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*A*meliorating Role of *Lepidium sativum* Seeds Extract on Cardiac Muscle Impairment in Diabetic Albino Rats

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Abstract: Hypoglycemic effects of *Lepidium sativum (Garden cress)* seed powder (20 mg/kg) was evaluated on diabetes and oxidative stress built up in streptozotocin-induced diabetic male Wistar albino rats. Divided experimental animals into four groups comprising of ten animals each. Diabetes has been induced by single injection of STZ (40 mg/kg body weight) intraperitoneally. Streptozotocin induced diabetes resulted in a significant increase in blood glucose levels, MDA, cardiac enzymes and significant decrease in insulin levels, and (SOD, GSH, catalase) antioxidant. Garden cress treated rats (20 mg/kg) showed a significant decrease in blood glucose levels (FBG), cardiac enzymes and MDA. A significant increase in insulin levels, and (SOD, GSH, catalase). *Lepidium sativum* seed powder thus shows effectiveness for diabetes mellitus prevention and management and associated complications.

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Key words*:* *Lepidium sativum*, Garden Cress, STZ, oxidative stress, glucose, Insulin, Histopathological of cardiac muscle.

1. Introduction

Diabetes mellitus (DM) represents a serious public health problem worldwide. The number of diabetic patients is expected to exceed 300 million by 2025 **(Wild et al., 2004 and Somaratne et al., 2011).** Diabetes mellitus is a chronic metabolic disorder characterized by abnormally elevated blood glucose level due to a pancreatic β-cell deficit in insulin secretion and/or resistance to the action of antidiabetic hormone insulin correlated with carbohydrate, lipid and protein metabolism disorders leading to macro and microvascular dysfunction and long-term complications in health **(Patel et al., 2008)**. People suffering from diabetes cannot produce or properly use insulin, and so they persistently have high blood glucose. Diabetes affects on vital organs including the heart, kidneys, eyes and peripheral nerves **(Valko et al., 2005)**. Throughout the past decade, oxidative stress and its possible role in diabetogenesis, diabetic complications development, atherosclerosis and associated cardiovascular disease have been of significant interest. The leading cause of mortality in diabetes patients is cardiovascular disease. Myocardiacl infarction and stroke are the cause of death in up to 80 %t of people with type 2 diabetes **(Giugliano and Ceriello, 1996).**

Diabetes mellitus is a major cardiovascular disease risk factor (CVD), like diabetic cardiomyopathy **(Parmer et al., 2012)**. Diabetic cardiomyopathy (DCM) describes structural and functional changes in diabetes-associated myocardium **(Liu et al., 2014).** Among diabetic patients the most common complications are cardiomyopathy **(Forouhi and Wareham, 2014)**. Various therapeutic strategies have been involved in the treatment of diabetic cardiomyopathy (DCM) such as exercise, administration of antioxidants, anti-diabetic medicines, medicinal plants and their active ingredients. **(Liu et al., 2014 and Adegate et al., 2010)**.

Modern and orthodox drugs which are currently available for diabetes mellitus management and treatment have severe side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea, and hypoglycemia **(Singh et al., 2008 and Fujisawa et al., 2005).** Because of this fact, researchers are constantly seeking high efficacy antidiabetic agents and low side effects for managing and treating diabetes. Treatment of diabetes mellitus with insulin has the underlying effect of hypoglycemic shock, which can lead to death **(Frier, 2014)**. The use of conventional plant-based medicine in many parts of the world is very common, for the reasons mentioned above. 21,000 plants were listed by the WHO **(World health organization, 2002)** that is used for medicinal purposes around the world.

Lepidium sativum (L.S) is a fast-growing, edible herb **(Sharma et al., 2012)** of the Brassicaceae family **(Prajapati et al., 2014)**. The seeds are used in folklore medicine and have many biological activities. In nature, the seed is thermogenic, depurative, aphrodisiac, ophthalmic, diuretic, abortive, and contraceptive in nature **(Dugasani et al., 2009 and Gokavi et al., 2004)**. *Seeds of Lepidium sativum* are rich sources of phytochemicals including phenolic compounds, terpenoids, alkaloids, flavonoids, and compounds of organosulfur. This also includes phytosterols and their derivatives which are considered to have antioxidant potential, anti-cancer, anti-inflammatory, and cardio-protective activity **(Hudaib et al., 2008; Hardman et al., 2001 and Conforti et al., 2008)**. It was found that aqueous L.S extract exhibits hypoglycemic activity in rats with diabetes **(Eddouks et al., 2005)**. The present research was performed to assess the effect of aqueous L.S extract on hypoglycemics in normal and diabetic rats induced by streptozotocin (STZ).

