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COMPARATIVE STUDY OF PHYSIOLOGICAL EFFECT OF LEMON JUICE AND AQUEOUS EXTRACT OF PUMPKIN FRUITS ON DIABETIC FEMALE MICE

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Abstract

This study was directed to a comparison study of physiological effect of lemon juice and aqueous extract of pumpkin fruits on diabetic female mice. Results Showed that body weight of G3, G4 and G5 groups were improved significantly ($p<0.05$) than G2 group. There is a significant ($p<0.05$) increase in RBCs and related values in G3, G4 and G5 groups in comparing with G2 group .While G5 group was significantly higher ($p<0.05$) in PCV values than G4 group. Results showed that G2 group was significantly ($p<0.05$) high in WBC, Monocytes, Granulocytes than all other groups. While, no significant ($p<0.05$) differences between all groups in Lymphocytes values. G3, G4 and G5 groups showed a remarkable ($p<0.05$) reduction of glucose, lipid proportions except in HDL level when compared to G2 group. In mice G3, G4 and G5 groups, also found a notable ($p<0.05$) reduction in AST, ALT, Creatinine and Urea results in contrast of G2 group.

Key word: *C. limon*, *Cucurbita maxima,* Alloxan, Hematological parameters, **S**erum parameters.

Introduction

Diabetes mellitus (DM) is a metabolic disorder associated with elevated level of blood glucose and dyslipidemia. Insulin resistance or reduce secretion of insulin is main causes of diabetes (Hughs *et al*., 1984; Defronzo, 1997).

It has been found a number of synthetic antidiabetic therapeutic preparations of notable ability to act as glycemic control agents (Grover *et al*., 2002; Khan *et al*., 2003).

Pumpkins (genus; *Cucurbita*) belong to the family of *Cucurbitaceae*. Pumpkins contains phytochemical, including (tetra cyclic triterpens, carbohydrates, saponins, fibers, proteins, minerals, manganese, zinc, copper, iron, etc.) (Lazos and Evangelos, 1986; Kazemi *et al*., 2011). Pumpkin fruit powder have hypoglycemic properties and antidiabetic effect (Chaturvedi and Padmaja, 2012). Several studies that pumpkins fruits ability to inhibit benign prostatic tumor hypolipidemia, hepatoprotective, antibacterial, anti-carcinogenic, anti-diabetic properties, anti-oxidant, antiulcer activities and anti-inflammatory (Pan *et al*., 2005; Caili *et al*., 2006; Makni *et al*., 2008).

Citrus Limon (lemon) belongs to the family *Rutaceae* that is the most main fruit tree produce in the world. Lemon fruit is used as medicinal power and is utilized in many different ways (Kuster, 2003). Lemon has health benefits due to its many nutrient like vitamin B, ascorbic acid, polysaccharide, minerals and proteins. Phenolic compounds is found in Lemon fruit as well as, dietary fiber, minerals, vitamins, essential fatty acid and carotenoids. *C. limon* fruit extract contains numerous active nutrient such as total flavonoids, pectins, vitamin C, and hesperidin (Luzia, 2009).

Citrus limon (lemon) containing antibacterial, antiviral, antioxidant, antifungal, analgesic, and anti-inflammatory properties (Luzia, 2009). Lemon juice is reported to possess good hypolipidemic effect and prevent atherosclerosis (Bergmann *et al*., 2010). Therefore, the aim of this study was to compare the anti-diabetic effect of pumpkin fruits and lemon juice on diabetic female mice.

Material and Methods

The plant that examined in current research, *Cucurbita maxima* and *Citrus limon* fruits were gathered from native vend of Al-Fajur District and were identified

and authenticated by the Department Soil and water resources, College of agriculture, University of Sumer, were kept for future reference.

