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Effect of Ethyl acetate Fraction of Silybum marianum and Ficus Carica Leaves Extract on Glucose Hemostasis, Oxidative Stress Biomarkers and Inflammatory markers in Streptozotocin-induced T2DM Rats

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KEYWORDS
Silybum marianum, Carica Leaves, Glucose Hemostasis, Oxidative Stress Biomarkers

ABSTRACT:

Background: Considering diabetes mellitus type two (T2DM) is a persistent metabolic syndrome, it can produce hyperglycemia, which in turn can develop substantial adverse effects such as retinopathy, neuropathy, nephropathy and cardiovascular disease. Plant-based medicine has a significant role to play since it is safe, affordable, and readily available. Synthetic drugs used for diabetic treatment also have identifiable negative side effects and worsened the diabetic condition, and since they are costly in developing countries due to poverty as well as limited availability of medical care in these countries, phytotherapy has an important role to play. The aim of this work was to evaluate the anti-diabetic, anti-oxidant and anti-inflammatory effects of ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract in Streptozotocin-induced T2DM Rats.

Methods: eighty (80) apparently healthy male Wister rats weighing between (180-250 gm.) were utilized in the current study which was stared at July, 2022. To induce type 2 diabetes mellitus (T2DM), animals were fed a high-fat diet (HDF) for 2 weeks before injected intraperitoneally with 40 mg/kg of Streptozotocin (except healthy control group). Rats were divided into 8 groups (each group include 10 rats) and used for 4 weeks as following G1: Healthy control group , G2: Negative control group (induced T2DM; rats were left without any treatment) , G3: Positive control group (induced T2DM, rats were treated with 22.5 mg/kg metformin) , G4: Rats were treated with 250 mg/kg ethyl acetate fraction of Silybum marianum , G5: Rats were treated with 250 mg/kg ethyl acetate fraction of Silybum Marianum + 22.5 mg/kg metformin. G6: Rats were treated with 250 mg/kg ethyl acetate fraction of Ficus carica, G7: Rats were treated with 250 mg/kg ethyl acetate fraction of Ficus carica + 22.5 mg/kg metformin, G8: Rats were treated with 250 mg/kg ethyl acetate fraction of Silybum marianum + 250 mg/kg Ficus carica leaves extract Blood samples were taken for the assay of biochemical parameters, oxidative stress and inflammatory markers measurements.

Results: Phytochemical investigation of the current study show that silymarin being a predominant in ethyl acetate fraction of silybum marianum extract and Isoqurcetin is being predominant in ethyl acetate fraction of Ficus carica using HPLC analyses these bioactive compounds regulates blood glucose and improves insulin sensitivity, demonstrating positive effects in the prevention and therapy of associated disorders; which mean these extracts used for treatments were have effectiveness in reduced hyperglycemic state by enhancement insulin secretion from regeneration cells in pancreas. Also ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract improved oxidative stress state in Streptozotocin-induced T2DM

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rats; where CAT, SOD and GSH were significantly increased in treated groups to provide antioxidant effect with significant reduced in MDA levels. While IL-6 and TNF-α were significantly higher in negative control group with significant dropping in treated groups; hence in the present study investigations indicate that ethyl acetate fraction of silybum marianum and ficus carica leaves extracts both had varying capacity in improve enzymatic activity, anti-diabetic, anti-oxidant and anti-inflammatory effects and aid in limiting free radicals in blood plasma.

Conclusion: The ethyl acetate fraction of silybum marianum and ficus carica leaves extract as a plant-derived product may be of major value in the treatment of diabetes mellitus and its consequences due to its anti-hyperglycemic, anti-inflammatory and antioxidant characteristics. Further experimental research is recommended to improve the transport and absorption of these plant extracts to increase their medicinal efficacy.

1. Introduction

Diabetes mellitus (DM) define as a chronic metabolic disorder characterized by hyperglycemia which caused by impaired insulin secretion or peripheral insulin resistance [1]. Insulin is a hormone that reduced blood glucose levels by allowing glucose to enter the cells for convert it to energy [1]. Diabetes is one of the fastest growing global emergencies of the 21st century An estimated 463 million adults have diabetes in 2019, with that number expected to rise to 578 million by 2030 and 700 million by 2045, according to the International Diabetes Federation (IDF) [2]. Diabetes Mellitus if left uncontrolled will contribute to a various serious health problems known as diabetic complications which effects on both small and large blood vessels [3]. Traditional treatments for DM, such as oral medications and insulin injections, may have certain side effects such as hypoglycemia (especially insulin) and the symptoms of hypoglycemia may include sweating, confusion, anxiety and dizziness [4]. Phytochemicals found in medicinal plants have been found to have hypoglycemic properties, suggesting they may be useful in reducing the risk of hyperglycemia or may be useful in glycemic control [5]. Alternative medicine, which includes herbal treatments, is a growing field. Humans are intrigued by the idea that they may be able to better control their blood glucose levels without resorting to pharmaceutical medicines or insulin injections. [6]. Polyphenols are a group of compounds found in plants that have been shown to have numerous health benefits, including potential benefits for individuals with T2DM [5-7]. Silybum marianum belongs to the family Asteraceae and is enriched with a variety of bio ingredients, in particular silymarin which play a major role in treating liver problems [8]. Originally from Asia and Southern Europe, Silybum marianum has now spread globally [9]. The seeds of plant contain a flavonoid complex called silymarin, which is believed to have medicinal properties [8]. Silymarin is known for its potential hepatoprotective properties and may also have antioxidant and anti-inflammatory effects [10]. Although the exact mechanisms by which silymarin promotes liver regeneration and blocks the deposition of collagen fibers that can lead to cirrhosis remain unknown, it is clear that silymarin acts in a variety of ways, including antioxidant and anti-inflammatory activities [11]. Against hepatotoxic substances like psychotropic medications and the toxic compounds generated by Amanita phalloides, Silybum marianum and its main flavonoid, silymarin, are efficient and well-tolerated antidotes [11]. Additionally, silymarin may protect the kidneys from nephrotoxic substances; Silybum marianum has been studied extensively for its effects on the liver, but it also offers a wide range of additional health benefits, including hypoglycemic activity and lowering insulin resistance in people with T2DM and associated consequences [8-12].Traditional diabetic management often includes the use of medicinal plants. As a result, several studies have been carried out on medicinal plants in search of hypoglycemic compounds such as Ficus Carica (F.carica) plant [13]. The World Health Organization (WHO) recommends traditional plants as a potential great candidate for the treatment of diabetes due to their activity, nontoxicity, and few side effects. [14]. Because medications produced from natural ingredients are less expensive and have less adverse

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effects than conventional pharmaceutical therapies, their popularity and use as alternative therapeutics is on the rise. Moreover, in the later stages of T2DM, a combination therapy of synthetic pharmaceuticals and natural molecules may improve treatment outcomes [15]. The current study's goals were to assess the hypoglycemic, anti-inflammatory, and antioxidant effects of the ethyl acetate fraction of Silybum Marianum and Ficus carica leaves extracts on high-fat diet /Streptozotocin-induced T2DM rats.

