectively; Rat in group (6) were fed basal diet supplemented 5 % of all flower powders together. At the end of experiment, after 28 days of feeding, blood samples were withdrawn from eye plexuses and all serum samples were analyzed. The weights of the rats were also recorded, and relative internal organs weight were presented. The kidney and liver were removed for histopathological examination. **Results:** The results in control positive group showed significant decrease of BWG and internal organs. Also significant elevation in the serum blood glucose, VLDL, LDL, TG, uric acid, ALP, ALT, urea, AST, and creatinine as compared to the control positive group. The results improved in diabetic rats treated groups 3,4,5 and 6 where BWG increased, internal organ weight , also decreased Serum blood glucose, TG, VLDL, LDL, ALP, AST, ALT, uric acid, urea and creatinine decreased. Well improvement was detected histologically in kidney and liver during examination of all diabetic rats treated groups. The most excellent results were detected in group 6 (5% mix from all tested plants).

**Key words:** diabetic rats, *Lippia citriodora*, *Tilia argentea*, *Cucurbita pepo L.* powder, Biochemical blood parameters, Histopathological examination.

## Introduction:

Diabetes mellitus (DM) includes metabolic disorders collection described by increased blood glucose levels induced by decreased insulin secretion, action, or interaction between both. Several organs, including the nerves, eyes, heart, kidneys and blood vessels, are irreparably damaged or become dysfunctional as a chronic hyperglycemia result caused by DM. Multiple pathogenic processes contribute to the onset of DM. Potential pathological conditions involve the autoimmune pancreas B-cells destruction, which culminates in insulin deficiency, or abnormalities that give rise to resistance to the insulin action. DM is characterized by abnormalities in carbohydrates, fats, and proteins metabolism due to insulin deficiency on target tissues. Due to decreased tissue responses to insulin or insufficient insulin secretion at one or more nodes in the intricate hormone and, insulin action pathways may be inadequate **[1].**

DM can be broadly categorized into three distinct types: type I, type II, and gestational. Type I DM (T1DM) is characterized by insufficient insulin production by the ß cells of the pancreas. Type II DM (T2DM) is the most prevalent form of the disease, affecting around 90-95% of all cases. It typically originates from insulin insensitivity, a condition characterized by impaired insulin response by fat, liver, and muscle cells in response to food intake. Eventually, the pancreas ceases to adequately secrete and produce insulin in response to food intake. Gestational DM may arise due to insulin deficiency or hormonal changes that occur during pregnancy **[2].** Several decades ago, many methods are recommended for DM treatment which including changes in lifestyle, such as exercise, weight management, and diet therapy, in addition to glucose-lowering drugs **[3].**

*Lippia citriodora* (LC) (lemon verbena) is a plant belonging to the family Verbenaceae. Besides its primary application as a spice, this plant also possesses a range of medicinal properties, including anticancer, anesthetic, antimicrobial, antispasmodic, digestive, anti-inflammatory, antioxidant, antipyretic, anxiolytic, and neuroprotective. Traditionally, leaf infusions have been used for the treatment of various disorders including fever, colic, cold, rheumatism, acne, and insomnia. **[4].**

*Tilia argentea* (TA) is believed to alleviate fever, treat infectious diseases, and promote sweat by acting as a diaphoretic., also, it is occasionally employed as an antispasmodic, stomachic, diuretic, and sedative; however, experimental proof is required for these effects **[5].**

Zucchini (*Cucurbita pepo* (CP). *L*) flowers, are mineral-rich and an excellent source of phytochemicals, vitamins B1, B2, essential amino acids and folic acid **[6,7,8,9],** in addition to ascorbic acid, carotenoids, and polyphenols, which are antioxidant compounds **[10]**, as well as being frequently prescribed for pregnant women and those who are anemic

or lethargic. Zucchini flowers are a preferred component in various regions due to their brilliant yellow hue, silky texture, and delicate, slightly sweet taste **[11].**

## Materials and Methods

### Materials:

### Vegetable and other materials:

Verbena (LC), Tilia (TA), Zucchini flowers *(*CP L.) are obtained from the Ministry of Agriculture at Shibin EL-kom, Menoufia, Egypt.

### Chemicals: -

Intraperitoneal injection of streptozotocin was at (150 mg/kg) body weight. DM was induced in male albino rats that were in good health. This drug was obtained from Sigma, 29 Mawardi Street, Qasr Al-Aini, Cairo, Egypt). kits were delivered by Bio Diagnostics Company, Cairo, Egypt.

### Rats:-

Thirty (30) adult albino rats weighting (140± 10g) were purchased from the National Center for Research Cairo, Egypt.

### Methods:

**Basal diet composition of tested rats**:

The experimental diet consisted of the following components: Methionine (0.3 %), casein (10%), choline chloride (0.2 %), vitamin mixture (1%), salt mixture (4%), cellulose (5 %), corn oil (10%), and corn starch (69.5 %) according to **[12]** vitamins and salt mixture were prepared according to **[13,14].**

**Preparation of tested plants**:

Each plant was sun-dried and sorted before being milled to a fine powder utilizing an electric grinder. The resulting powder was then sealed in dusky-stoppered glass bottles and stored in a dry, cool place until it was utilized according to **[15].**

**Induced DM for rats:**

Healthy, normal male albino rats were administered with streptozotocin (125 mg/kg) body weight intraperitoneally to stimulate DM in accordance with the procedure defined by **[16].** One week after the streptozotocin injection, fasting blood samples were collected in order to evaluate fasting serum glucose. In rats, DM was diagnosed when serum glucose levels were above 200 mg/dL **[17].**

**Experimental design and animal groups:**

Under standard laboratory conditions, rats were kept in wire cages, and being fed a basal diet for one week to facilitate adaptation. Six groups were established, with five rats

in each group. All rats' groups were maintained in wire cages at ambient temperature (25 $c^0$) and in suitable health conditions. The rats were categorized as follows:

**Group (1):** Control negative group (-), in which normal rats were fed on basal diet.

**Group (2):** Control positive group (+), in which diabetic rats were fed on basal diet.

**Group (3):** DM rats fed on basal diet with 5% Verbena LC powder.

**Group (4):** DM rats fed on basal diet with 5% Tilia TA powder

**Group (5):** DM rats fed on basal diet with 5% Zucchini flowers CP *L*. powder.

**Group (6):** DM rats fed on basal diet with 5% powder composed of a mixture of 5% of herbs (LC - TA and CP *L*), where the percentage of each herb is 1.66%.