The fruits of *Cucurbita maxima* (Pumpkin) were cleaned well with water and cut into small pieces and desiccated in a n exceedingly a darken site. Once finished desiccation, the fruits had been pulverized and 100g of pulverized plant was immersed with 2.5L of distilled water and therefore the mixture was agitated for ten hours with mechanical shaker. The combination (extract) had been sieved by a cotton or glass wool. The method was perennial thoroughly for finishing extraction. The extract separate out was then concentrated across a water bath temperature of 40-45^{0}C.

Citrus limon was cut and squeezed by hand to yield fresh juice which was filtered immediately before use.

Female Swiss mice (25.20–27.60g), with 2 months of age, were used throughout this study divided in randomized groups (n = 10 per group). The experimental animals were nourished with normal laboratory diet and water. The experimental animals had been deprived from diet for overnight before the experiment but had free gain on to water.

Female Swiss mice were used throughout this study divided in randomized groups (n = 10 per group). Mice were made diabetic by single administration of alloxan (120mg/kg B.W.) was intraperitoneal injected to overnight fasted mice. The first group was considered as control (drink with a normal saline 0.75 ml/kg body weight). The second subgroup was kept as diabetic control. The third subgroup was kept as Diabetic mice received (0.75 ml/kg B.W./mice/day) lemon juice and the fourth subgroup Diabetic mice given Aqueous extract of *Cucurbita maxima* fruits, 300 mg/kg B.W. Which was given orally by gavage for four weeks. Fifth subgroup Diabetic mice given a combination of both lemon juice (0.75 ml/kg B.W./mice/day) and Aqueous extract of *Cucurbita maxima* fruits (300 mg/kg B.W.). Prior to administration of alloxan, the animals were fasted for 12h with free access drinking water. The blood samples were collected from cardiac puncture. The body weight was measured at the end of study.

The standard methods were used for limitation of each Red blood cells (RBC), Hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), differential WBC count.

The samples of Serum were taken and maintained at -20°C up to testing for biochemistry values (Parasuraman *et al*., 2010). While blood glucose had been measured by enzymatic kit (Elabscience Company, India) demand for enzymatic colorimetric way (Obaid, 2017).

Total cholesterol (TC) and triacylglycerol (TAG) detected by using of total cholesterol and triacylglycerol kits (Elabscience Company, India) (Obaid, 2017). HDL-C kit (Elabscience Company, India) had been used for Determination of High-density lipoprotein cholesterol (HDL-C), But LDL-C level was measured by (Friedewald *et al*., 1972).

Statistical analysis: Results had been mentioned like mean ± SEM; n=10 experimental animals in even group; statistically significant from diabetic control were expressed as $P<0.05$; and Statistical inspection had been done by SPSS software (version 22). With One way ANOVA had been used.

Results and Discussion

The G3, G4 and G5 groups, the body weights were improved than G2 group (Table 1).

There is a significant increase in RBC, Hb, PCV, and MCH in G3, G4 and G5 groups in comparing with G2 diabetic. While G5 group was significantly higher in PCV values than G4 group, in addition to G3 group, showed significantly higher than G2, G4 and G5 groups in concerning with MCH values (Table 2).

While G2 group was significantly high in WBC, Monocytes, Granulocytes than all other groups (Table

Table 1: Effect of oral administration of the treatments on the body weight of female mice Mean ± SE.

Unit Group	B.W. (Kg)
G1	31.40 ± 0.84 ^{a}
G2	18.50 ± 0.84 ^{c}
G3	28.20 ± 0.84 ^{b}
G4	26.90 ± 0.84 ^{b}
G5	30.10 ± 0.84 ^{a}

Table 2: Effect of treatments on Hb, PCV, RBC, MCV, MCH and MCHC of mice Mean ± SE.

