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Protective Effects of Rigla, Metformin, or their Combination on STZ-diabetic Hepatotoxicity and Nephrotoxicity in Male Rats

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Abstract:

The rigla (*Portulaca oleracea*) is a cosmopolitan species with a diverse variety of biological functions including antioxidant, anti-inflammatory, analgesic and neuroprotective actions. In this research, the aerial parts of *Portulaca oleracea* were harvested, air dried and powdered. Chemical tests were carried out on the ethanolic extract and the powdered specimen to determine the phytoconstituents using standard procedures. Also, the present study was undertaken to investigate the protective and therapeutic effects of ethanolic extract of *Portulaca oleracea* on a model of diabetic rats were fed a high-fat diet and injected with a single dose of streptozotocin (STZ) (35mg/kg i.p). Liver enzymes activity, kidney function, lipid profile and glucose level were determined. Diabetic rats were fed a high-fat diet (HFD) exhibited a significant increase in enzymes liver activity ($p<0.05$), also, the parameters of kidney function recorded a significant increase ($p<0.05$). In additional, total triglycerides, total cholesterol and glucose level revealed a significant increase ($p<0.05$). The diabetic rats treated with ethanolic extract of *Portulaca oleracea*, metformin (antidiabetic drug) or their combination exhibited a significant decrease ($p<0.05$) in liver enzymes activity, kidney function, total triglycerides, total cholesterol and glucose level. The present results confirmed that *Portulaca oleracea* exhibited a protective effect against oxidative stress.

Keywords: Rigla, HFD, STZ, Liver, Kidney.

1- Introduction

Overconsumption of nutrients in the form of high dietary intake of fats is associated with various somatic disorders, such as type 2 diabetes mellitus, obesity, metabolic syndrome and cardiovascular diseases.

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HFD is commonly used to develop models for metabolic syndrome and their associated complications (Brendan ret al., 2017). However, these models require expensive diet and lengthy feeding regimens before any detectable decline of mass of β-cells. In one model, this barrier is overcome using (STZ) which depletes β-cell mass experimentally after development of diet-diabetic insulin resistance (Monte et al., 2009; Binh et al., 2013). In addition, HFD lowers glucose uptake and inadequately suppresses hepatic glucose production stimulated by insulin (Ghibaudi et al., 2002) and stated to impair glucose metabolism in rat skeletal muscle (Sreekumar et al., 2002).

Portulaca oleracea L. is a succulent annual herbaceous plant that thrives in warm climates. It is commonly known as rigla (Egypt), purslane (USA and Australia), Ma-Chi-Xian (China), pourpier (France) and pigweed (England) (Elkhayat et al., 2008). Rigla has been utilised in a variety of locations around the world as folk medicine and traditional food since ancient times (Chugh et al., 2019; Xiang et al., 2005). Its use as a medicinal plant is reported from almost all the continents suggesting its huge importance in the healthcare of the indigenous communities. Recent advancements in the quantitative tools for the analysis of phytochemicals have led to the identification of several hundred metabolites from various parts of the rigla (Mohamed and Hussein, 1994; Negi, 2018; Okafor et al., 2014; Uddin et al., 2014).

The present study aimed to evaluate the beneficial effect of rigla on hepatic and renal effect that could make it one of the most significant diets in the future..

MATERIAL AND METHODS

Plant materials:

Preparation of plant extract (ethanolic extract):

The extract of tested plant was prepared according to (Ezeabara et al., 2014) with some modifications. The plant components (leaves and stems) were air dried at room temperature for four weeks and mangled with sterilized mortar and pestle. Hammer mill was used to grind the plant parts into powder, each of the powdered plant materials was stuffed into a Soxhlet apparatus and extracted exhaustively with 70% ethanol for 8 hours. The ethanol was evaporated using the (Rotary evaporator) and then left overnight at laboratory temperature for evaporation of the remaining ethanol, these yielded semi-solid extracts was lyophilized by Vacuum freeze dryer. The lyophilized powder of plant extract was then stored at -20ºC until further analysis.