**Biological evaluation:**

Weekly body weight assessments and daily dietary records were maintained throughout the experimental period. The body weight gain (BWG g) was calculated utilizing the equations outlined by Chapman et al. (1959).

BWG (g) = Final weight - Initial weight

**Organs:**

Testes, kidney and liver were removed, cleaned, and weighed.

**Determination of Biochemical Blood Parameters:**

Following a 12-hour fasting period, blood samples were obtained through the abdominal aorta at the conclusion of the experiment. While under ether anesthesia, scarification was performed on the rats. After collecting blood samples into sterile and dry centrifuge tubes, the samples were centrifuged at 3000 r.p.m. for 10 minutes to separate the serum after clotting at room temperature. Serum was aspirated with caution, transferred to sterile cuvette tubes, and frozen at -20 °C in accordance with the protocol for biochemical analysis **[18].** The following parameters were determined for every serum sample:

Urea concentration was determined utilizing an enzymatic method of **[19]**, the creatinine measurement was performed utilizing the kinetic method of **[20]** and uric acid was determined utilizing an enzymatic colorimetric test of **[21].** Alanine amino transferase (ALT) and aspartate amino transaminase (AST) were performed utilizing the method of **[20, 22].** According to the method of **[23]**, alkaline phosphatase (ALP) was quantified. The evaluation of total cholesterol (TC) was performed utilizing method of **[24],** earning considt high density lipoprotein cholesterol (HDL-c) according to **[25].** The low-density lipoprotein cholesterol (LDL-c) evaluation was conducted utilizing the method of **[26]** and triglyceride (TG) **[21],** HDL and TC according to(24 m / 25) respectively chemical kits were utilized to measure serum glucose in accordance with **[27].**

**Histopathological Examination:**

In the experiment, small kidney and liver samples were collected from each group and fixed with 15% neutral buffered formalin, dehydrated in ethanol at increasing concentrations (70, 80, and 90%)., embedded in paraffin after undergoing xylene

clearance. Preparation of sections measuring 4-6 µm in thickness, followed by staining with Hematoxylin and Eosin according **[28].**

**Statistical Analysis:**

By means of one-way ANOVA, the data were statistically analyzed utilizing a computerized costat program. Means S.D. are used to represent the results. P≤0.05 was utilized to delineate significant differences among treatments. **[42].**

**Results and Discussion:**

Total phenolic compounds proved that TA was the best planet, while this could not be detected in biochemical analysis, since TA occupied the second position (²nd best planet) after LC, a mixture of three plants was not analyzed for phenolic to know the effect of combination. Finally, it should be noted that not only phenolic compounds are the potent antioxidant effect such as level of selenium which may be variable in different examined plants. Nevertheless, TA have the ²nd position as hypoglycemic and highest phenolic compounds.

**Table (1): Phenolic compounds of TA powder**

| Sample 1 | |
|---|---|
| **Compound** | **Conc. (µg/g)** |
| **Gallic acid** | **486.19** |
| **Chromogenic acid** | **937.91** |
| **Catechin** | **1458.76** |
| **Methyl gallate** | **419.06** |
| **Coffeic acid** | **193.75** |
| **Syringic acid** | **106.90** |
| **Pyro catechol** | **0.00** |
| **Rutin** | **29.92** |
| **Ellagic acid** | **40.53** |
| **Coumaric acid** | **110.67** |
| **Vanillin** | **138.76** |
| **Ferulic acid** | **77.76** |
| **Naringenin** | **778.51** |
| **Daidzein** | **93.88** |
| **Querectin** | **23.35** |
| **Cinnamic acid** | **17.38** |
| **Apigenin** | **0.00** |
| **Kaempferol** | **20.16** |
| **Hesperetin** | **0.00** |
| **Total** | **4933.49** |

**Table (2): Phenolic compounds of LC powder**

| Sample 2 | |
|---|---|
| **Compounds** | **Conc. (µg/g)** |

| | |
|---|---|
| **Gallic acid** | **177.17** |
| **Chlorogenic acid** | **78.47** |
| **Catechin** | **12.05** |
| **Methyl gallate** | **0.00** |
| **Coffeic acid** | **21.95** |
| **Syringic acid** | **67.22** |
| **Pyro catechol** | **0.00** |
| **Rutin** | **0.00** |
| **Ellagic acid** | **15.85** |
| **Coumaric acid** | **2.21** |
| **Vanillin** | **15.78** |
| **Ferulic acid** | **14.81** |
| **Naringenin** | **18.61** |
| **Daidzein** | **6.05** |
| **Querectin** | **4.17** |
| **Cinnamic acid** | **1.99** |
| **Apigenin** | **0.00** |
| **Kaempferol** | **0.00** |
| **Hesperetin** | **0.00** |
| **Total** | **436.33** |

## Table (3): Phenolic compounds of CP L powder

| **Sample 3** | |
|---|---|
| **Compounds** | **Conc. (µg/g)** |
| **Gallic acid** | **274.05** |
| **Chlorogenic acid** | **673.76** |
| **Catechin** | **729.43** |
| **Methyl gallate** | **51.45** |
| **Coffeic acid** | **47.15** |
| **Syringic acid** | **6.99** |
| **Pyro catechol** | **14.18** |
| **Rutin** | **172.34** |
| **Ellagic acid** | **155.01** |
| **Coumaric acid** | **21.40** |
| **Vanillin** | **3.39** |
| **Ferulic acid** | **11.24** |
| **Naringenin** | **39.64** |
| **Daidzein** | **8.19** |
| **Querectin** | **17.35** |
| **Cinnamic acid** | **2.27** |
| **Apigenin** | **146.09** |
| **Kaempferol** | **0.00** |
| **Hesperetin** | **0.00** |
| **Total** | **2373.93** |

As shown in table 4, the mean value of BWG was significantly lower in the control (+) group (17.37±1.34 vs. 39.59±1.05) g) than in the control (-) group (P≤0.05). Significant differences were observed in the mean values of rats injected with STZ on different diets when compared to the control group (+). The group of rats fed 5% LC, TA, and CP powder had the highest BWG, followed by group (5) rats fed CP, in comparison to the control group (+).