2. Materials and methods

2.1. Plant material

The work was done in the pharmacognosy laboratory of Department of pharmacology in College of Pharmacy / Baghdad University. The herbs included in the present study were identified and authenticated by pharmacognosy department, College of Pharmacy, Baghdad University, Iraq), then the aerial part of Silybum marianum and Ficus carica leaves were collected and dried aerial whole plant were crushed and grounded in to fine powder using a manual mortar to fine powder then it was sequentially extracted by macerated with 85% ethanol using Soxhlet ,Then the mixture filtered using whatman No.1(18.5cm) filter paper [16] (Whatman International Ltd., Maidstone, England). The filtrate was evaporated by rotary evaporator vacuum at 40℃ until ethanol free extract was remained that contained the total constituents of active ingredients. After that the extract was fractionated by adding distilled water and ethyl acetate using separatory funnel with continuous shaking and after separation ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract has was isolated and ethyl acetate evaporated by left it exposed at room temperature then plant weighed then it has been stored for further use.

2.2. Phytochemical assay

Crude ethyl acetate extract of Silybum marianum and Ficus carica leaves aerial parts were subjected to analyze for checking the existence of Phenolic and other bioactive compounds in it.

2.2.1 Investigation of Bioactive compounds in plant Extract using HPLC analyses

Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a SYKMAN HPLC chromatographic system equipped with a UV-detector, Chemstation, a Zorbax Eclipse Plus- C18-OSD 25 cm, 4.6 mm column [17]. The column temperature was 30 °C the gradient elution method, with eluent A (methanol) and eluent B (1 % formic acid in water (v / v)) was performed, as follows: Initial 0-4 min, 40% B; 4-10 min, 50 % B; and flow rate of 0.7 mL/min. The injected volume of samples 100 µL and standards was 100 µL and it was done automatically using auto sampler. The spectra were acquired in the 280 nm [17].

2.3 Animals

Eighty (80) apparently healthy male Wister rats weighing between (180-250 gm.) were utilized in the current study which was started at July, 2022. The experiments were approved by the Institutional Review Board (IRB NO.90/ 25-1-2022) at the College of Medicine /AL Nahrain University, Baghdad / Iraq. These animals were supplied by animal house at private animal house –Baghdad city. These animals were kept under standard conditions at temperature between (25±2) °C and relative humidity of 50-60%, with 12/12 hr. were applied. Animals were subjected to acclimatization for 2 weeks prior to staring the work .During the acclimatization period, as well as during the whole experimental period, animals were fed standard laboratory diet mixed with peanut butter as high fat diet in addition to free access to tap water ad libitum [18] . Animals were randomly allocated into eight groups (each group contained ten animals; n= 10).

2.4. Induction of T2DM

To generate a rat model mimicking human T2DM with increased adiposity follows [19].Protocol with simple modification. Each group of rat (except G 1 / healthy control group) was fed for 2 week before induction High Fat Diet (30% fat (cholesterol powder and peanut butter) + 70% standard chow) was added increase adiposity. Rats were fasted for 8-10 hours and then injected single intraperitoneal (IP) Streptozotocin at a dose of 40 mg/kg [20]. Dissolved in freshly prepared 0.1M citrate buffer, PH (4.57) [21] was given for immediate use within 10 min in dark [22]. During first 24 hour after injection of STZ to overcome the fatal hypoglycemia; the rats were given 5% glucose solution.

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2.7 Experimental design

Eighty adult male rats were randomly divided into 8 groups with ten animals in each group and the experimental animal groups received the followings: G1 (Healthy Control group):10 rats were IP injected by citrate buffer alone. Diabetes will be induced in the rest 70 rats, by IP injection 40mg/kg of Streptozotocin , and will be sub-divided as follows: G2 (Negative Control group): DM induced rats group and left without any treatment . G3 (Positive control group): rats were treated with a standard drug (rats administered orally with metformin (22.5 mg/kg) [23] daily, for 4 weeks. Group 4: Rats will be treated orally with ethyl acetate Silybum marianum extract (250 mg/kg per day) [24] for 4 weeks. Group 5: Rats were orally administrated with ethyl acetate Silybum marianum (250 mg/kg per day) + metformin (22.5 mg/kg daily) for 4 weeks G6: Rats were treated with ethyl acetate fraction of Ficus carica(250 mg/kg per day) [25], G7: Rats were treated with 250 mg/kg ethyl acetate fraction of Ficus carica + 22.5 mg/kg metformin, G8: Rats were treated with 250 mg/kg ethyl acetate fraction of Silybum marianum + 250 mg/kg Ficus carica leaves extract . Fasting blood glucose (FBG) of the rats was measured using a glucometer (Roche, Switzerland). At the end of study, all groups were fasted (water is not restricted) overnight before blood collection. The blood was collected by the heart puncture and put in gel tube to allowed clotting process occur after that sample centrifuged to obtained serum and then put it in eppendorf for frozen until used to measurements of biochemical parameters.

2.8 Blood/ Serum Collection

Blood samples were collected by cardiac puncture using 5ml syringes and serums were obtained by centrifugation at 3000rpm for 10 minutes which were used for serum creatinine , blood urea , GOT ,GPT ,Insulin , Fructosamine , Glutathione , MDA , Catalase and SOD,TNF-α IL-6 measurements. In general, cardiac puncture is recommended for terminal stage of the study. Blood samples were taken slowly from the heart, preferably from the ventricle slowly [25].

2.9 Determination of fasting serum rat insulin level (FSI), serum Fructosamine and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

Rat insulin level was determined according to the enzyme-linked immunosorbent assay (ELISA) using a commercial kit (MYBioSource, USA). HOMA-IR was calculated using the formula: HOMA – IR = (Fasting Insulin mIU/L * Fasting Glucose mg/dL) / 405[26]. Fructosamine was determined by ELISA method using a commercial kit (Human, Germany).

2.11 Oxidative stress Biomarkers analyses

Rat malondialdehyde (MDA) levels, rat superoxide dismutase (SOD) and rat catalase (CAT) activities were evaluated by ELISA method using a commercial kit from MYBioSource, USA. Furthermore Glutathione (GSH) was determined in serum using standard kits from Fine Test, China.

2.12 Inflammatory Markers analyses

Rat Interleukin 6 (IL-6) and Rat Tumor Necrosis Factor Alpha (TNF-α) determined by ELISA in serum using commercial kits from MYBioSource, USA.