2. Materials and Methods

2.1. Plant Material and Preparation of aqueous Extract

Lepidium sativum L. seeds were collected from a local commercial suppliers, Alexandria, Egypt. Subsequently, the seeds were thoroughly washed under running tap water, then with distilled water; and the shade was dried and ground to powder. The aqueous extract was prepared by boiling 1 g of dried powdered LS seeds in 100 ml of distilled water for 10 min in a standardized manner. It was left to infuse for another 15 min, then cooled and filtered. The filtrate was lyophilized and the required dose (milligram of lyophilized aqueous LS extract per kilogram of body weight) was then prepared and reconstituted just before oral administration in 10 ml of distilled water per kilogram of body weight. The oral dose of aqueous extract was 20 mg / kg body weight daily. (**Eddouks et al., 2005).**

2.2. Experimental Design

The experiment was conducted on 40 male Wistar albino rats (eight weeks old) weighing 150 ± 30g. Two weeks before the study all rats were placed under the same environmental conditions, they were retained in a temperature controlled room with a light / dark schedule of 12 h/12 h , and supplied with standard rat pellet diets and water. All animal experiments were under taken with the approval of Ethical Animal research by Public Health Guide for the care and use of laboratory animals National Institute of Health (NIH). **Induction of diabetes mellitus in rats**: Animals were fasted overnight, then injected with a single dose of streptozotocin (STZ) intraperitoneally (i.p) (40 mg/kg body weight) obtained from (Sigma –Aldrich hemical, Steinheim, Germany), dissolved in a 0.1 M citrate buffer freshly prepared (pH 4.5) **(Sharma et al., 2011)**

The rats were divided into four groups, each with 10 rats as follows: ***Group I****: Normal* Control group**:** rats received orally distilled water only for four weeks. ***Group II****: Lepidium sativum* group: rats received orally *Lepidium sativum extract* (20 mg/kg) for four weeks.

Group III: Diabetic control: rats were injected with a single dose of streptozotocin intraperitoneally (40 mg/kg body).

Group IV: Diabetic - treated group: diabetic rats received orally *Lepidium sativum* extract (20 mg/kg) for four weeks.

2.3. Biochemical Assays

The rats were sacrificed at the end of the treatment period, under a light anesthesia. Blood samples were taken through heart puncture into sterile vacuum tubes with and without anticoagulant (EDTA), the serum and plasma tubes were taken for biochemical assays. Pieces of heart that were immediately isolated from the rats after their scarification and washed with cold saline solution. In histological studies, cardiac tissue pieces have been fixed to 10% of neutral buffered formalin, and the others parts of heart tissues were stored at -20 ^{o}C until analyzed **(Lulat et al., 2016)**. The Separate sera were used to measure the following parameters:

Serum glucose levels were determined according to enzymatic colorimetric method **(Tietz., 1995),** Rat insulin ELISA kit (Millipore USA) is used for the non-radioactive quantitative quantification of insulin in rat serum **(Weyer et al., 2002),** Serum cardiac enzymes assay: Determination of Creatine Kinase (CK-MB) by **(Young, 1997)** method and Determination of Lactate dehydrogenase (LDH) by **(Van der heiden et al., 1994)** method.

Antioxident parameters: superoxide dismutase (SOD), malondialdehyde (MDA), glutathione reductase (GSH), catalase (CAT) were determined in accordance with the methods described by **Nishikimi et al., (1972); Ohkawa et al., (1979); Beutler et al., (1963)**; **Aebi, (1984)**, respectively.

2.4. Statistical analysis

Using IBM SPSS software package version 20.0, USA, data were fed to the computer and analyzed. Using numbers and percentages + S.D. data were analyzed. Comparisons between the studied groups were analyzed using F-test (ANOVA) and Post Hoc test (LSD) for the normally distributed data. Significance was achieved at levels $p < 0.05$, $p < 0.01$ or $p < 0.001$ **(Kotz et al., 2006)**.

3. Results

Biochemical analysis:

Glucose concentration: In this study, the anti-hyperglycemic impact of the aqueous extract of Lepidium sativum (L.S) was investigated using diabetic rats induced by streptozotocin. In the streptozotocin-induced diabetic rats, substantial reductions in blood glucose levels were observed after oral administration of aqueous L.S extracts for 28 days. Blood glucose was significantly higher in diabetic rats than in normal control rats ($P \leq 0.001$) (table, Figure1). Oral administration of aqueous L.S extract (20 mg/kg of body weight) in diabetic rats significantly decreased blood glucose ($P \leq 0.001$).