In fact, DM induced by STZ is associated with weight loss resulting from excessive protein breakdown and muscle mass atrophy caused by insulin deficiency this is likely due to the extract's ability to enhance insulin secretion, which inhibits muscle mass atrophy and the reduced glucose uptake, and metabolism in adipose tissue may also contribute to this effect. Due to the fact that insulin can stimulate glucose oxidation and uptake in adipocytes, it may increase leptin levels due to its valuable impact on β-cells, which are responsible for insulin secretion **[29, 30].**

The mean value of feed intake FI in the control (+) group was found to be significantly lower (10.34 ±0.22) in comparison to the control (-) group (14.4 ±0.12). A notable disparity was detected in the average values of rats injected with STZ on different diets in comparison to the control group (+). Group 6, consisting of rats injected with STZ and fed a mixture of plants, exhibited the highest FI value, which was significantly lower than that of the control group (-).

Numerous medicinal plants are documented to have antioxidant properties, which may give protection against the onset of DM. T2DM that is left untreated is characterized by weight loss due to protein and fat breakdown, as well as insufficient insulin secretion caused by aging and genetic factors. A reduction in feed intake may have contributed in part to the weight loss that was observed. Feeding on CP *L*. led to low blood glucose levels and improved appetites which may be due to a regenerative effect that certain compounds from the CP *L* could have acted on pancreatic β-cells. Several phytochemicals in CP *L* possess antioxidant properties, which contribute to its therapeutic efficacy **[9].** These findings agree with **[31]** who found that, in contrast to the control group, diabetic rats whose diets contained 5% Verbena consumed more food (g/day).

Phytoconstituents, such as flavonoids and phenolics, are said to be responsible for the antioxidant properties exhibited by plants. The observed antioxidant scavenging activity indicates that flower extracts may contain phytochemicals possessing antioxidant properties. By increasing insulin secretion, they decreased the rate of protein degradation, resulting in an equivalent increase in animal body weight to that of the non-diabetic control group **[32].**

Table 4 additionally shows the mean value of FER for rats that were injected with STZ and were provided with varied diets. The data indicates that the mean value of FER was significantly reduced in the control (+) group (0.060±0.001) than in the control (-) group (0.098±0.002). Contrast to the (+) control group, the mean values of all rats injected

with STZ and fed on various diets differed significantly. Group 6 (rats injected with STZ and fed a mixture of plants) exhibited the highest FER value, revealing a statistically significant difference in contrast to the control group (+). The effect of CP *L* is attributed to its varied phytochemical constituents that possess antioxidant properties, thereby enhancing feed intake, body weight, and FER., **[9,31]** reported that, the diet intake, body weight (g/day) and the diet efficiency of diabetic rats increased, as contrast to control group when rats fed on Verbena at the level 5%.

**[32].** found that TA have flavonoids, phenolics which have antioxidant scavenging activity and enhance the diet efficiency by improving the animal body weight.

**Table (4): Effects of LC, TA and CP powder (P) on BWG, feeding intake (FI) and feed efficiency ratio (FER) of diabetic rats**

| Parameter / Groups | BWG (g/28d) | | FI (g/day) | | FER | |
|---|---|---|---|---|---|---|
| | Mean± SD | %Change of control (+)positive | Mean ±SD | %Change of control (+)positive | Mean ±SD | %Change of control (+)positive |
| (G 1) control -ve | 39.59 a±1.05 | 1.279 | 14.4 $^{a}$±0.12 | 39.26 | 0.098$^{a}$±0.002 | 63.33 |
| (G2) control +ve | 17.37 f±1.34 | _ | 10.34 $^{f}$±0.22 | _ | 0.060$^{f}$±0.001 | _ |
| (G3) *LCP* (5%) | 26.75 c±2.21 | 54.001 | 12.35 $^{b}$±0.18 | 19.49 | 0.077$^{c}$±0.003 | 28.33 |
| (G4) *TAP* (5%) | 23.13 d±2.11 | 33.16 | 11.54 $^{d}$±0.32 | 10.73 | 0.072 $^{d}$±0.01 | 20 |
| (G5) *CPP* (5%) | 20.25 e±2.05 | 16.58 | 11.07 $^{e}$±0.15 | 7.059 | 0.068 $^{e}$±0.003 | 8.33 |
| (G6) *MixP* (5% | 30.33 b±1.99 | 74.61 | 11.99 $^{c}$±0.03 | 15.95 | 0.090 $^{b}$±0.001 | 50 |
| LSD | 2.55 | - | 0.35 | - | 0.004 | - |

The results presented in table 5 indicate that, in many cases there was no significant difference in the weights of the kidneys, liver, lungs, heart, and spleen between diabetic rats and normal control rats. The most effective treatment was observed in mix group 6, as opposed to the control group (+).