2.13 Statistical analysis

Data were analyzed by using SPSS (Statistical package for social scientists) version 24 and Microsoft office excel. Post hoc tukey test (ANOVA analysis) was used to significant compare between means of groups in this study , data were expressed as mean ± standard Error (M ± SE), and the P values ≤ 0.05 were considered as statistically significant.

3. Results

3.1. Total polyphenols and flavonoids content

3.1.1 Silybum marianum

According to the identification of bioactive compounds in ethyl acetate fraction of Silybum marianum by using HPLC technique and In comparison of ethyl acetate fraction of Silybum marianum sample with the standards the results in figure (3.1) show a peak with retention time (tR) equal to 9.5 which belongs to the standard compound Tannic acid, another peak with tR at 4.2 which belongs to the standard compound silymarin, another peak with tR at 6.3 which belongs to the standard chlorogenic acid, another peak with tR at 11.79 which belongs to the standard compound Gallic acid and another peak with tR at 13.89 which belongs to the standard compound Vanilic acid as illustrated in following table (3.1)

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Table (3.1) Results chromatography of ethyl acetate fraction sample of Silybum marianum

No.	tR [min.]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W0S	Compound name	Concentration mg/gm
1	4.2	12458.58	950.15	30.59	31.25	0.35	Silymarin	**25.1**
2	6.3	1205.65	410.22	15.49	15.88	0.1	Chlorogenic acid	**14.3**
3	9.5	3568.98	633.59	20.59	20.15	0.16	Tannic acid	**2.4**
4	11.79	850.15	340.16	12.59	12.66	0.08	Gallic acid	**1.1**
5	13.89	4521.58	620.19	18.55	18.49	0.2	Vanillic acid	**7**
	Total	22604.89	2954.18	100	100			

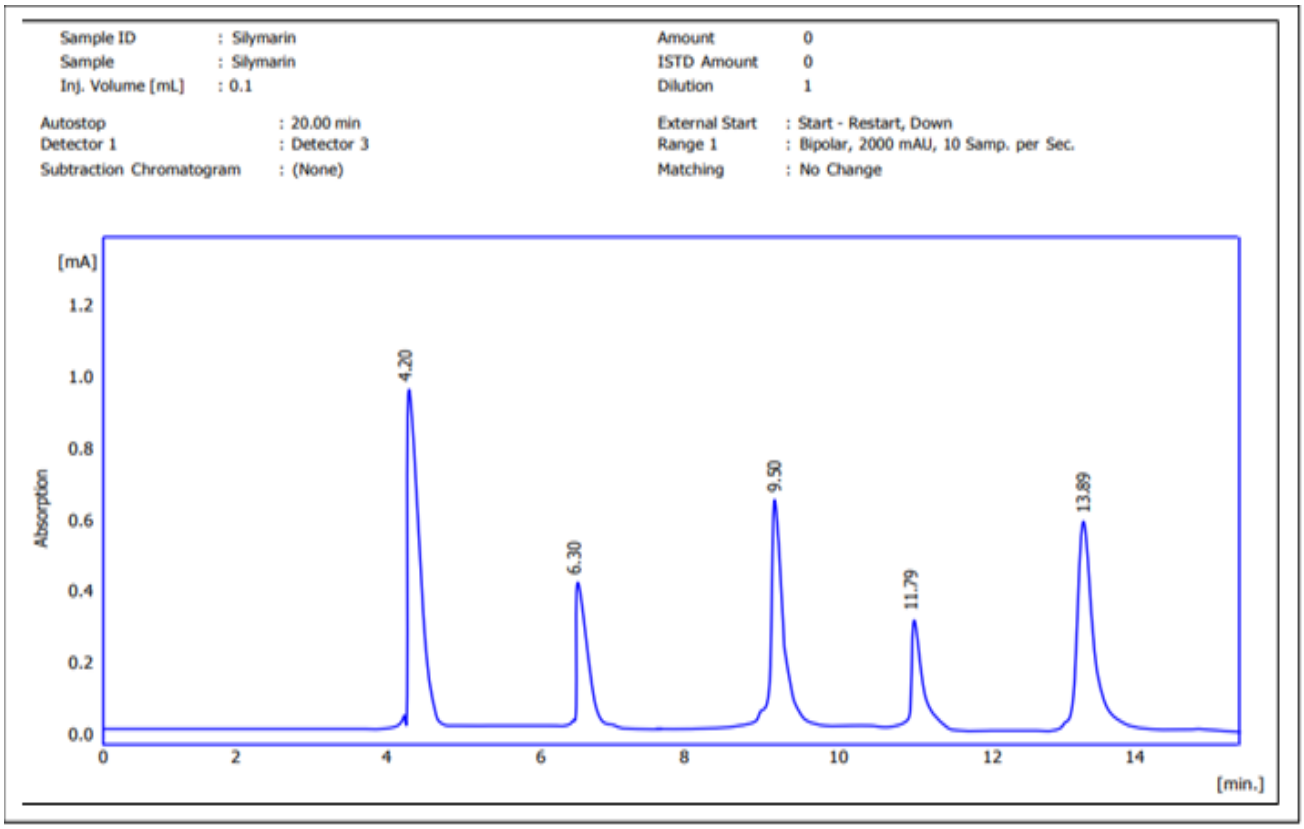

Figure (3.1) Retention time of ethyl acetate fraction of Silybum marianum

3.1.2 Ficus Carica leaves

According to the identification of bioactive compounds in ethyl acetate fraction of Ficus Carica Leaves by using HPLC technique and in comparison of ethyl acetate fraction of Ficus Carica leaves sample with the standards the result in figure. (3.2) below show a peak with retention time equal to 11.97 min. which belongs to the standard compound Gallic acid , another peak with tR at 8.08 which belongs to the standard compound Isoqurcertin , another peak with tR at 6.29 min. which belongs to the standard chlorogenic acid , another peak with tR at 10.04 min. which belongs to the standard compound Luteolin , another peak with tR at 5 min. which belongs to the standard compound Qurcetine and another peak with tR at 3.75 min. which belongs to the standard compound Rutin as illustrated in following and table (3.2) below:

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Table (3.2) Results chromatography of ethyl acetate fraction sample of Ficus carica leaves extract

No.	tR [min.]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W0S	Compound name	Concentration mg/gm
1	3.79	3652.49	820.16	20.11	20.14	0.25	Rutin	5.3
2	5	1254.11	433.65	12.58	12.89	0.1	Qurcetine	1.7
3	6.29	4256.98	740.25	18.98	18.49	0.16	Chlorogenic acid	5
4	8.09	5412.28	745.98	19.05	19	0.16	Isoqurcetin	7
5	10.10	812.08	310.22	10.25	10.15	0.08	Luteolin	4
6	11.97	1542.99	520.49	16.59	16.66	0.13	Gallic acid	2
	Total	16930.09	3570.14	100.0	100			