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Table (1): Effect of *Lepidium sativum* seed extract on plasma glucose levels (mg/dl)

Glucose level	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	110.0	108.0	267.40^{a***}	119.80^{b***}
SD.	7.91	5.70	5.94	4.55

a: Statistically significant with **Normal group,** b: Statistically significant with **Control Diabetic group**
***: Statistically significant at $p \leq 0.001$

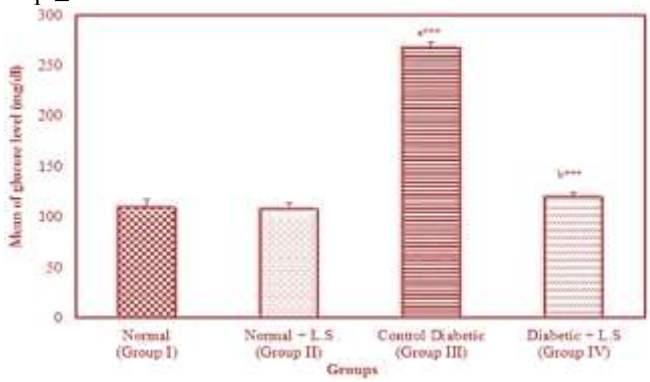

Figure (1): Effect of *Lepidium sativum* seed extract on plasma glucose level (mg/dl)

Insulin concentration

Insulin levels have been significantly decreased ($P \leq 0.001$) in rats with diabetes relative to the normal control rats. Significant increase was observed after administration of LS extract in diabetic rats ($P \leq 0.01$) (table, Figure 2).

Table (2): Effect of Effect of *Lepidium sativum* seed extract on serum insulin level (µIU/ml)

Insulin level (µIU/ml)	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	13.56	13.52	5.08^{a***}	12.48^{b**}
SD.	0.49	0.26	0.55	0.28

a: Statistically significant with **Normal** group, b: Statistically significant with **Control Diabetic** group
: Statistically significant at $p \leq 0.01$ *: Statistically significant at $p \leq 0.001$

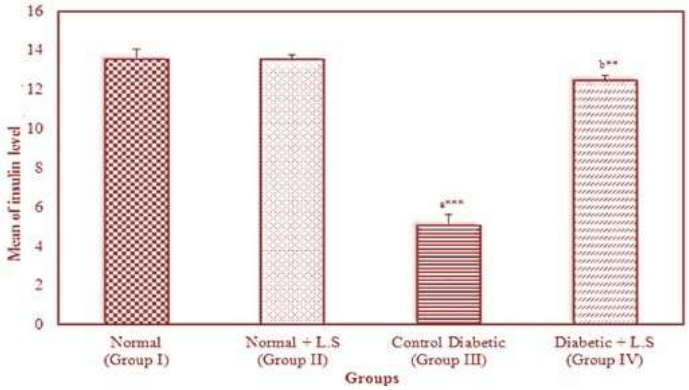

Figure (2): Effect of *Lepidium sativum* seed extract on serum insulin level (µIU/ml)

Cardiac enzymes

From the results of this study, it is evident that STZ injection in diabetic rats produced a significant increase in CK-MB and LDH serum levels ($P \leq 0.001$) compared to normal control rats. Oral administration of L.S. aqueous extract gave a substantial decrease ($P \leq 0.001$) in serum levels of these enzymes in the diabetic treated group compared to diabetic rats (table, Figure 3,4).

Table (3): Effect of L.S treatment on the Serum CK-MB (U/l)

Serum CK-MB	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	38.22	36.94	190.20 a***	60.58 b***
SD.	1.53	1.49	1.69	1.66

a**:** Statistically significant with **Normal** group, b**:** Statistically significant with **Control Diabetic** group
***:** Statistically significant at $p \leq 0.001$

Table (4): Effect of *Lepidium sativum* seed extract **on the Serum LDH (U/l)**

Serum LDH	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	60.90	57.82	239.26 a***	139.64 b***
SD.	3.38	1.68	1.87	2.35