The liver is a vital organ in regulating carbohydrates metabolism. The liver is responsible for regulating both the maintenance of blood glucose levels and the distribution of blood glucose to other organs. Glucose intolerance has the potential to induce hepatic damage. In addition, our findings revealed that hyperglycemia directly caused an increase in the relative weight of the liver in the diabetic group. Changes in the TG output of hepatocytes in animals with DM may result in hepatic fat accumulation, hepatic lipogenesis induction, and increased intrahepatic fat synthesis. These factors potentially contribute to the atypical weight of the liver. Reactive oxygen species (ROS)

generated because of DM in numerous target organs can induce significant cellular damage and apoptosis in the absence of cytoprotective molecules, as has been observed in the kidney and liver. **[33, 34].**

The obtained findings was in the same line of **[35]** who reported that oxidative stress significantly contributes to the organ damage progression in individuals with DM. Elevated levels of glucose lead to the generation of free radicals, which subsequently cause the development of heart failure. Hyperglycemia, a hyperosmotic condition, contributes to an increase in the water content of the myocardium, which subsequently results in cardiac dysfunction and affects the weight of the heart.

Pneumonia, tuberculosis, asthma, fibrosis, and numerous other incapacitating pulmonary complications can result from DM by increasing oxidative stress, which may result in the development of complex lung tissue disorders. Cancer cell proliferation and development in the lungs are aided by elevated blood sugar levels. Prolonged-term DM has the potential to induce blood vessels damage. It led to swell in legs and fluid building up in lungs, making it hard to breathe **[36].**

Histological examinations of the spleen 60 days after induction of DM revealed abnormal splenic architecture and an increase in collagen fibers. **[36].** Similar findings regarding splenic fibrosis, thickened capsules, and trabecular structures in diabetic model plant flowers were reported previously. It stimulates the production of cytokines and cellular respiration, in addition to increasing T-cell activity and production. According to studies, compounds found in plant flowers enhance insulin sensitivity **[9,31].**

**Table (5): Effects of LC, TA and CP powder (P) on organs weight of diabetic rats**

| Parameter / Groups | Liver (g) | | Heart (g) | | Kidneys (g) | | Lungs (g) | | Spleen (g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean± SD | %Change of control (+) | Mean ±SD | %Change of control (+) | Mean ±SD | %Change of control (+) | Mean ±SD | %Change of control (+)positive | Mean ±SD | %Change of control (+)positive |
| (G 1) control -ve | 4.35 $^{e}$±0.05 | -26.25 | 0.64 $^{b}$±0.009 | -7.24 | 1.34 $^{e}$±0.025 | -23.86 | 1.63 $^{b}$±0.004 | -2.97 | 0.47 $^{c}$±0.02 | -20.33 |
| (G2) control +ve | 5.92 $^{a}$±0.004 | – | 0.69 $^{a}$±0.017 | – | 1.76 $^{a}$±0.032 | – | 1.68 $^{a}$±0.003 | – | 0.59 $^{a}$±0.011 | – |
| (G3) *LC P* (5%) | 4.71 $^{d}$±0.20 | -20.43 | 0.65 $^{b}$±0.11 | -5.79 | 1.48 $^{d}$±0.003 | -15.91 | 1.64 $^{b}$±0.005 | -2.38 | 0.49 $^{c}$±0.004 | -16.94 |
| (G4) *TAP* (5%) | 4.99 $^{c}$±0.12 | -15.17 | 0.67 $^{a}$±0.013 | -2.89 | 1.56 $^{c}$±0.004 | -11.36 | 1.66 $^{a}$±0.01 | -1.19 | 0.50 $^{c}$±0.021 | -15.25 |
| (G5) *CP P* (5%) | 5.25 $^{b}$±0.02 | -11.32 | 0.68 $^{a}$±0.011 | -1.45 | 1.62 $^{b}$±0.007 | -7.95 | 1.67 $^{a}$±0.16 | -.59 | 0.55 $^{b}$±0.002 | -6.77 |
| (G6) *mix powder* (5% | 4.48 $^{e}$±0.001 | -24.32 | 0.65 $^{b}$±0.002 | -5.79 | 1.38 $^{e}$±0.04 | -21.59 | 1.63 $^{b}$±0.11 | -2.97 | 0.48 $^{c}$±0.001 | -18.64 |
| LSD | 0.22 | _ | 0.02 | _ | 0.04 | _ | 0.02 | _ | 0.03 | _ |

Table 6 displays the average serum glucose level (mg/dL) among rats that were diagnosed with hyperglycemia and were subjected to different dietary conditions. A significant difference was noted between the mean values of the control group (–) and the positive control group. A significant reduction in average values was noted in all diabetic rats that were provided with different diets, in comparison to the control group (+). Group (6) (Mix 5%) rats exhibited the most significant reduction of -44.32%, followed by group (3) rats nourished with LC P 5%. Group (5) exhibited the least reduction of -11.35% expected. The most effective treatment was observed in mix group 6, as opposed to the control group (+).

Insulin resistance is corrected by the hypolipidemic and antioxidant properties of these components. T2DM, which is the most prevalent metabolic syndrome, is a complex ailment that places a significant strain on both individuals and families. For the induction of T2DM in this investigation, we utilized STZ-nicotinamide, a compound that has been extensively employed in experimental studies by other scientists. Streptozotocin (STZ) is a nitrosourea compound that is produced by *Streptomyces achromogenes*. It exerts its specific effect on insulin-producing β-cells via the induction of DM and the generation of free radicals and DNA strand breaks within these cells **[36].**

Also, nicotinamide protects pancreatic β-cells from the cytotoxic effects of STZ by scavenging free radicals and acting as an antioxidant. Subsequently, STZ induced minor harm to these cells. STZ reaches the β cell by a glucose transporter (GLUT2) and induces DNA damage by producing numerous ROS. Insulin resistance and β-cell dysfunction are mediated by the generated ROS, which deactivate the signal pathway connecting the insulin receptor and the glucose transporter system. The β cells are subjected to necrosis as a consequence of the STZ action **[33].**