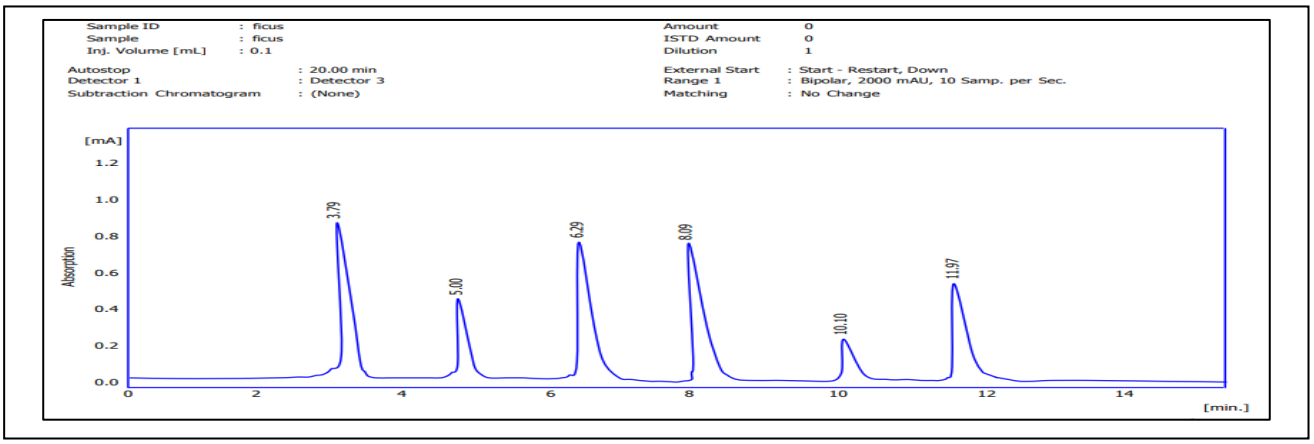

Figure (3.2) Retention Time of Ethyl acetate fraction of Ficus Carica leaves.

The concentration of each bioactive component in (mg/gm) of ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract have been calculated as the results illustrated in table (3.1 & 3.2) using following formula:

$$\mathbf{C\ sample} = \frac{\mathbf{C\ standard * A\ sample}}{\mathbf{A\ standard}} * \frac{\mathbf{D.F}}{\mathbf{Wt.sample}}$$ [17]

Where; **C** $_{\mathbf{sample}}$**:** Concentration of bioactive component in sample in mg /gm, **C** $_{\mathbf{standard}}$**:** Concentration of Standard in ppm and equal 10 ppm in present study, **A** $_{\mathbf{sample}}$**:** Area of Sample in mAU.s, **A** $_{\mathbf{standard}}$**:** Area of standard in mAU.s, **D.F**: Dilution Factor and equal to 25 ml in present study, **Wt.** $_{\mathbf{sample}}$**:** weight of sample after using for HPLC after extraction and fractionation and it was equal to 0.2 gm for each of ethyl acetate fraction of silybum marianum and ficus carica leaves extract.

3.3 Evaluation Glycemic parameters (FBS, Insulin, HOMA-IR and Fructosamine):

Regarding to serum FBS levels, serum levels [M±SD (mg/dl)] of FBS seen in table (3.3) show that there are highly significant differences ($p \leq 0.01$) among the studied groups, where G2 (negative control group) had higly significant elevation ($p \leq 0.01$) in comparison with G1, G3, G4, G5, G6, G7 and G8.While the least mean concentration of FBS levels equal to (85.78 ±7.68) seen in G1 (healthy control group). On the other hand the means of FBS concentration in rest groups were as following (293.35 ±25.74), (291.37 ±4.48), (244.12 ±40.84), (150.12 ±16.14), (114.62 ±088), and (111.16 ±3.07) for G6 (Ficus carica extract), G7 (Ficus carica + metformin), G8 (Silybum + ficus) G4 (Silybum extract) and G3 (positive control) respectively. On the other hand means with the same letter mean non-significant differences.

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Additionally serum Insulin levels, serum levels [M±SD (Mμ/L)] of Insulin seen in table (3.4) below show that there are highly significant differences (p≤0.01) among the studied groups, where G1 (healthy control group) had highly significant elevation in comparison with G2, G3, G4, G5, G6, G7 and G8 with mean concentration of Insulin equal to (73.65 ±5.53) While the least Insulin concentration show in G5 (silybum+ metformin) equal to 28.45 ±0.23. Furthermore the means of Insulin concentration in rest groups were as following (68.95 ±2.45), (46.23 ±8.65), (46.01 ±2.34), (45.10 ±1.56), (41.03 ±0.66), (32.18 ±1.68) and (28.45 ±0.23) for G8 (silybum +ficus), G2 (negative control), G6 (Ficus extract), G7 (Ficus carica + metformin), G3 (positive control) and G4 (silybum extract) respectively. On the other hand means with the same letter mean non-significant differences.

While serum Fructosamine levels, serum levels [M±SD (μmol/L)] of Fructosamine seen in table (3.4) below show that there are highly significant differences (p≤0.01) among the studied groups, where the G2 had highly significant elevation in comparison with G1, G3, G4, G5, G6, G7 and G8 with mean concentration of Fructosamine equal to (720.74 ±46.83) While the least Fructosamine concentration show in G6 (ficus extract) equal to (378.62 ±36.38). Furthermore the means of Fructosamine concentration in rest groups were as following (684.67 ±22.5), (667.52 ±18.78), (611.47 ±72.63), (574.44 ±27.18), (461.26 ±72.97) and (378.62 ±36.38) for G3 (positive control), G7 (Ficus carica + metformin), G1 (healthy control), G5 (silybum+ metformin) and G4 (silybum extract) respectively. On the other hand means with the same letter mean non-significant differences.

Also serum HOMA-IR levels, serum levels [M±SD] of HOMA-IR seen in table (3.4) below show that there are highly significant differences (p≤0.01) among the studied groups, where the G2 had highly significant elevation in comparison with G1, G3, G4, G5, G6, G7 and G8 with mean concentration of HOMA-IR equal to (46.34 ±8.98) While the least HOMA-IR levels show in G5 (silybum+ metformin) equal to (7.93 ±0.06). Furthermore the means of HOMA-IR levels in rest groups were as following (41.11 ±6.92), (34.19 ±3.95), (32.16 ±3.17), (16.17 ±2.53), (11.83 ±1.23 and (11.28 ±0.45) for G8 (silybum +ficus), G6 (Ficus extract), G7 (Ficus carica + metformin), G1 (healthy control) and G4 (silybum extract) respectively. On the other hand means with the same letter mean non-significant differences.