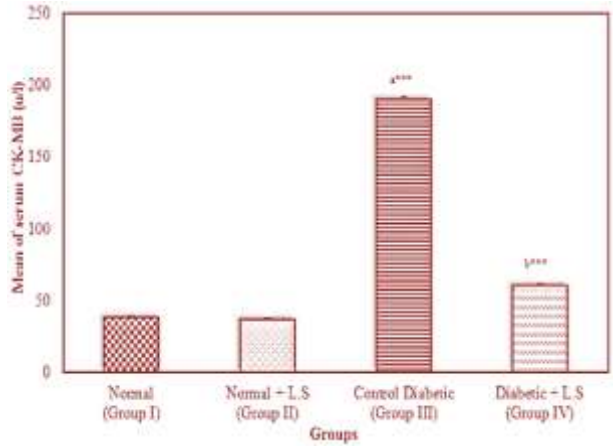

Figure (3): Effect of L.S treatment on serum CKMB

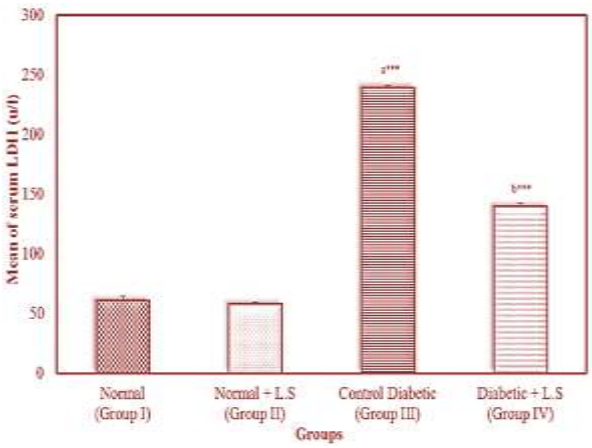

Figure (4): Effect of L.S treatment on serum LDH

Antioxidant parameters

Marked oxidative stress was associated with STZ-induced diabetes, demonstrated by a substantial increase in serum MDA and a decrease in SOD, CAT and GSH levels, compared to the normal control group. Oral administration of aqueous extract of L.S treatment showed a marked antioxidant effect with a substantial decrease in serum MDA and increased levels of SOD, CAT and GSH in the diabetic group treated, relative to diabetic rats (table, figure 5,6,7,8). This study showed no substantial difference between normal group and normal group treated with L.S. extract in all investigated parameters.

Table (5): Effect of L.S treatment on the SOD in blood (U/ml)

SOD in blood	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	6.20	6.28	3.04 a***	4.58 b***
SD.	0.43	0.26	0.48	0.61

Table (6): Effect of *Lepidium sativum* seed extract on serum MDA (n mol/ml)

MDA in Serum	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	23.53	24.82	45.62 ^{a***}	20.38 ^{b***}
SD.	2.50	2.68	2.34	0.69

a**:** Statistically significant with **Normal** group, b**:** Statistically significant with **Control Diabetic** group
***:** Statistically significant at $p \leq 0.001$