**Table (6): Effects of** LC **powder , TA powder and CPP powder on serum glucose (mg/dl) of diabetic rats**

| Groups | Glucose (mg/dl) Mean ± SD | %Change of control (+) |
|---|---|---|
| (G 1) control -ve | 100.67 f ±3.76 | -66.36 |
| (G2) control +ve | 299.25 a ±9.65 | _ |
| (G3) LCP (5%) | 188.68 d ±6.73 | -36.95 |
| (G4) TAP (5%) | 220.6 c ±4.52 | -26.28 |
| (G5) CPP (5%) | 265.29 b±5.02 | -11.35 |
| (G6) Mix powder (5%) | 166.62 e±5.33 | -44.32 |
| LSD | 10.87 | _ |

The findings presented in Table 7 indicate that diabetic rats exhibited significant elevations in serum TG, TC, VLDL-c, and LDL-c, levels in comparison to the control group of normal rats. In comparison to diabetic rats fed various diets, the mean values of all diabetic rats analyzed decreased significantly. Also, serum HDL-c levels of diabetic rats indicated significant decreases compared to normal control rats. In comparison to diabetic rats,

HDL-c levels increased significantly in all diabetic rats fed a variety of diets. The best treatment was recorded for mix group 6 when compared to control (+) group.

This trail observed a significant elevation in serum cholesterol levels among rats that had developed DM induced by STZ, which is consistent with **(37).** The findings of this trail revealed that rats diagnosed with DM revealed a significantly increased level of total cholesterol, which occurred concurrently with a rise in blood glucose levels. This correlation was attributed to the reduced activity of lipoprotein lipase (LPL) resulting from insulin deficiency **(38).** In accordance, diabetic rats administered LC showed a significant decrease in serum lipid levels, which was likely the result of an increase in insulin levels. The occurrence of hyperlipidemia in patients with DM is widely acknowledged. In fact, a decrease in insulin level results in the inability of lipoprotein lipase to be activated, which prolongs the lipolysis cycle, causes hypertriglyceridemia, and elevates the concentration of free fatty acids in the plasma **(39).**

Diabetic dislipidemia is predominantly caused by an increase in the plasma concentration of LDL-c and a reduction in the removal of triglycerides (TGs) from fat depots. An increased lipid metabolism from adipose tissue to the plasma is the consequence of insulin secretion dysfunction. Furthermore, it induces numerous disruptions in lipid metabolism, resulting in the buildup of lipid substances such as TGs and TC in patients with DM **(38).** However, **(40)** reported that elevated levels of serum lipids in the diabetic subject are primarily the finding of increased free fatty acid mobilization from peripheral fat depots. The elevated levels of serum TGs observed in the diabetic animals involved in this study could potentially be attributable to reduced clearance and elevated synthesis of the primary transporters responsible for endogenously synthesized TGs.

HDL-c concentration reduced in a highly significant manner subsequent to the initiation of DM. These outcomes are consistent with the findings of **(38)** who found a significant decrease in HDL levels in the serum of rats with DM and IDDM patients.

On other hand, **[29]** found that an increase in HDL-cholesterol is extremely significant in rats with alloxan DM. The potential factors related to the decrease in serum cholesterol levels include a range of mechanisms, such as enhanced bile salt reabsorption, obstruction of cholesterol oxidation to bile salts, prevention of bile salt reabsorption, and inhibition of cholesterol synthesis.

The accumulation of cholesterol in the bloodstream during DM may be attributed to an increase in intestinal cholesterol synthesis or absorption rate, which increases the input into the body. Conversely, a reduction in the number of LDL receptors hinders the synthesis of bile salts, which in turn causes a deceleration in the clearance of cholesterol-rich LDL particles, which decreases the output. In contrast, there was a significant elevation in LDL-cholesterol levels within the serum of rats with DM. The increased risk to cardiovascular disease is certainly influenced by this anomaly. Elevated levels of LDL-cholesterol may result from VLDL overproduction by the liver or impaired hepatic VLDL and LDL elimination **[29].**

**Table (7): Effects of LC, TA and CP powder (P) on organs weight on TC, TG , HDL-c, LDL-c, VLDL-c of diabetic rats**

| Parameter / Groups | T.C(mg/dl) | | T.G(mg/dl) | | HDL(mg/dl) | | LDL(mg/dl) | | VLDL(mg/dl) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean± SD | %Change of control (+) | Mean ±SD | %Change of control (+) | Mean ±SD | %Change of control (+) | Mean ±SD | %Change of control (+)positive | Mean ±SD | %Change of control (+)positive |
| (G 1) control -ve | 96.09 $^{f}$±3.76 | -60.45 | 90.09 $^{e}$±2.05 | -45.74 | 50.33 $^{a}$ ±6.88 | 57.82 | 27.74 $^{f}$±0.19 | -84.41 | 18.02 $^{d}$±1.49 | -45.74 |
| (G2) control +ve | 242.98 $^{a}$ ±5.43 | _ | 166.03 $^{a}$ ±1.95 | _ | 31.89 $^{c}$±5.22 | _ | 177.88 $^{a}$ ±1.11 | - | 33.21 $^{a}$ ±2.60 | _ |
| (G3) LC P (5%) | 116.13 $^{d}$±4.22 | -52.20 | 115.66 $^{d}$±5.93 | -30.33 | 45.09 $^{a}$ ±0.06 | 41.39 | 47.91 $^{d}$±3.65 | -73.066 | 23.13 $^{c}$±1.81 | -30.35 |
| (G4) TAP (5%) | 200.4 $^{c}$±6.04 | -17.67 | 143.53 $^{c}$±3.85 | -13.55 | 38.32 $^{b}$±0.68 | -20.16 | 133.37 $^{c}$±2.91 | -25.022 | 28.71 $^{b}$±0.78 | -13.55 |
| (G5) CP P (5%) | 228.39 $^{b}$±4.55 | -6.004 | 157.89 $^{b}$±2.11 | -4.902 | 33.12 $^{b}$±1.57 | 2.41 | 163.69 $^{b}$±0.07 | -7.977 | 31.58 $^{a}$ ±2.11 | -4.91 |
| (G6) Mix powder (5% | 105.77 $^{e}$±3.28 | -56.4 | 96.42 $^{e}$±4.32 | -41.92 | 49.02 $^{a}$ ±2.19 | 53.71 | 37.47 $^{e}$±0.55 | -78.935 | 19.28 $^{d}$±1.37 | -41.95 |
| LSD | 8.34 | - | 6.73 | _ | 5.99 | _ | 9.06 | _ | 2.78 | _ |

Serum concentrations of creatinine, urea, and uric acid were significantly elevated in rats with DM contrast to healthy rats, as shown in Table 8. When comparing the control group (+) to the DM rats that were fed various diets, a significant decrease in mean values was observed. The most effective treatment was observed in mix group 6, as opposed to the control group (+).