Table 3.3: Comparison between difference groups in Glycemic parameters

Group	Mean ± SE			
	FBS (mg/dl)	Insulin (Mμ/L)	HOMO-IR	Fructosamine (μmol/L)
Healthy control	85.78 ±7.68 **c**	73.65 ±5.53 **a**	16.17 ±2.53 **c**	611.47 ±72.63 **ab**
Negative control	419.41 ±44.06 **a**	46.23 ±8.65 **b**	46.34 ±8.98 **a**	720.74 ±46.83 **a**
Positive control	111.16 ±3.07 **c**	41.03 ±0.66 **bc**	11.28 ±0.45 **c**	684.67 ±22.54 **a**
Silybum extract	150.12 ±16.14 **c**	32.18 ±1.68 **cd**	11.83 ±1.23 **c**	461.26 ±72.97 **bcd**
Silybum + metformin	114.62 ±088 **c**	28.45 ±0.23 **d**	7.93 ±0.06 **c**	574.44 ±27.18 **abc**
Ficus carica extract	293.35 ±25.74 **b**	46.01 ±2.34 **b**	34.19 ±3.95 **ab**	378.62 ±36.38 **d**
Ficus carica + metformin	291.37 ±4.48 **b**	45.10 ±1.56 **b**	32.16 ±3.17 **b**	667.52 ±18.78 **a**
Silybum extract + ficus carica	244.12 ±40.84 **b**	68.95 ±2.45 **a**	41.11 ±6.92 **ab**	441.76 ±92.46 **cd**
LSD value	68.094 **	11.097 **	12.765 **	156.04 **
P-value	0.0001	0.0001	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).				

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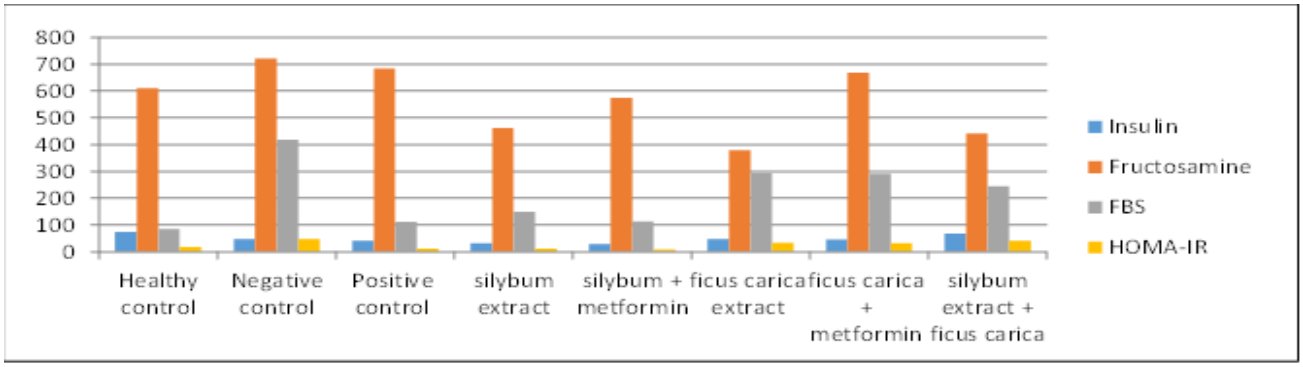

Figure (3-3): Comparison of Glycemic parameters among the Studied Groups

3.4 Evaluation of Oxidative stress Biomarkers (GSH, CAT, SOD and MAD):

Regarding to serum GSH levels, serum levels [M±SD (ug/ml)] of GSH seen in table (3.4) show that there are highly significant differences ($p \leq 0.001$) among the studied groups, where the G2 (negative control) had highly significant decrease ($p \leq 0.01$) in comparison with G1 and G8 with significant decrease ($p \leq 0.05$) in comparison to G3, G5, G6, and G7 and non-significant difference ($p \geq 0.05$) in comparison to G4. The highest mean concentration of GSH equal to (48.98 ±3.94 µg/ml) While the least GSH concentration show in G2 (negative control) equal to (11.83 ±0 µg/ml). On the other hand the means of GSH concentration in rest group were as following (38.46 ±4.03), (27.86 ±4.52), (26.89 ±1.95), (20.56 ±0.57), (19.29 ±0.68), and (18.19 ±1.36) for G1 (healthy control), G6 (Ficus carica extract), G7 (Ficus carica + metformin), G6 (Silybum + metformin) and G4 (Silybum extract) respectively. On the other hand means with the same letter mean non-significant differences.

Additionally serum MDA levels, serum levels [M±SD (nmol/ml)] of MDA seen in table (3.4) below show that there are highly significant differences ($p \leq 0.01$) among the studied groups, where the G2 had highly significant elevation in comparison with G1, G3, G4, G5, G6, G7 and G8 with mean concentration of MDA equal to (4.36 ±0.69) While the least MDA concentration show in G8 (0.860 ±0.02). Furthermore the means of MDA concentration in rest groups were as following (1.57 ±0.02), (1.43 ±0.03), (1.37 ±0.02), (1.31 ±0.01), (1.27 ±0.02), (0.950 ±0.04) and (0.860 ±0.02) for G3 (positive control), G4 (silybum extract), G5 (silybum+ metformin), G7 (Ficus carica + metformin), G1 (healthy control) and G6 (Ficus extract) respectively. On the other hand means with the same letter mean non-significant differences.

While serum CAT levels, serum levels [M±SD (Miu/ml)] of CAT seen in table (3.4) below show that there are highly significant differences ($p \leq 0.01$) among the studied groups, where the G1 had highly significant elevation in comparison with G2, G3, G4, G5, G6, G7 and G8 with mean concentration of CAT equal to (3431.26 ±351.13) While the least CAT concentration show in G2 (495.74 ±11.24). Furthermore the means of CAT concentration in rest groups were as following (1088.07 ±81.64), (861.86 ±87.09), (706.28 ±1.39), (628.50 ±16.08), (617.32 ±1.41), and (501.46 ±17.93) for G3 (positive control), G4 (silybum extract), G5 (silybum+ metformin), G6 (Ficus extract), G7 (Ficus carica + metformin) and G8 (Silybum+ Ficus) respectively. On the other hand means with the same letter mean non-significant differences.

Also regarding to serum SOD levels, serum levels [M±SD (U/ml)] of SOD seen in table (3.4) below show that there are highly significant differences ($p \leq 0.01$) among the studied groups, where the G1 had highly significant elevation in comparison with G2, G3, G4, G5, G6, G7 and G8 with mean concentration of SOD equal to (330.25 ±22.03) While the least SOD concentration show in G2 (62.84 ±2.69). Furthermore the means of SOD concentration in rest groups were as following (206.83 ±2.29), (203.33 ±4.53), (196.75 ±1.981), (157.87 ±8.63), (152.45 ±1.12), and (128.53 ±15.58) for G4 (silybum extract), G3 (positive control), G5 (silybum+ metformin), G6 (Ficus extract), G7 (Ficus carica + metformin) and G8 (Silybum+ Ficus) respectively. On the other hand means with the same letter mean non-significant differences.