Animals:

Sixty- four male Wistar rats were bought from the National Research Center, Alexandria. The experimental animals were conducted in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept in polypropylene cages (four animals per cage) covered with metallic grids in a room maintained at proper environmental conditions of temperature and humidity with a 12-hours light-dark cycle. Drinking water and standard chow diet were provided. All the animals were adapted for 2 weeks before the start of the experiment. Appropriate measures were needed to minimize pain, stress or discomfort of the animals and only the minimum number of animals was used that was crucial for the generation of trustworthy scientific data.

Experimental design:

Animals were divided into eight main groups:

Group I: **control group**, (n=8) rats of this group were fed a regular normal diet (low fat diet, Table 1).

Group II: **rigla group**, (n=8) fed on normal diet and received orally 100 mg/kg body weight, ethanolic extract of rigla for four weeks.

Group III: **Metformin group**, (n=8) fed on normal diet and received orally 100 mg/kg body weight, metformin for four weeks**.**

Group IV: **Combination group**(n=8) fed on normal diet and orally received 50mg/kg body weight ethanolic extract of rigla and 50mg/kg body weight of metformin for four weeks.

Group V: **Diabetic Group** (n=8): fed on high fat diet (Table 1) for two weeks and received a single dose of 35mg/kg body weight STZ intraperitoneally

Group VI: **rigla Treated Group** (n=8): fed on high fat diet for two weeks, received a single dose of 35mg/kg body weight STZ intraperitoneally and treated with orally 100 mg/kg body weight ethanolic extract of rigla for four weeks.

Group VII: **metformin Treated group** (n=8) fed on high fat diet for two weeks, received a single dose of 35mg/kg body weight STZ intraperitoneally and treated orally with100 mg/kg body weight metformin for four weeks.

Group VIII: **combination treated group** (n=8) group fed on high fat diet for two weeks, received a single dose of 35mg/kg body weight STZ intraperitoneally and treated orally with 50mg/kg body weight metformin and 50mg/kg body weight with rigla extract (50mg/kg) for four weeks.

Table 1: High fat diet (HFD) and low-fat diet (LFD) compositions (El-Sayed et al., 2013).

Component	**High fat diet (g%)**	**Low fat diet (g%)**
Casein	19.61	17.32
Corn starch	36.24	56.39
Sucrose	10.75	12.37
Cellulose	7	6.18
Butter oil	19	3
Soybean	1	1
Vitamin mix	1.27	1.24
Cystine	0.25	0.22
Acetyl choline	0.27	0.3
Cholesterol	0.59	---

All animals were anesthetized at the end of the study and blood samples were removed from the retro-orbital plexus (Shermer, 1968). Blood samples were collected in heparin tubes with anticoagulant for plasma separation. Plasma was separated using centrifugation at 4 ˚C for 10 min at 1000×g. Plasma samples were collected in eppendorf tubes and stored at -80 ˚C until analysis**.**

Determination of total phenol contents (TPC)

The total phenolic content was carried out using the Folin-Ciocalteu reagent, following the method of **(**Singleton et al., 1999; Dewanto et al., 2002). A weight of **(**1 mg**)** extract was dissolved in 1ml deionized water and 200µl of dissolved sample was taken and added to 600µl of distilled water and 100µl of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 2 ml of 2% Na_2CO_3. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, from 0-100 µg/ml).