UnitGroup	RBC (10^6/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (pg)
G1	9.24 ±0.22 ^{a}	13.36 ± 0.22 ^{a}	43.02 ± 1.13 ^{a}	46.52 ± 0.75	14.48± 0.35 ^{a}	31.17 ±1.24
G2	7.60 ±0.13 ^{c}	9.98 ± 0.15 ^{d}	34.34± 0.85 ^{c}	45.19 ± 1.08	13.23± 0.25 ^{b}	29.11 ± 0.76
G3	8.68 ± 0.13 ^{ab}	12.60±0.33 ^{bc}	41.60 ± 0.92 ^{ab}	47.98± 1.46	14.52 ± 0.44 ^{a}	30.21 ± 1.23
G4	8.54 ± 0.25 ^{b}	12.16± 0.11 ^{c}	39.86 ±0.88 ^{b}	46.72 ±0.75	14.29±0.47 ^{ab}	30.56 ± 0.76
G5	9.12 ±0.16 ^{a}	13.10 ± 0.17 ^{ab}	42.12± 0.58 ^{ab}	46.26 ± 1.10	14.36±0.27 ^{ab}	31.08± 0.22

Table 3: Effect of treatments on WBC, Monocytes, Granulocytes and Lymphocytes of mice, Mean ± SE.

UnitGroup	WBC 10^3/ µL	MON (%)	AGRA (%)	LYMPH (%)
G1	10.62±0.33 [b]	4.86± 0.56 [b]	21.62± 1.08 [b]	73.52± 1.10
G2	14.44 ± 0.35 [a]	6.78 ± 0.41 [a]	32.72 ± 1.50 [a]	60.50±1.35
G3	11.04±0.37 [b]	5.02± 0.42 [b]	23.32 ± 1.34 [b]	71.66± 1.41
G4	11.68 ± 0.29 [b]	5.14 ± 0.40 [b]	24.64±1.74 [b]	70.02 ± 1.66
G5	10.84± 0.32 [b]	5.46± 0.38 [b]	5.14 ± 0.40 [b]	72.46± 1.11

3).

All the other groups showed a significant decrease of blood glucose, cholesterol, triglyceride, VLDL, LDL by increased peripheral uptake of glucose (Vaag *et al*., 1992).

The outcome of investigated animals that dosed with essential oil revealed a high decrease in triglyceride, LDL and cholesterol (Huang *et al*., 2014). The oil extract of *Citrus Limon* leaves were decreased the lipid oxidation and nitrite values (Huang *et al*., 2014). High reduction in serum cholesterol, serum triglycerides, LDL, VLDL and blood glucose in dose related method that caused by extract of *Citrus medica* seeds (Huang *et al*., 2014).

Table 4: Effect of treatments on serum blood glucose and lipid profile Mean ± SE.

Unit Group	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
G1	84.20 ± 0.33 [d]	95.28±1.57 [d]	71.00± 1.58 [d]	45.78± 1.03 [a]	14.20 ± 0.31 [d]	35.30± 0.87 [d]
G2	300.60±7.91 [a]	126.60±1.69 [a]	104.20± 2.45 [a]	31.66± 1.33 [d]	20.84± 0.49 [a]	74.18 ± 1.65 [a]
G3	142.20±3.44 [b]	110.80±1.20 [b]	87.00± 2.09 [b]	40.50 ± 0.68 [c]	17.40± 0.41 [b]	52.82± 1.18 [b]
G4	122.60±2.50 [c]	109.00±0.83 [b]	81.60 ± 1.88 [bc]	41.24± 0.71 [bc]	16.32± 0.37 [bc]	51.44 ± 0.97 [b]
G5	110.40±2.76 [c]	101.60±1.80 [c]	76.20 ± 1.52 [cd]	44.08± 0.91 [ab]	15.24± 0.30 [cd]	41.92 ± 2.45 [c]

level except for HDL level when compared to G2 group. While G1 group was high significantly than G3, G4 and G5 in HDL level. In addition to, G5 group showed low

Table 5: Effect of treatments on serum AST, ALT, Creatinine and Urea Mean ± SE.