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Table 3.4: Comparison between difference groups in Oxidative stress marker

Group	Mean ± SE			
	Glutathione (ug/ml)	CAT (Miu/ml)	SOD (U/ml)	MDA (nmol/ml)
Healthy control	38.46 ±4.03 **b**	3431.26 ±351.13 **a**	330.25 ±22.03 **a**	0.950 ±0.04 **bc**
Negative control	11.83 ±0.63 **f**	495.74 ±11.24 **c**	62.84 ±2.69 **e**	4.36 ±0.69 **a**
Positive control	26.89 ±1.95 **cd**	1088.07 ±81.64 **b**	203.33 ±4.53 **b**	1.57 ±0.02 **b**
***Silybum* extract**	18.19 ±1.36 **ef**	861.86 ±87.09 **bc**	206.83 ±2.29 **b**	1.43 ±0.03 **bc**
***Silybum* + metformin**	19.29 ±0.68 **def**	706.28 ±1.39 **c**	196.75 ±1.981 **b**	1.37 ±0.02 **bc**
***Ficus carica* extract**	27.86 ±4.52 **c**	628.50 ±16.08 **c**	157.87 ±8.63 **c**	1.27 ±0.02 **bc**
***Ficus carica* + metformin**	20.56 ±0.57 **cde**	617.32 ±1.41 **c**	152.45 ±1.12 **cd**	1.31 ±0.01 **bc**
Silybum* extract + *ficus carica	48.98 ±3.94 **a**	501.46 ±17.93 **c**	128.53 ±15.58 **d**	0.860 ±0.02 **c**
LSD value	7.703 **	372.43 **	29.038 **	0.696 **
P-value	0.0001	0.0001	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).				

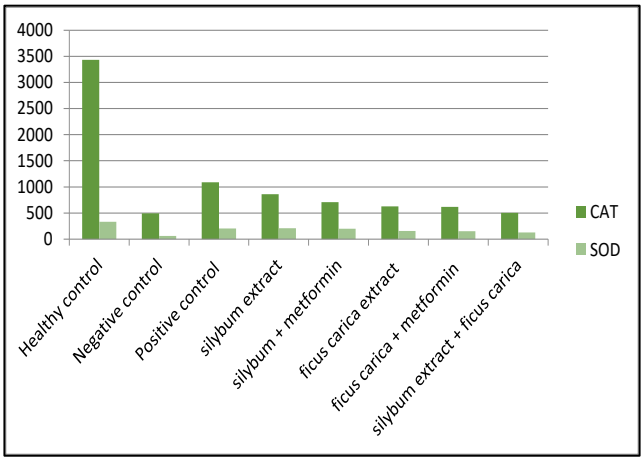

Figure (3-5): Comparison of CAT levels and SOD among the Studied Groups

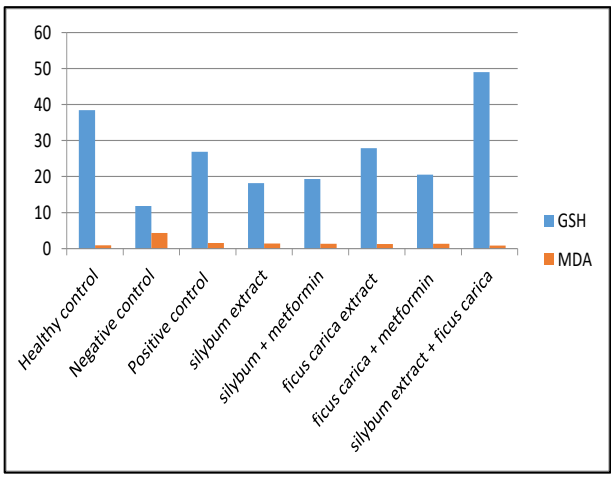

Figure (3-4): Comparison of GSH levels and MDA among the Studied Groups

3.5 Evaluation of inflammatory markers (TND- α and IL-6):

Regarding to serum IL-6 levels, serum levels [M±SD (pg/ml)] of IL-6 seen in table (3.5) and figure (3.6) below show that there are highly significant differences (p-value ≤ 0.01), where G2 (negative control group) had highly significant elevation in comparison with G1, G3, G4, G5, G6, G7 and G8 with mean concentration of IL-6 equal to (508.38±46.98 pg/ml). While the least IL-6 concentration show in G8 (silybum extract + ficus extract) equal to (95.92±1.47). On the other hand the means of IL-6 concentration in rest group were as following (112.29±1.24), (135.31±22.96), (143.18±23.85),(242.31±5.43),(247.56±19.93) and (254.13 ±1.29) for G7 (Ficus carica + metformin) , G1(healthy control) , G6 (Ficus extract) , G4 (silybum extract), G3 (positive control) and G5(silybum+ metformin) respectively.

Also regarding to serum TNF-α levels, serum levels [M±SD (pg/ml)] of TNF-α seen in table (3.5) and figure (3.6) below show that there are highly significant

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differences (≤ 0.01) among the studied groups, where G2 had highly significant elevation in comparison with G1, G3, G4, G5, G6, G7 and G8 with mean concentration of TNF-α equal to (3198.18 ±319.35 pg/ml) While the least TNF-α concentration show in G1 (632.64 ±24.48). Furthermore the means of TNF-α concentration in rest group were as following (807.63 ±7.87), (833.07 ±2.58), (862.86 ±14.74), (987.62 ±3.16), (1098.47 ±77.56) and (1622.19 ±8.57) for G8 (silybum extract + ficus extract), G7 (Ficus carica + metformin), G6 (Ficus extract), G5 (silybum+ metformin), G4 (silybum extract) and G3 (positive control) respectively. On the other hand means with the same letter mean non-significant differences.

Table (3.5): Comparison between difference groups in inflammatory markers

Group	Mean ± SE	
	IL-6 (Pg/ml)	TNF (Pg/ml)
Healthy control	135.31 ±22.96 c	632.64 ±24.48 d
Negative control	508.38 ±46.98 a	3198.18 ±319.35 a
Positive control	247.56 ±19.93 b	1622.19 ±8.57 b
Silybum extract	242.31 ±5.43 b	1098.47 ±77.56 c
Silybum + metformin	254.13 ±1.29 b	987.62 ±3.16 c
Ficus carica extract	143.18 ±23.85 c	862.86 ±14.74 cd
Ficus carica + metformin	112.29 ±1.24 c	833.07 ±2.58 cd
Silybum extract + ficus carica	95.92 ±1.47 c	807.63 ±7.87 cd
LSD value	61.223 **	330.64 **
P-value	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