Determination of total flavonoid contents (TFC)

Total flavonoid content of the plant extract was determined by a modified colorimetric method described by (Sakanaka et al., 2005**)**, using catechol as a standard at concentrations of (20 – 200 µg/ ml). Extract or standard solutions (250µl) were mixed with distilled water (1.25 ml) and 75µl of 5% sodium nitrite ($NaNO_2$) solution followed by the addition of 150µl of 10% aluminum chloride ($AlCl_3$) solution after 5 min later. After 6 min, 0.5 ml of 1 M sodium hydroxide (NaOH) and 0.6 ml distilled water were added. The mixture was then mixed and absorbance was measured at 510 nm.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of plant extracts was measured by the DPPH method as proposed by (Brand-Williams *et al*., 1995), with some modifications. A solution of 0.2 mm DPPH in methanol (0.0078 g/100 ml) was prepared and 1 ml of this radical solution was added to 1 ml of sample or standard solution at different concentrations (1:1 *V/V*). The mixture was incubated for 30 min in the dark at room temperature and then the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid solutions as standards in the concentration range of (5-200 µg/ml) were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g dried sample.

Blood parameters determination

Blood chemistry analyses was estimated in collected plasma automated blood chemistry analyzer:

1. Liver enzymes activity: AST and ALT were determined according to the method of (Reitman and Frankel, 1957), albumin was determined according to (Reinhold, 1953) and Alkaline phosphatase (ALP) according to (Han et al., 2015).

2. Kidney function tests: Urea was estimated by the method of (Fawcett and Scott, 1960). Also, creatinine was determined according to (Schirmeister, 1964).

3. Lipid profile: Triglycerides were estimated using (Sidney and Bernard, 1973). Total cholesterol determined according to (Zak et al., 1954).

4. blood glucose level was estimated as manufacturing Instructure.

Statistical analysis:

The obtained data were stated as means ± standard deviation (SD). Differences between means were analyzed by one-way analysis of variance ANOVA followed by the student-new man

Keuls T-test using Minitab 12 software so that the data found can be compared and statistically estimated. Statistical significance was considered when it comes to ($p < 0.05$).

Results and Discussion:

Total phenolic and flavonoid contents:

Phenolic compounds are a wide class of secondary plant metabolites that play an important role in oxidative defense processes. Phenolic compounds have antioxidant properties and can take up the role of free radical scavengers, hydrogen donators and singlet oxygen quenchers (Croft, 1999)**.** The total phenolic content was completed using a standard gallic acid curve. Furthermore, the results are presented by the milligrams equivalent of gallic acid (mg GAE/g) ratio, as shown in **Figure 1**. The results of the TPC can be seen in Table 2. The calibration curve using gallic acid as a standard has a line equation, namely y = 0.002x + 0.0874 (R2 = 0.9608).

Flavonoids are a class of flavonoid phenolic that are vital in the prevention and treatment of a variety of ailments. The antioxidant activity of flavonoids could be used to prevent and minimise fat accumulation in the body to treat obesity and its risk factors (Manna and Jain, 2015; Abdali et al., 2015). Metabolic compounds such as flavonoids, ellagic acid, saponins and tannins have antioxidant activity by reducing the level of oxidative stress in adipocytes to prevent obesity (Zhang et al., 2015). The flavonoids included in rigla consist of 5 types, namely quercetin, apigenin, kaempferol, luteolin, and myricetin (Xu et al., 2006). The results of the total flavonoid content of rigla herb extract can be observed in Table 2. The standard curve is constructed using quercetin and the results can be noticed in **Figure 2** with the line equation y = 0.2639x + 0.1313 (R2 = 0.9075).

Table 2: Total phenolics and total flavonoids contents of plant extract

Herbal extracts	Total phenolics (mg/g)	Total flavonoids (mg/g)
Rigla	75.35±0.07	22.81±2.04

- Reported values are the mean ± SD of three replicates. Total phenolics was formulated as gallic acid equivalents (GAE) mg/g sample. Total flavonoids were formulated as mg Quercetin/g sample.