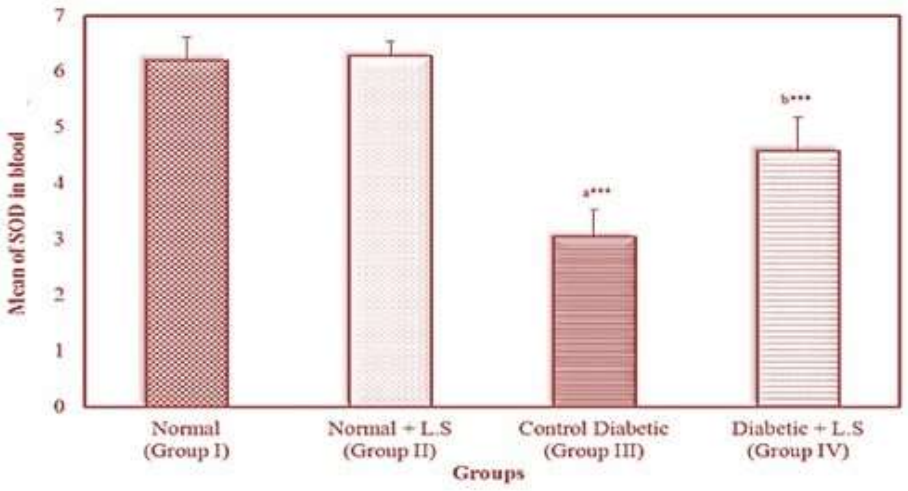

Figure (5): Effect of *Lepidium sativum* seed extract **on blood SOD (U/ml)**

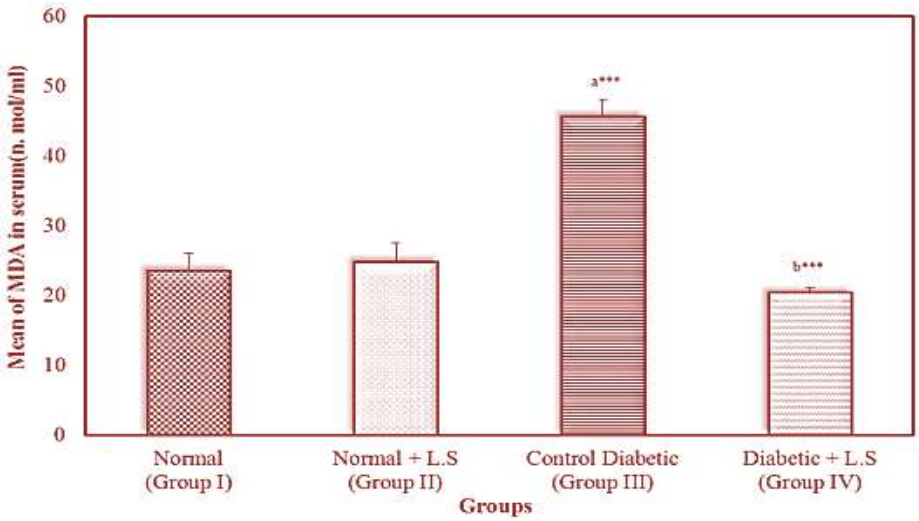

Figure (6): Effect of *Lepidium sativum* seed extract **on serum MDA (n. mol/ml).**

Table (7): Effect of L.S treatment on GSH in blood (mg/dl)

GSH in blood	Normal (Group I)	Normal + L.S (Group II)	Control Diabetic (Group III)	Diabetic + L.S (Group IV)
Mean	5.29	5.08	1.94 ^{a***}	4.78 ^{b***}
SD.	0.45	0.26	0.51	0.38

a**:** Statistically significant with **Normal** group, b**:** Statistically significant with **Control Diabetic** group
***:** Statistically significant at $p \leq 0.001$

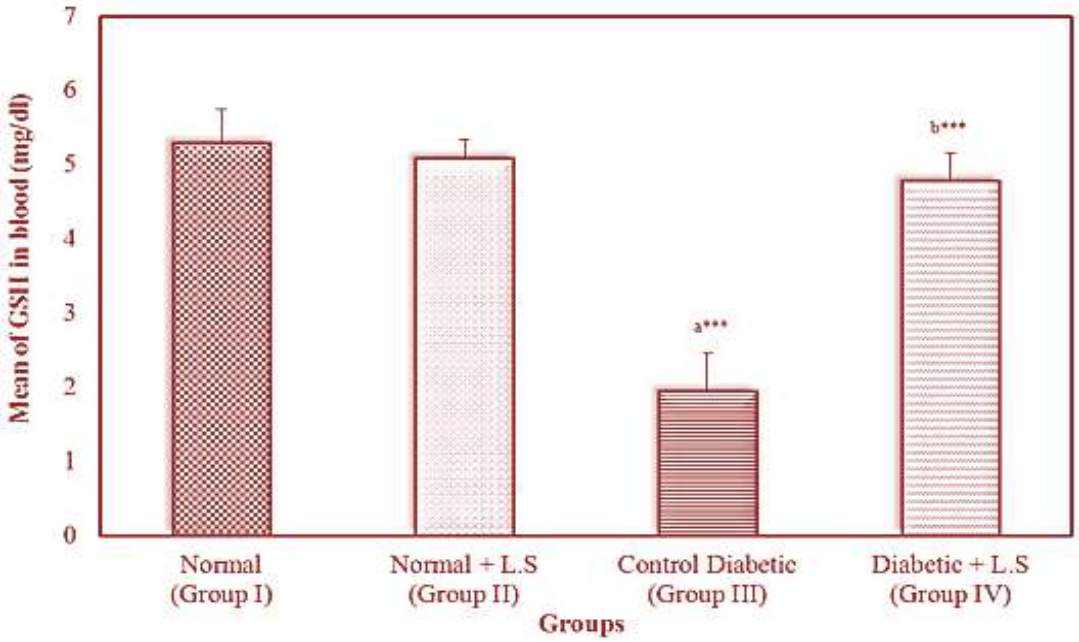

Figure (7): Effect of L.S treatment on the GSH in blood (mg/dl).

Table (8): Effect of L.S treatment on the Catalase in plasma (U/l)

Catalase in plasma	**Normal (Group I)**	**Normal + L.S (Group II)**	**Control Diabetic (Group III)**	**Diabetic + L.S (Group IV)**
Mean	12.10	12.16	4.82 [a***]	9.88 [b***]
SD.	0.47	0.30	0.63	0.58

a**:** Statistically significant with **Normal** group, b**:** Statistically significant with **Control Diabetic** group
***:** Statistically significant at $p \leq 0.001$