UnitGroup	AST (IU/L)	ALT (IU/L)	Creatinine	Urea
G1	55.46±1.63 [d]	62.40±1.86 [d]	0.70 ± 0.03 [c]	32.44 ± 1.29 [c]
G2	152.28±3.50 [a]	218.00±5.14 [a]	1.77 ± 0.08 [a]	45.00± 1.04 [a]
G3	81.46 ± 2.51 [b]	90.82±3.07 [b]	0.88 ± .067 [bc]	37.36 ± 0.76 [b]
G4	84.00±1.70 [b]	93.68±2.01 [b]	0.91 ± 0.05 [b]	36.34 ± 1.05 [b]
G5	72.80±1.77 [c]	80.68 ± 1.88 [c]	0.85 ± 0.04 [bc]	34.72 ± 1.41 [bc]

significantly than G3 and G4 in all values except in HDL (Table 4).

In mice G3, G4 and G5 groups, they were found a significant reduction in values of AST, ALT, Creatinine and Urea values if compared with G2 group (Table 5).

Hesperidin is an ample and cheap byproduct of Citrus growth (Allen *et al*., 2005; Kakadiya *et al*., 2010). It was documented the anti-diabetic activity of hesperidin and naringin with fat rich diet/diabetic rats (Kakadiya *et al*., 2010). The increased levels of blood glucose in concerning with group of diabetic control animals is decreased by treating with variable extracts of *Cucurbita maxima* and the viewed results reveal that notably. The extract of *Cucurbita maxima* reduce glucose level by increase the insulin effect of plasma through increasing the pancreatic release of insulin from â-cells of islets of Langerhans or through secretion from bound insulin or

The (SGOT) and (SGPT) enzymes values were increased to high levels in diabetic group that mention the active liver injury or may be inflammatory hepatocellular disorders (Panda *et al*., 2010). Treatment with *citrus Limon* restored the elevated enzyme levels to normal level in a dose-dependent manner, extract of *Cucurbita maxima*. Leads to a high decrease in the levels of each serum aspartate transaminase (AST), serum alanine transaminase (ALT), Creatinine and Urea to the normal values, giving the beneficial activity of the extract (Kundusen *et al*., 2011). Increased values of the two liver enzymes mentioned above decreased by the administration of *Cucurbita maxima* that may be outcome of the fixation of plasma membrane in addition to decrease hepatic tissue damage that induced by alloxan (Fortson *et al*., 1985). The *Cucurbita maxima* extract can cause noticeable decrease in alkaline phosphate enzyme and bilirubin, suggested an enhancement in secretory activity. Many experiments that done suggested the variable of *Cucurbita maxima* extracts may be contain flavonoids, carbohydrates, phenolics, saponins and tannins. Stimulation of pancreatic β-cells and subsequent secretion of insulin are included with saponins (Fortson *et al*., 1985; Mohamed *et al*., 2009).

The values of RBCs and its parameters had been considerably increased. It will supply with a symptom that mentioned flavonoids might induce the creation or

releasing of erythropoietin hormone that induces stem cells of bone marrow to manufacture of RBCs (Ashour *et al*., 2009). Induction of this hormone encourages the fast creation of red blood cells that is accompanied by enhanced level of MCH and MCHC (Mansi *et al*., 2008). Flavonoids were attributed to the ability for decrease of lipid peroxidation, which leads to hemolysis of RBCs.

The WBC count was given to be accompanied with type two diabetes, insulin resistance (Mohamed *et al*., 2009), coronary artery disease (Ashour *et al*., 2009) and diabetes micro- and macrovascular obstacles (Tong *et al*., 2004). Diabetes in mice may causes moderate neutrophilic leukocytosis and elongated circulation times of monocytes and neutrophils, and a decreased circulation time of lymphocytes that elevates the susceptibleness to infection as described by (Kozlov *et al*., 1995; Hamzah and Hasso, 2019). Elevated leukocytes count is reflect the slight inflammation. This may explains the raised leukocyte count. The reduction in the leukocytes count, in diabetic treated female mice mention the anti-inflammatory process of both juices of *Citrus limon* and extracts of *Cucurbita maxima*.

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