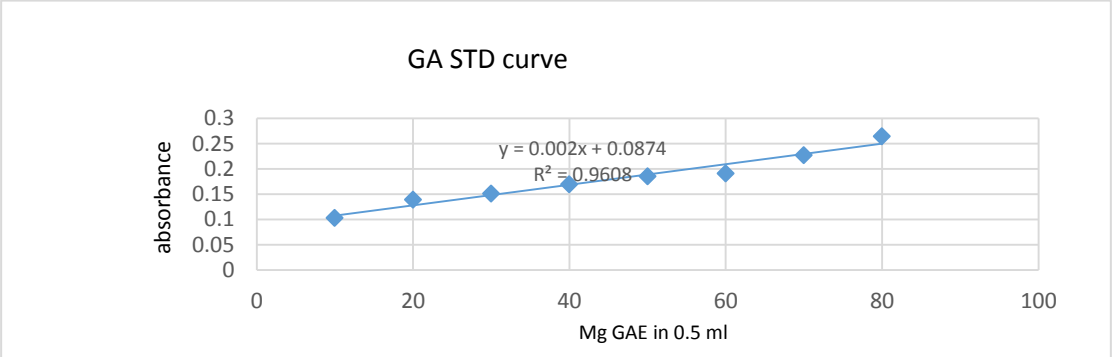

Fig. 1: The calibration curve of standard gallic acid for determination of total phenolic contents.

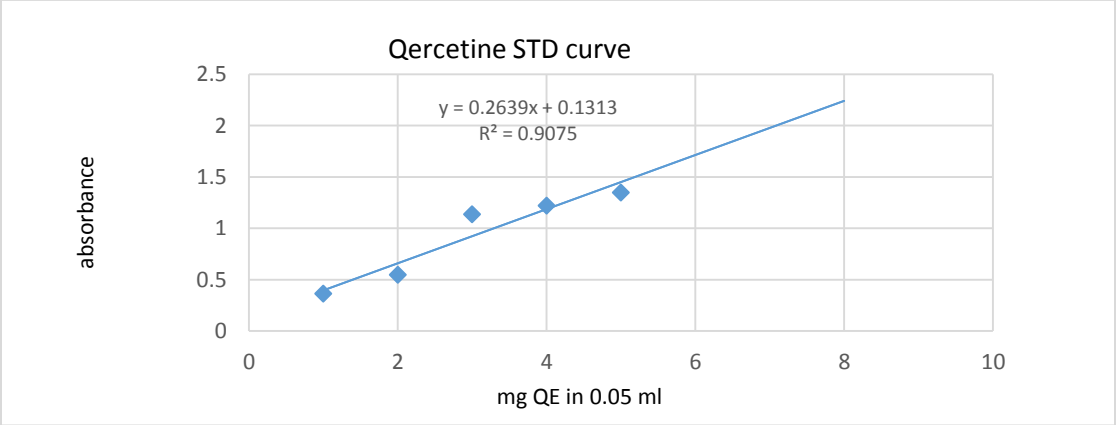

Fig. 2: The calibration curve of standard quercetin for determination of total flavonoid contents.

Determination of 2,2-diphenyl-1-picrylhydrazyl DPPH scavenging activity:

DPPH is often utilized to test how far compounds can quantify antioxidants in complex systems and act as free radical scavengers or hydrogen donors. DPPH is a stable, free radical and accepts an electron or hydrogen radical to become a stable, diamagnetic molecule produces purple solution in methanol and converts to pale when it responds to antioxidant molecules and the DPPH radical which results in the scavenging of the radical by hydrogen donation (Elmastasa et al., 2007). Results tabulated in table 3 show that rigla ethanolic extract acting as antioxidants where it prevented the DPPH autoxidation with IC50 of 50.3 ug/ml which was higher than that of vitamin C 11 folds approximately (table 3). This antioxidant properties could be due to the presence of phenolic and flavonoids in this extract where It is well knowledge that extracts rich in phenolic and flavonoid composites are potent antioxidants (Lopez -Velez et al., 2003).