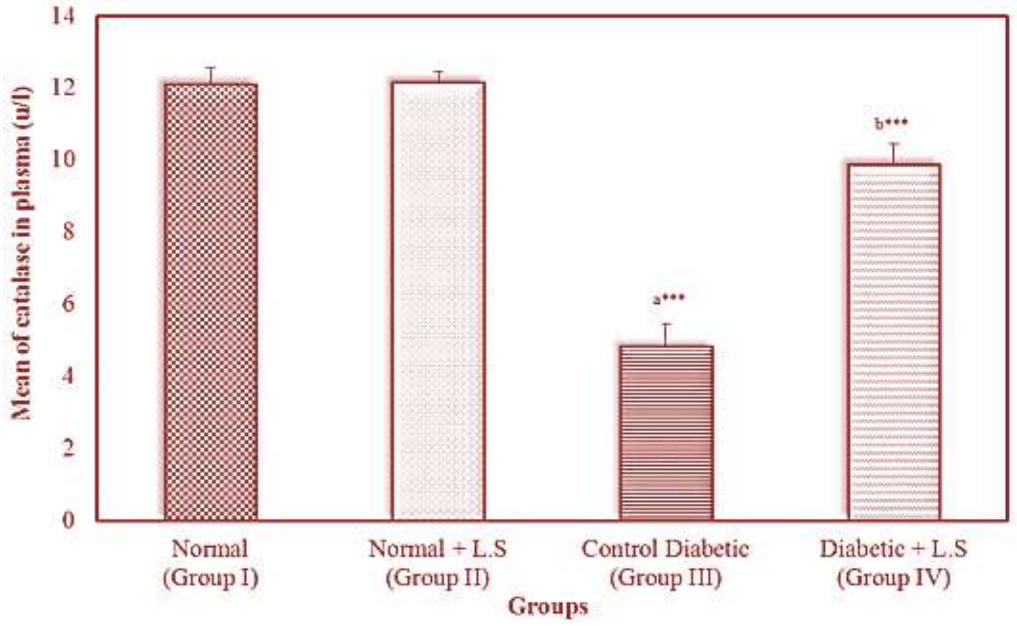

Figure (8): Effect of L.S treatment on the Catalase in plasma (U/l).