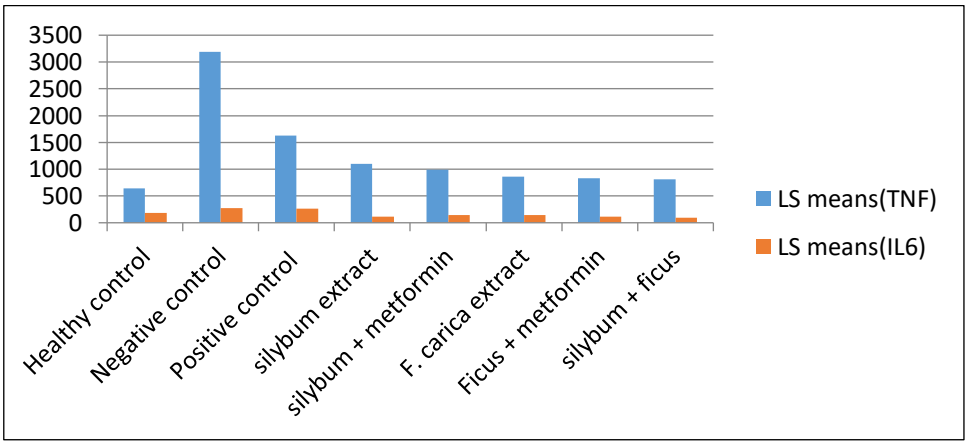

Figure (3-6): Comparison of TNF levels and IL-6 among the Studied Groups

4. Discussion

Since T2DM is a chronic metabolic syndrome causes hyperglycemia which in long term period lead to serious complications such as neuropathy, nephropathy, retinopathy and cardiovascular disease [27]. Synthetic drugs used for diabetic treatment have measurable adverse and worsened the diabetic condition and because of the high cost and limited availability of healthcare in developing nations in these countries hence phytotherapy plays an important part in treatment of DM and it is safe, less expensive, readily available [28]. Flavonoids were found in a large amount in plant ethanol extracts may play a role in diabetic complication as hypoglycemic role by enhancement of

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endogenous insulin excretion via inhibition of intestinal R-glycosidase [29].

4.1. Investigation of ethyl acetate Silybum Marianum and Ficus carica leaves extract by HPLC:

In this study according to the results in table (3-1) and table (3-2) show that the total of nine bioactive compounds (Silymarin , Tannic acid , Vanillic acid , Chlorogenic acid , Gallic acid , Isoqurectin , Luteolin , Qurcetine and Rutin) were determined in the whole extract of ethyl acetate fraction of Silybum marianum and ficus carica leaves extracts by HPLC , with silymarin being a predominant in silybum marianum extract and Isoqurcetin is being predominant in Ficus carica. Silymarin and Isoqurectin and other phenolic compounds present in extracts of the current study have been used traditionally in alternative medicine, through modulation of blood glucose and lipid metabolism and the development of insulin sensitivity, these bioactive substances have been shown to have therapeutic effects in the prevention and treatment of associated disorders [27]. In addition , as discussed above , bioactive compounds present in the current study can improve oxidative stress by preventing the glycation of antioxidant enzymes such as SOD , CAT and GSH ,facilitating pancreatic islets regeneration through the protection of β-cells from free radicals insults and hence enabling insulin secretion [27] . Furthermore , flavonoids and phenolic acids based on their features and structural characteristics may be play a role in stabilize free radicals through their ability to donate hydrogen ion from hydroxyl group and by chelation mechanisms of metal ions by both hydroxyl and carboxyl groups [27]. According to the results in table (3.1 & 3.2) with figure (3.1 & 3.2) the chemical constituent of the extracts displays the phytochemical analysis of two distinct ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract in quantitative terms, by comparing these phenolic content in the two extracts the results revealed that Silybum marianum had the highest concentration of phenolic compound combined in mg/gm.

4.2 Effects of Different Treatments used for Streptozotocin- induced T2DM rats on Glycemic Markers (FBG, Insulin, Fructosamine and HOMA-IR):

Result in table (3-3) shows the effect of orally administered of ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract on fasting blood glucose levels in STZ- induced diabetic rat and healthy control group rats for 4 weeks of treatment. In the current study, Results of FBS levels revealed a highly significant increased (p-value < 0.01) in negative control group in comparison with healthy control group and in comparison with positive control group and in comparison with all other studied groups that mean STZ has toxicity effects on β-cell in pancreas lead to hyperglycemia by induced damage in pancreas islets through free radicals generation and DNA damage. Also table(3.3) shown that the levels of FBS were reduced by 73.4%, 72.67%, 64.2%, 41.8%, 30.5% and 30% for G3, G5, G4, G8, G7 and G6 respectively. This result shows very clearly that ethyl acetate fraction of Silybum marianum extract demonstrated a high level of efficiency in reducing FBG levels, which could result from the improvement of β- cell functions in the islets of Langerhans to secret insulin for the subsequent augmentation of insulin signal transduction to increase glucose intake in vivo. Previous studies found that some plant extract having an abundance of phenolic chemicals that have an impact on ATP-sensitive K^+ channels and enhancement insulin secretion from regenerated cells and due to regulated blood glucose levels [30]. Doostkam, A. et al. 2022 , results were agreed with our study which have been proposed Silybum marianum has hypoglycemic effect by preserving or repairing pancreatic β-cells , decrease insulin resistance or by inhibition of alpha – amylase action[8] . These results were inconsistent with Oliveira, M. L et al, 2020 where they noticed that milk thistle has no significant effect on the basal glucose level and an oral glucose tolerance test [31]. The variations in the outcomes obtained may be due to the different experiment conditions such as doses applied or duration of treatment. Ramadan, S. et al. 2021 ; Roy, T. et al. 2021did an experiment to evaluate the effect of ficus carica leaves on hyperglycemia state , the results in their studies showed a reduced in FBG levels in STZ-diabetic rats given ethanolic extracts of ficus carica intraperitoneal [12,32]. Ficus carica leaves contain

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flavonoids and polyohenol that reported as antidiabetic agents [32].

Fructosamine reflect the average levels of blood glucose over the past 2-3 weeks [33]. In the current study, Based on the results showed in table (3-3) of the analysis, there are significant differences between the fructosamine levels of different groups in comparison with G1 (healthy control). The increased in serum fructosamine levels in studied group is associated with prolonged hyperglycemia in rats during the current experiment due to T2DM induced by STZ. The animal experiment relieved that there are a variation in fructosamine levels among studied groups that mean there are different responses to treatments used among affected groups where result in table (3.3) appears that fructosamine levels were reduced by 47.5%, 38.7%, 36%, 20.3%, 7.38% and 5% for G6, G8, G4, G5, G7 and G3 respectively.