Table 3: The inhibition concentration values (IC_{50}) value of the plant extract

Sample	DPPH (IC_{50}) µg/ml
Ascorbic acid	4.23^{a}
Rigla	50.3^{b}

- Means in the similar column after that different lower-case letters are significantly different ($p<0.05$) IC_{50} (µg/ml): inhibitory concentrations at which 50%of DPPH radicals are scavenged.

Liver function test:

As shown in Table (4), the present data revealed that the consumption of HFD produced a significant increase in the activities of AST, ALT, ALP and bilirubin (total and direct) compared with control group. The diabetic group fed on (HFD) and treated with ethanolic rigla extract, metformin or the combination exerted a significant decrease in the activities ($p<0.05$) of plasma AST, ALT, ALP and bilirubin (total and direct) as compared to diabetic group. Similar observations reported by Fernández et al. (2012) who indicated that the increase of liver enzymes values may be symptomatic of some liver impairment or probably damage. The same author showed that liver damage resulting from underlying cellular death is often associated with cholestasis, drug-diabetic injury and obesity. These results are in harmony with the studies carried out by Am (2012) and Panchal et al. (2011) who observed that consumption of HFD

rapidly exacerbates the progress of fatty liver disease that occurs with chronically elevated glucocorticoids.

The present results are consistent with Dkhil et al. (2011) who mentioned that the decrease in liver function showed the protective role of rigla against liver damage. Rigla contains antioxidant compounds (Ivo et al., 2009) that protect against oxidative stress (Cherukui et al., 2013). The decreased activity of the liver enzymes, AST, ALT, ALP and bilirubin (total and direct) in rigla treated group, indicates its protective role against liver damage.

Although metformin has been used for the treatment of diabetes for >50 years, it has currently become one of the first-line treatment options in the management of diabetes and is recommended by several international guidelines and consensus (Stumvoll et al., 1995). Regarding the liver enzymes, there were many studies assessing the effect of metformin on ALT and AST. Overall, they showed a statistically significant reduction in ALT and AST levels in the metformin group. Certain reviews (Chavez-Tapia et al., 2006; Adams and Angulo, 2006) have shown improvements in liver enzymes only in the single-arm trails.

Table 4: Effect of rigla, metformin and their combination on liver function of different experimental rat groups treated with high fat diet and STZ.

Parameter /groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T. Bilr (mg/dl)	D.Bilr (mg/dl)
Control	35.47 ± 2.6	76.67 ± 2.4	145.22 ± 1.63	1.54 ± 0.05	0.6 ± 0.014
Rigla	40.93 ± 2.8	85.23± 2.10[a]	158.33± 2.80[a]	1.65 ± 0.03	0.705 ± 0.015
Metformin	65 ± 2.49[a]	94.33± 3.19[a]	165.6 ± 2.69[a]	1.76 ± 0.02[a]	0.83 ± 0.040
Rigla + Met	44.9 ± 2.15[a]	87.30± 2.33[a]	156.80± 3.83[a]	1.68 ± 0.03	0.735 ± 0.026
Diabetic group	142 ± 2.93[a]	155.1± 3.37[a]	283.37± 3.50[a]	3.70 ± 0.07[a]	1.86 ± 0.031[a]
Rigla - treated	79.1 ± 1.99[b]	104.7± 3.05[b]	224.37± 2.00[b]	2.72 ± 0.05[b]	1.555 ± 0.032[b]
Met-treated	83 ± 2.46[b]	116.6± 2.66[b]	246.33± 2.71[b]	2.97 ± 0.12[b]	1.745 ± 0.045[b]
(Rigla + Met)-treated	78.8 ± 3.40[b]	107.9± 2.26[b]	227.5 ± 2.97[b]	2.82 ± 0.04[b]	1.635 ± 0.025[b]

Values are Means ±S.D. of 5 rats in each group.
([a]) Significant (P<0.05) different from control.
([b]) Significant P<0.05) different from diabetic group.