Histopathological observations

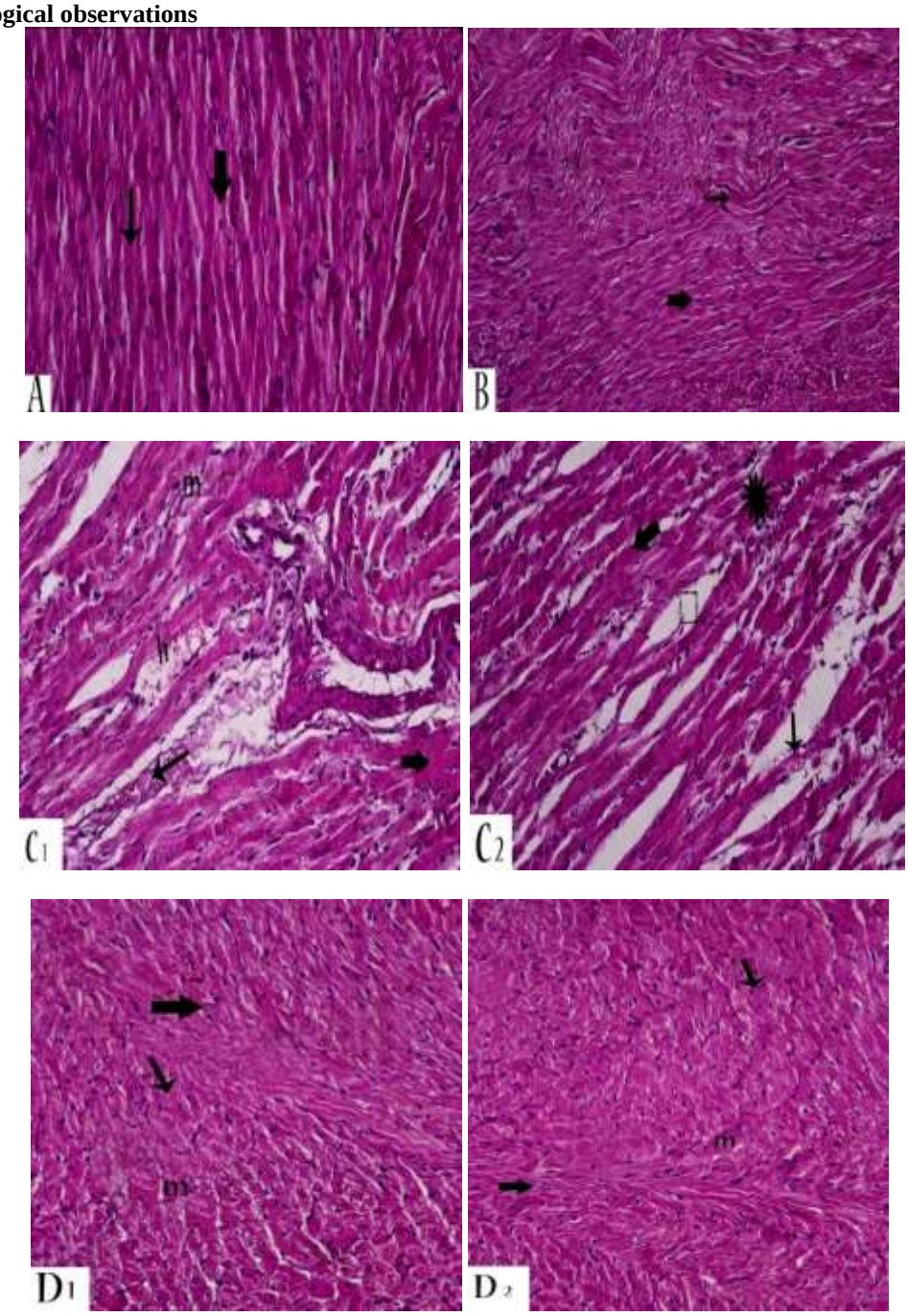

Figure (9): Histopathological analyzes of the ventricular heart tissue of rats stained with Hematoxylin and Eosin (magnification=400).

A): Control normal group: normal heart architecture, normal myocardial cell morphology with normal myocardial fibers (thin arrow) and centrally located oval nuclei (thick arrow).

B): Normal group treated with L.S extract: the myocardium was striated and arranged in a linear array that branched (thin arrow) and nuclei is clear and centrally located (thick arrow).

C1,C2) STZ-diabetic group: destruction and atrophy of myocardial fibers (thin arrow), wide intercellular space (square), absence of nuclei (thick arrow), mononuclear cellular infiltration (star), extravasated red blood cells in between the muscle fibers (Hemorrhages (h), in addition to severe degree of diffuse myolysis (m).

D1, D2) Diabetic rat treated with L.S extract: improvement of histopathological changes is evident such mild degree of myolysis (m) and a near normal structure of cardiac muscle fibers (thin arrow) with central oval vesicular nuclei (thick arrow).

4. Discussion

It is well known that diabetes mellitus (DM) is a major health problem **(Ng et al., 2014).** Mainly, diabetic patients with poor glycemic control are at risk of various diseases, morbidity and mortality among people with diabetes mellitus are mostly triggered by cardiovascular disease **(American Diabetes Association, 2010)**. Cardiomyopathy is the most common complications in diabetic patients **(Forouhi and Wareham, 2014).**

Experimental diabetes produced by low STZ dose combined with high energy intake is regarded as a general strategy to obtain type 2 diabetes animal model, since it simulates the real course of human type-2 diabetes mellitus. The injection of low dose STZ makes partial dysfunction of beta cell to suppress insulin secretion **(Wang et al., 2007 and Ti et al., 2011).**The toxic effect of STZ is due to its selective uptake into β-cells via its low affinity glucose transporter (GLUT2) present in the plasma membrane, leading to inhibition of insulin secretion of beta cells, damaging the pancreas and glucose metabolism **(Eleazu et al., 2013 and Ghosh et al., 2015)**

Insulin and sulphonylureas, are used most commonly for the treatment of diabetes, which may increase the risk of hypoglycemia and lead to fatal consequences. Hence, there is a need to search for safer and more effective alternatives **(Frier, 2014)**. **Shukla *et al*., (2012)** previously studied L.S extract antidiabetic activity in type-1 diabetic rats. Phytochemicals studies of *Lepidium sativum* is documented to possess flavonoids, sulphur glycosides, sterols coumarins, triterpenes, and various imidazole alkaloids are some of the phytochemicals present in L.S **(Radwan et al., 2007)**.These Alkaloids, Flavonoids and Phenolic compounds are known for their hypoglycemic and antioxidative properties **(Kanter et al., 2005 and Yao et al., 2012)**. Blood glucose level significantly increased in rats treated with STZ compared to normal controls. Treatment with L.S aqueous extract (20mg / kg) (daily oral administration) of these diabetic rats has restored the blood glucose to almost normal level. The glucose lowering action might also be the result of flavonoids and glycosides that present in L.S extract stimulating the surviving β-cells of islets of Langerhans to release more insulin and enhance glucose metabolism **(Saravanan et al., 2012)**. Aqueous L.S extract has been reported to affect glucose homeostasis by inhibiting endogenous glucose production in the liver **(Eddouks et al., 2003),** reabsorption through the alteration of renal glucose transporter (SLGT1) expression in the kidneys **(Adachi et al., 2000),** increasing the uptake of both glucose **(Luo et al., 1998)** and glucose intracellular metabolism in the muscle and adipose tissues **(Zhang and Tan, 2000) and (Peungvicha et al., 1998)**. Finally, the inhibition of intestinal glucose absorption could also be involved in this observed hypoglycemic activity of L.S **(Patel and Srinivasan, 1997)**.