**[33]** reported that there is an association between serum uric acid and diabetic nephropathy, indicating that uric acid potentially contributes to the pathogenesis of the disease. An association was observed between elevated levels of normal serum uric acid and renal dysfunction in patients with T1DM, as determined by serum cystain-C-based estimations of glomerular filtration rates.

**Table (8): Effects of LC, TA and CP powder (P) on kidney function of DM rats**

| Parameter / Groups | Urea(mg/dl) | | Creatinine(mg/dl) | | Uric acid(mg/dl) | |
|---|---|---|---|---|---|---|
| | Mean± SD | %Change of control (+) | Mean ±SD | %Change of control (+) | Mean ±SD | %Change of control (+) |
| (G 1) control -ve | 22.22 $^{d}$ ±3.43 | -52.74 | 0.62 $^{d}$ ±0.04 | -54.74 | 3.27 $^{c}$ ±0.65 | -46.56 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **(G2) control +ve** | **47.02 [a] ±6.51** | **_** | **1.37 [a] ±0.08** | **_** | **6.12 [a] ±0.48** | **_** |
| **(G3) LC P (5%)** | **32.58 [c] ±3.77** | **-30.71** | **0.90 [c] ±0.03** | **-34.31** | **4.11 [c] ±0.62** | **-32.84** |
| **(G4) TAP (5%)** | **38.75 [b] ±1.06** | **-17.59** | **1.08 [b] ±0.05** | **-21.17** | **4.81 [b] ±0.33** | **-21.41** |
| **(G5) CP P (5%)** | **42.24 [b] ±6.28** | **-10.16** | **1.14 [b] ±0.06** | **-16.78** | **5.02 [b] ±0.11** | **-17.97** |
| **(G6) Mix powder (5%** | **24.38 [d] ±3.8** | **-48.15** | **0.75 [d] ±0.002** | **-45.25** | **3.34 [c] ±0.007** | **-45.42** |
| **LSD** | **3.96** | **-** | **0.14** | **-** | **0.88** | **-** |

Table 9 shows that diabetic rats had significantly higher levels of serum ALP, , AST and ALT in contast to healthy rats. Compared to diabetic rats, the serum concentrations of ALT, ALP, and AST decreased significantly in all diabetic rats fed a variety of diets. The most effective treatment was observed in mix group 6, as opposed to the control group (+).

The higher incidence of liver function tests abnormalities has been associated with individuals with T2DM than individuals without T2DM. The ALT was elevated in 40.4% of the diabetic population, while the AST and ALP were increased only in 17% and 16% of the diabetic population, respectively **[30]**

**Table (9): Effects of LC, TA and CP powder (P) on liver function of diabetic rats**

| **Parameter / Groups** | **AST(U/L)** | | **ALT(U/L)** | | **ALP(U/L)** | |
|---|---|---|---|---|---|---|
| | **Mean± SD** | **%Change of control (+)** | **Mean ±SD** | **%Change of control (+)** | **Mean ±SD** | **%Change of control (+)** |
| **(G 1) control -ve** | **36.86 [e]±1.62** | **-46.001** | **32.72 [d]±3.98** | **-46.84** | **123.58 [e]±4.22** | **-37.57** |
| **(G2) control +ve** | **68.26 [a]±7.33** | **_** | **61.56 [a]±2.09** | **_** | **197.98 [a]±5.62** | **_** |
| **(G3) LC P (5%)** | **52.66 [d]±3.04** | **-22.58** | **35.25 [d]±9.62** | **-42.73** | **152.66 [d]±4.52** | **-22.89** |
| **(G4) TAP (5%)** | **58.22 [c]±4.77** | **-14.71** | **40.31 [c]±4.06** | **-34.52** | **163.22 [c]±5.86** | **-17.55** |
| **(G5) CP P (5%)** | **63.61 [b]±2.99** | **-6.81** | **45.85 [b]±6.71** | **-25.52** | **182.98 [b]±5.97** | **-7.57** |
| **(G6) Mix powder (5%)** | **49.48 [d]±1.04** | **-27.51** | **31.96 [d]±2.65** | **-48.08** | **146.92 [d]±4.76** | **-25.79** |
| **LSD** | **3.55** | **-** | **4.98** | **-** | **7.03** | **_** |

## Histopathological examination (liver)

The rats' liver sections in group 1 (control negative) exhibited normal histoarchitecture of the hepatic lobule upon microscopic examination (Figure 1). In contrariwise, rats' liver from group 2 (control positive) exhibited histopathological lesions characterized by Kupffer cells activation, hepatocellular steatosis, fibroplasia in the portal triad associated with inflammatory cells infiltration (Figure 2). In contrast, central veins congestion and inhibition of Kupffer cells were observed in the livers of rats in group 3 (5% LC) (Figure 3). Furthermore, rats' hepatic sections in group 4 (*5%* TA) detected infiltration of inflammatory cells, Kupffer cells activation, the central vein congestion, and small focal hepatocellular necrosis (Figure 4). Conversely, the livers of rats in group 5 (*5%*

CP *L*) showed sporadic hepatocytes necrosis, Kupffer cells activation and central vein congestion (Figure 5). Likewise, liver tissue of rats from group 6 (mix 5%) demonstrated minor vacuolization. of some hepatocytes, central vein congestion and Kupffer cells activation (Figure 6).