On the other hand results of insulin levels in table (3-3) show there are a diminished in insulin levels among diabetic rat groups due to the poisoning effect of STZ on β-cell of pancreas and this is indicate that the dose of STZ (40 mg/kg) dependent in this experiment was suitable for generation of T2DM rat model. Regarding to results in table (3.3) insulin concentration was elevated by 49.14% only in G8 in comparsion with G2 (negative control). Hassan et al, 2022 demonstrated that the polyphenol contents that are responsible for increased insulin secretion from beta-pancreatic cells, glucose oxidation, and the lipid synthesis pathway may be responsible for the polyherbal mixture's considerable hypoglycemic and hypolipidemic effect [34]. Aslam, B., et al, 2023; showed that flavonoids cause c-AMP phosphodiesterase inhibition that modulate insulin secretion , which explain the significant decline in FBG levels and elevate insulin level in diabetic rats[35]. The HOMA-IR shows that human liver, muscle, and adipose cells are less sensitive to insulin., leading to hyperglycemia , thus the pancreas make an extra effort to secret more insulin this causes hyperinsulinemia to occur [27]. In the current study according to results in table (3-3) shows that HOMA-IR diminished by 82%, 75.6%, 74.5%, 72.5%, 26.23 and 11.3% for G5, G3, G4, G8, GG6 and G7 respectively which mean these treatments according to the mentioned order were have most effectiveness in reduced hyperglycemic state by enhancement insulin secretion from regeneration cells in pancreas.

4.3 Effects of Different Treatments used for Streptozotocin- induced T2DM rats on Oxidative Stress Biomarkers (GSH, CAT, SOD and MDA):

Looking at the results presented in table(3-4) and figures (3.4 & 3.5) we can see that the G2 (negative control) has significantly lower Glutathione levels than most other groups. Table (3-4) summarizes the results of a statistical analysis performed on the Catalase enzyme in blood samples of different groups of rats that were given different drugs revealed that G1 (healthy control) has higher levels of CAT , while G2 (negative control) has lower levels of CAT . Also based on the results (table 3-4) showed that G2 (negative control) has higher levels of SOD .Furthermore table (3-4) shows the differences between groups of rats that were given different drugs and their blood was tested for MDA and revealed that G2 (negative control) has higher MDA levels , while G8 has lower MDA levels. This is may be attributed to the oxidative stress state occurring in the rats that were given different drugs. Oxidative stress is a major risk factor in pathogenesis of most human disease; oxidative stress is a result of inequality between oxidants represented by reactive oxygen species (ROS) and antioxidants in body such as Glutathione, catalase and SOD [36]. In diabetes mellitus poorly controlled blood glucose level directly related with elevation of ROS, these species are very reactive can cause cell membrane damage through their reaction with protein, DNA, lipid and biomolecules [37]. Antioxidant compounds suggested that pancreaticβ -cells would be protected by lowering FBG and fructosamine levels, accompanied by elevation fasting plasma insulin levels through a reduction in the levels of thiobabituric acid reactive substrate in pancreas IL-1β and $NO^{\cdot}$ as well as an elevation in the antioxidant compounds such as SOD and GSH [36]. In the current study table (3.4) it appears that GSH levels increased by 314%,135.5%, 127%, 73.8%,63% and 54% for G8, G6 , G3, G7, G5 and G4 respectively .In addition to that table (3.4) shows that CAT levels were elevated by 119.5%, 73%, 42%, 27%, 24.5% and 1.15% for G3, G4, G5, G6, G7 and G8 respectively. On the other hand SOD levels regarding to table (3.4) were increased by 229%, 223%, 213%, 151%, 142.6% and 104% for G4, G3G5, G6, G7 and G8 respectively. While the levels of MDA were dropped by 80.2%, 70.8%, 69.95%, 69%, 67% and 64% for G8, G6, G7, G5, G4and G3 respectively. The peroxidation of unsaturated fatty acids is thought a major cause of tissue injury [38]. In addition

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to lipid peroxidation lead to significant injury in cell membrane and dysfunction of membrane [39]. Jaishree, V. et al. 2020 [29] results agreed with that obtained from present study where they found that the concentration of CAT , SOD and GSH were significantly decrease due to the free radicals induced inactivation of glycosylation in diabetic rat in comparison with a normal control group [40]. Several anti-diabetic studies reports have indicated that flavonoids broadly confirm a protective effect against decreasing levels of CAT, SOD and GSH [40]. Our study results revealed that CAT, SOD and GSH were significantly increased in treated groups to provide antioxidant effect, hence in the present study investigation Silybum marianum and Ficus carica both discovered to improve enzymatic properties and aid in preventing the action of free radicals in pancreatic and plasma. Hyperglycemia can also cause generation of free radicals which in turn lead to increase lipid peroxidation and decrease in cellular antioxidants [40]. These results are in agreement with some previous work by Abd EL-Fattah et al. 2018 carried out on the model of diabetes induced using HFD/STZ results agreed with obtained from the current study [41].

4.4 Effects of Different Treatments used for Streptozotocin- induced T2DM rats on Inflammatory markers (IL-6 and TNF-α):

Table (3.5) and figure (3.6) shows the higher levels of IL-6 and TNF-α G2 (negative control), obviously T2DM are prone to the elevation of oxidation stress, resulting in reduced antioxidant activity [27]. Production of ROS stimulates the generation of pro-inflammatory cytokines such as IL-6 and TNF –α [40]. Al-Qabbaa, S. M. et al. (2023) showed that rats with diabetes produced significantly higher levels of IL-6 and TNF –α , suggesting the involvement of pro-inflammatory cytokines in the development of diabetic complications[41]. These results are in agreement with that obtained from the current study. Table (3.5) show that the levels of IL-6 were reduced by 81.13% , 77.91% , 71.83% , 52.34% and 51.3% for G8, G7, G6, G5, G4 and G3 respectively. In addition to Table (3.5) show that-α 74.74%, 73.95%, 73%, 69% and 49.27% for G8, G7, G6, G4, and G3 respectively.

5. Conclusion

The current study's phytochemical analysis reveals that silymarin, which predominates in silybum marianum extract, and isoqurcetin, which predominates in Ficus carica, are bioactive compounds that have positive effects on the prevention and treatment of abnormalities related to blood glucose as well as the improvement of insulin sensitivity. This means that the extracts used for treatments were effective in reducing hyperglycemia. Additionally, the ethyl acetate fraction of Silybum marianum and Ficus carica leaves extract reduced MDA levels and improved the state of oxidative stress in Streptozotocin-induced T2DM rats. As a result, in the current study, Silybum marianum and Ficus carica were both found to improve the enzymatic properties and have assisted in inhibiting the action of free radicals in both. As conclusion anti-hyperglycemic, anti-inflammatory, and antioxidant properties of ethyl acetate fraction of silybum marianum and ficus carica leaves extracts as a plant-derived product may be of major significance in the treatment of diabetes mellitus and its complications. It is encouraged to conduct further experimental research to improve theses extracts absorption and transport in order to increase its therapeutic effect.

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