A specific and extremely sensitive index of myocardial damage, necrosis or ischemia is claimed to be an abnormally high serum activity of LDH and CK-MB **(Howard-Alpe et al., 2006)**. Most of the studies reported increased LDH and CK-MB serum activity in STZ-induced DCM **(Akhtar et al., 2016; Wang et al., 2012 and Wang et al., 2013)**. According to findings of this research, STZ-induced diabetes has had a harmful effect on the heart due to elevated serum LDH and CK-MB activity in STZ diabetic rats relative to normal rats and L.S treatment has attenuated this. Increased levels of cardiac enzymes in diabetes were also noted in a study (**Fuleshwor et al., 2013)** which indicated that one of the factors that contributed to this damage was myocardial damage and atherosclerosis, as it contributes to the deposition of cholesterol and cholesterol esters, this narrows the coronary artery with a consequent decrease in the nutrients that the heart requires, that affects its work.

With increased free radical-induced lipid peroxidation activity, and accumulation of lipid peroxidation products, diabetic complications develop. Polyunsaturated fatty acids (PUFA) react to free radicals for the formation of peroxides, which degrade lipids and release malondialdehyde (MDA), a stable lipid peroxidation product and measured as a lipid peroxidation index **(Kousar et al., 2011)**.

Qusti et al., (2016) reported significant increase in GSH levels and significant decrease in serum MDA levels and kidney tissue homogenate in rats received methanolic L.S extract for 4 weeks. Also, these results were in correlation with the study of **Mohamed and Safwat, (2016)**. They reported that a diet supplemented with of L.S seed powder restored the levels of myocardial MDA and GSH. Its high content of antioxidants (vitamin C, E, carotenoids, polyphenols and flavonoids) has been attributed to this effect.

Treatment with L.S and reversal of these enzymatic antioxidants' activities that may result from reduced lipid peroxidation and/or reduced usage. L.S extract, by directly scavenging the free radicals in diabetic rats, thereby resulting in an increased GSH, SOD and CAT content in L.S treated diabetic rats **(Ramesh and Pugalendi, 2006).**

Hyperglycemia-associated diabetes mellitus and oxidative stress that generally causes significant damage to the tissue and consequently degenerative complications in several organs like the heart **(Jemai and Sayadi, 2015 & Yan et al., 2017)**. Diabetic

cardiomyopathy (DCM) identified as an increased risk of heart failure with ventricular dysfunction, is frequently seen in both humans and animals in the absence of hypertension, coronary artery and valvular heart disease **(Tarquini et al., 2011)**.

In the present study, microscopic observations are consistent with **Badole et al., (2015)** and **Ganugula et al., (2017)** who has detected a marked cardiac muscle degeneration and pancreas of STZ-diabetic rats. Histological changes in diabetic heart have shown massive heart muscle fiber necrosis in this study, increasing oxidative stress and hyperglycemia can lead to focal degeneration. And the myocardial injury in diabetic heart represented by vacuolation of myocardial fibers and marked myolysis were associated with myocardial fibers atrophy and separation from each other, extravasations of red blood cells, increased intracellular spaces between myocardial fibers, absence of nuclei, in addition, mononuclear cellular infiltration in between the muscle fibers of the diabetic rat group as compared with non-diabetic heart.

The cardioprotective potential of Garden cress seed powder (GSP) in albino rats against cardiotoxicity and oxidative stress induced by 5-fluorouracil (5-FU) has been evaluated recently. GSP pre and post treatment significantly enhances all (5-FU) modified parameters. The results show that (GSP) significantly affects the protection of the heart by preserving antioxidant and anti-inflammatory activities against (5-FU) -induced cardiotoxicity **(Mohamed and Safwat, 2016).**

Whereas L.S Extract (20mg/kg) of the diabetic group treated demonstrated pathological changes; that is the myocardial damage was obviously ameliorated with minute vacuolation and showed a marked decrease in myocardial injury compared with the diabetic group and minute myolysis was observed. *Lepidium sativum* offered protection to the heart at cellular level.

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