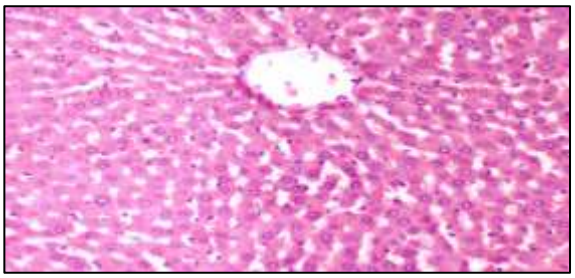

**Figure (1): Photomicrograph of liver of rat from group 1(-) showing normal histoarchitecture of hepatic lobule (H & E X 400).**

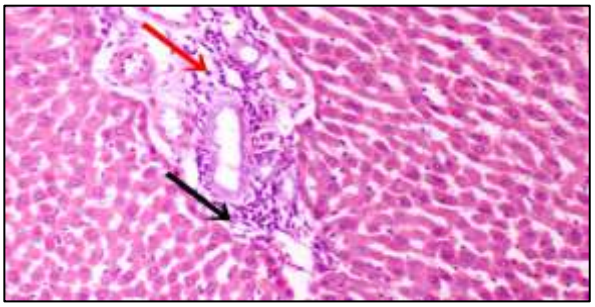

**Figure (2): Photomicrograph of liver of rat from group 2(+) showing fibroplasia in the portal triad (black arrow) associated with inflammatory cells infiltration (red arrow) (H & E X 400).**

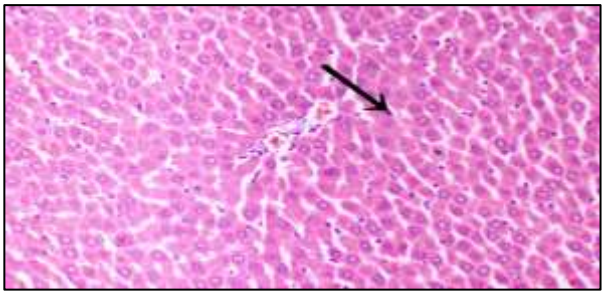

**Figure (3): Photomicrograph of liver of rat from group 3 (LC 5%) showing Kupffer cells activation (black arrow) (H & E X 400).**

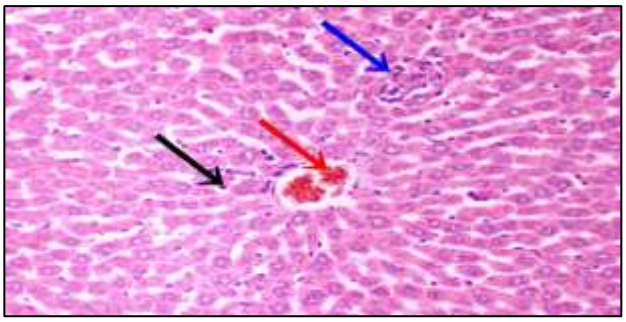

**Figure (4): Photomicrograph of liver of rat from group 4 (TA 5%) showing Kupffer cells activation (black arrow), congestion of central vein (red arrow) and small focal hepatocellular necrosis associated with inflammatory cells infiltration (blue arrow) (H & E X 400).**

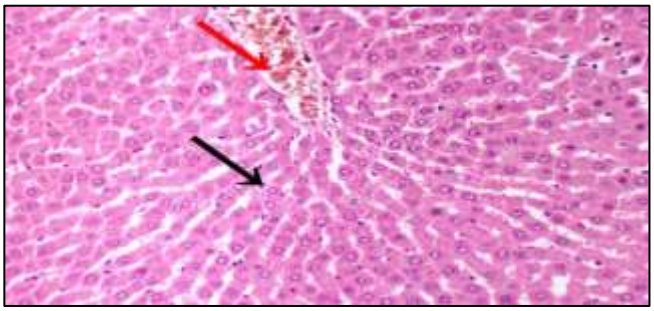

**Figure (5): Photomicrograph of liver of rat from group 5 (CP *L* 5% ) showing Kupffer cells activation (black arrow) and congestion of central vein (red arrow) (H & E X 400).**

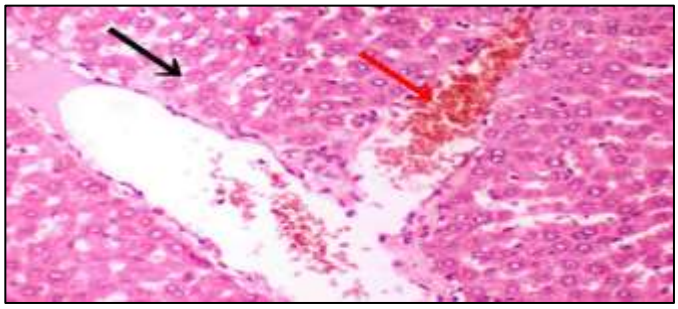

**Figure (6): Photomicrograph of liver of rat from group 6 (mix 5%) showing slight vacuolization of some hepatocytes (black arrow) and congestion of central vein (red arrow) (H & E X 400).**

## Kidneys:

Certain renal tissue sections obtained from rats in group 1, which was the negative group, exhibited a normal histological structure when observed under a microscope (Figure 7). Conversely, proteinaceous cast infiltration by periglomerular inflammatory cells, the epithelial lining vacuolar degeneration of the proteinaceous cast, and renal tubules, within the renal tubules lumen were observed in renal sections obtained from rats in group 2 (positive group) (Figure 8). Conversely, rats assigned to group 3 (*5%* LC) demonstrated vacuolar renal tubule epithelial lining degeneration of and mild congestion of the glomerular tuft (Figure 9). Conversely, specific sections obtained from group 4 (*5%*

TA) that underwent histopathological examination did not manifest any alterations. However, other sections indicated vacuolar degeneration of the epithelial lining of the renal tubules (Figure 10). In contrast, rats assigned to group 5 (*5%* CP *L*) exhibited marginal vacuolar degeneration of specific renal tubule epithelial linings, as well as the renal blood vessels and glomerular tuft congestion (Figure 11). Aside from that, the majority of kidney sections from group 6 (mix 5%) rats examined lacked histopathological lesions. However, a small number of sections displayed the vacuolar epithelial lining degeneration in sparse renal tubules (Figure 12).