Kidney function test: Results in Table (5)

The data of the present work showed that the diabetic group fed on (HFD) showed significantly (p<0.05) higher plasma levels of urea, creatinine and uric acid as compared to the control group. However, the treatment with ethanolic rigla extract, metformin (antidiabetic drug) or their combination induced significantly (p<0.05) lower plasma levels of urea, creatinine and uric acid.

The previous studies reported by by Gregor and Hotamisligil (2011) who suggested that elevation reactive oxygen species (ROS) and plasma level of free fatty acids and production of adipocytokines which adversely effect on adipose tissues. Obesity is along with low grade inflammation that finally leads to hepatic, renal and cardiovascular disorders, in addition to type 2 diabetes (Haslam and James, 2005; Brunt, 2010). Individuals with the metabolic syndrome (MetS) are in danger of developing chronic kidney disease (CKD), which is an independent risk factor for cardiovascular morbidity and mortality (Nashar and Egan, 2014; Zhang and Lerman, 2017). Also, continuous HFD intake causes a pre-diabetic state, leading to kidney impairment (Sasatomi et al., 2001; Zhang and Lerman, 2017).

Several researchers reported that the antioxidant and free radical scavenging activities of the rigla have already been established and linked to its components (Simopoulos, 2004; Xin et al., 2008). Moreover, multiple investigations have demonstrated that the aqueous extract of rigla has significant nephroprotective activity and may have a promising function in the treatment of diabetic acute renal injury caused by nephrotoxins (Priyamvada et al., 2008; Karimi et al., 2010; Ghara and Ghadi, 2018). Also, Shirwaikar et al. (2003) found that decreased levels of urea, uric acid and creatinine in the rigla treated animals, may be due to its antioxidant potential. These findings were found to be in the same line with our results.

The biguanide metformin is now the first-line oral medicine for treating type-2 diabetes mellitus, according to Gökçay Canpolat and Sahin (2021). Also, metformin creates its antidiabetic action through various mechanisms (Horakova et al., 2019; Tantisattamo et al., 2020; Mostafa et al., 2021). Metformin has also been reported to prevent fibrosis in several organs, including the kidneys (Satriano et al., 2013; De Broe et al., 2020).

Our present results suggested that metformin treatment decreased the plasma level of urea, creatinine and uric acid in diabetic rats compared with the diabetic group, which might run with the previous studies (Dimo et al., 2007; Hammoud et al. (2021).

Table 5: Effect of rigla, metformin and their combination on kidney function of different experimental rat groups treated with high fat diet and STZ.

Parameter /groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	30.27 ± 0.27	0.336 ±0.06	5.99 ± 0.110
Rigla	33.18 ±0.61	0.376 ± 0.06	6.43 ± 0.093[a]
Metformin	36.13 ± 1.44[a]	0.417 ±0.05[a]	6.68 ± 0.095[a]
Rigla + Met	34.06 ±.2.78	0.390 ± 0.03	6.50 ± 0.066[a]
Diabetic group	59.80 ± 1.01[b]	0.536 ± 0.06[a]	9.45 ± 0.130[a]
Rigla - treated	41.48 ± 3.00[b]	0.442 ± 0.04[b]	7.48 ± 0.046[b]
Met-treated	46.90 ± 2.69[b]	0.490 ± 0.03[b]	7.97 ± 0.114[b]
(Rigla + Met)- treated	43.62 ± 1.86[b]	0.472 ± 0.05[b]	7.62 ± 0.036[b]

Values are Means ±S.D. of 5 rats in each group.
([a]) Significant (P<0.05) different from control.
([b]) Significant (P<0.05) different from diabetic group.

Lipid parameters and glucose test:

The data on the levels of plasma lipid and glucose in all rats groups is summarized in Table (6). The present study showed a significant ($P<0.05$) high plasma lipid parameters (total triglycerides and total cholesterol) and glucose levels in diabetic group compared with the control group. On the other hand, the diabetic group treated with ethanolic rigla extract, metformin or their combination showed significantly ($P<0.05$) lower plasma lipid parameters and glucose levels versus to those of the diabetic group.