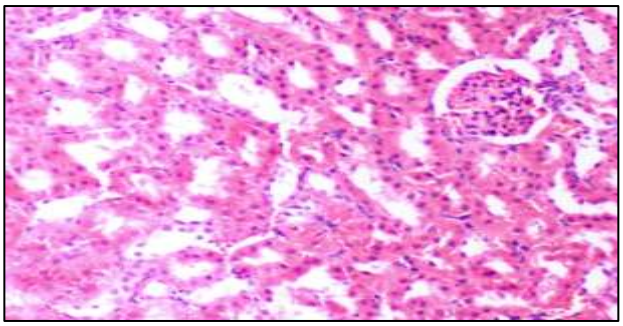

**Figure (7) Photomicrograph of kidney of rat from group 1(-) showing normal histological structure of renal tissue (H & E X 400).**

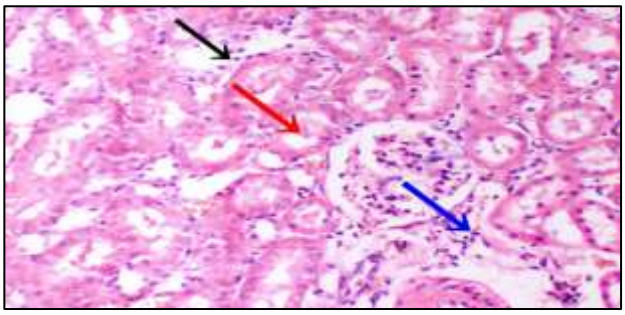

**Figure (8): Photomicrograph of kidney of rat from group 2(+) showing vacuolar degeneration of epithelial lining renal tubules (black arrow), proteinaceous cast in the lumen of renal tubules (red arrow) and periglomerular inflammatory cells infiltration (blue arrow) (H & E X 400).**

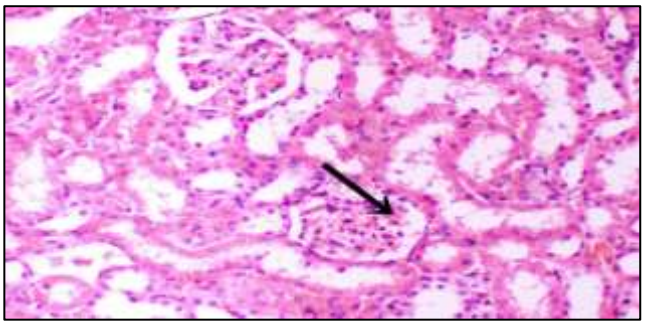

**Figure (9): Photomicrograph of kidney of rat from group 3 (LC 5%) showing slight congestion of glomerular tuft (arrow) (H & E X 400).**

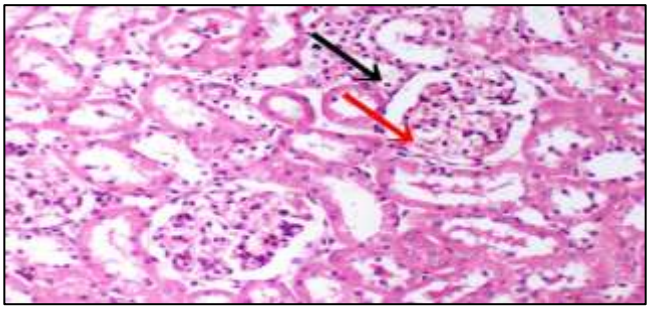

**Figure (10): Photomicrograph of kidney of rat from group 4 (TA 5%) showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) and slight congestion of glomerular tufts (red arrow) (H & E X 400).**

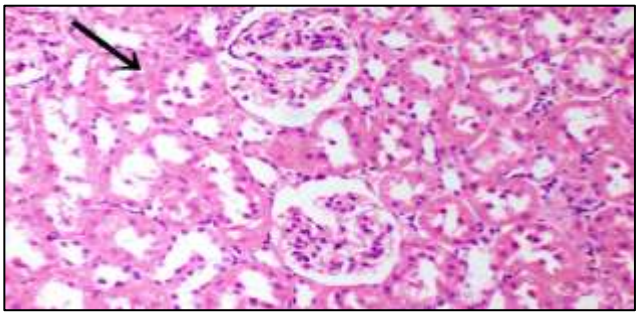

**Figure (11): Photomicrograph of kidney of rat from group 5 (CP *L* 5% ) showing vacuolar degeneration of epithelial lining sparse renal tubules (black arrow) (H & E X 400).**

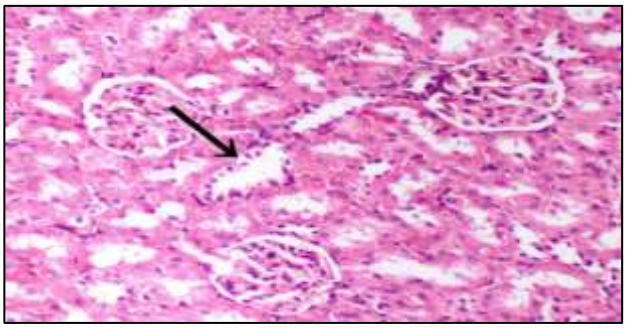

**Figure (12): Photomicrograph of kidney of rat from group 6 (mix 5%) showing vacuolar degeneration of epithelial lining sparse renal tubules (black arrow) (H & E X 400).**

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