Raised triglycerides and total cholesterol in diabetes have been found to be an important risk factor for cardiovascular diseases in many studies (Christian et al., 2011; Nagasawa et al., 2012; Riediger et al., 2017). During last few decades, mean triglycerides levels have increased in concert with the growing epidemic of diabetes mellitus and obesity (Kompoti et al., 2006; Flegal et al., 2010). Our results concerned with glucose level run with the results reported by Fukudome et al. (2008) who found that a high-fat meal mixed with numerous mild doses of STZ caused an increase in blood glucose and lipid levels in diabetic rats.

Simopoulos (2001) reported that rigla is nutritious plant representing not only a major source of nutrient but also contain many protective factors, the hypolipidemic effect of rigla may be attributed to melatonin content of crude extract. Similarly, Deriaz et al. (2007) reported that the effect of melatonin on lipid profile could be attributed to its direct scavenger of free radical. Additionally, Nishida (2005) reported the effect of melatonin on lipid profile could be due to indirect stimulation of the expression and activity of antioxidant enzymes.

Also, Kanter et al. (2006) found that rigla extract caused significant decrease in blood glucose without change in insulin level in streptozotocin diabetic rats, which may be due to its contents that improved the insulin resistance which might run with present data. Also, Ramadan et al. (2017) mentioned that *Portulaca oleracea* is a general tissue protecting and regenerative agent.

Metformin can correct lipid metabolic abnormalities in T2DM through a variety of routes (Malin et al., 2012; Han and Kaufman, 2016; Melmed et al., 2016). Also, Sima et al. (2010) found that metformin lowers the fraction of irreversibly glycated LDL-C that is eliminated less efficiently from the body by lowering plasma glucose levels. Metformin, as previously demonstrated in the UKPDS, not only regulates hyperglycemia but also lowers cardiovascular risk (American Diabetes Association, 2002). People with dyslipidemia who are ineligible for lipid-lowering therapy may benefit from metformin treatment despite the cost and potential side effects of lipid-lowering drugs such as statins (Raymond et al., 2014).

Table 6: Effect of rigla, metformin and their combination on total cholesterol, total triglyceride and glucose level of different experimental rat groups treated with high fat diet and STZ.

Parameter /groups	TC (mg/dl)	TG (mg/dl)	Glucose mg/dl
Control	49.5 ± 1.91	53.2 ± 2.27	73.63 ± 0.87
Rigla	50.5 ± 1.97	70.6 ± 1.75[a]	87.70 ± 1.91[a]
Metformin	61.6 ± 1.80[a]	93.3 ± 1.86[a]	74.87 ± 1.69
Rigla + Met	59.4 ± 2.00[a]	86.3 ± 1.95[a]	81.90 ± 0.56[a]
Diabetic group	182 ± 2.00[a]	123.8 ± 1.54[a]	196.53 ± 2.28[a]

Rigla - treated	76 ± 2.00[b]	99.5 ± 1.75[b]	144.97 ± 1.92[b]
Met - treated	88.8 ± 2.50[b]	103.4 ± 2.37[b]	141.88 ± 0.94[b]
(Rigla + Met)-treated	72.2 ± 1.95[b]	87.5 ± 1.80[b]	132.43 ± 2.97[b]

Values are Means ±S.D. of 5 rats in each group.
([a]) Significant (P<0.05) different from control.
([b]) Significant (P<0.05) different from diabetic group.

Conclusion:

According to the results of this study, ethanolic rigla extract appears to have a protective effect against oxidative stress. Our results from DPPH test confirmed the antioxidant activity of rigla in improvement of liver function, kidney function, lipid profile and glucose levels against high fat diet and streptozotocin. It is recommended to consume greater amounts of this plant regularly as naturally occurring phytochemicals are relatively nontoxic, inexpensive and available in an ingestive form.

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