Original Article

Histological and Immunohistochemical Study on the Effect of the Combined Vitamin C and Metformin versus Metformin Alone in Treating Diabetic Myopathy in Rat

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ABSTRACT

Background: Metformin is used in type 2 diabetes mellitus (T2DM). Vitamin C is a water-soluble vitamin with antioxidant and anti-inflammatory properties. Therefore, the effect of combining metformin and vitamin C in treating the diabetic myopathy was investigated in this research.

Methods: Forty rats were used divided into 4 groups; control group (group I), diabetic group (group II), metformin group (group III), and vitamin C and metformin group (group IV). Body weight was measured before and at the end of the experiment. Blood glucose levels and serum C-reactive protein (CRP) were measured. Tissue malondialdehyde (MDA) and superoxide dismutase (SOD) were measured. Sections of the skeletal muscle were stained with H&E, Masson trichrome, and caspase-3 and inducible nitric oxide synthase (iNOS) immnuostaining. Morphometric and statistical analyses for the results were performed.

Results: Body weight of rats of group I and group II were decreased then increased in group IV. Blood glucose levels were elevated in group II; and reduced in group III and group IV. MDA level were increased in group II, then lowered in both in group III and group IV.SOD activity was decreased in group II, then increased in group III and group IV toward normal level. Collagen deposition and immune reaction to caspase-3 were elevated in group II and group III and group IV. Immune reaction to iNOS was elevated in group II and group III, and nearly the same as group I in group IV.

Conclusions: Combined intake of vitamin C and metformin provided better results in treating diabetic myopathy than that obtained from metformin alone.

Key Words: Diabetic myopathy, Inflammation, Metformin, Oxidative stress, Vitamin C.

H2O2: hydrogen peroxide. **NO:** nitric oxide. **ROS:** Reactive oxygen species. **RNS:** reactive nitrogen species. **T2DM:** Type2 diabetes mellitus. **IGT:** impaired glucose tolerance. **IFG:** impaired fasting glucose. **AMPK:** AMP activated protein kinase. **iNOS:** inducible nitric oxide synthase. **NA-STZ:** Nicotinamide-streptozotocin. **O-GlcNAcase:** O- GlcNAc-selective N-acetyl-β-d-glucosaminidase.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) results from impaired insulin secretion by β- cells in the pancreas and insulin resistance; both factors induce hyperglycemia[1]. T2DM represents about 90 % of diabetes mellitus cases and by 2030, it is expected that nearly 8 % of the worldwide population will suffer from T2DM.The risk factors type 2 diabetes mellitus include sedentary lifestyles and increased adiposity[2, 3].

Diabetic myopathy is one of the most important complications of diabetes mellitus. It is characterized by a decreased muscle mass and physical capacity. The pathological and functional changes that occur in the skeletal muscle in T2DM affect also the blood glucose level[4]. Metformin is a first-line drug prescribed for patients with T2DM according to the current clinical guidelines. It reduces the blood glucose level without inducing hypoglycemia through enhancing the peripheral glucose uptake, decreasing the fasting plasma insulin levels, and increasing the insulin sensitivity. It also diminishes the intestinal glucose absorption. The most common adverse effects of metformin are nausea, and diarrhea and the most serious are lactic acidosis[5, 6].

Studies showed that metformin can also protect against vascular inflammation and treat the cardiac complications of diabetes. Additionally, metformin can effectively prevent or delay the occurrence of diabetes especially in those with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)[7, 8].

Vitamin C is a water-soluble vitamin. It functions as a cofactor for several enzymes including those

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involved in collagen hydroxylation, the synthesis of norepinephrine and serotonin, and hepatic production of bile from cholesterol. Vitamin C has many non-enzymatic actions; it protects low-density lipoproteins from oxidation, reduces the harmful effects of oxidants in the stomach, and promotes the iron absorption. Being water-soluble, vitamin C can neutralize the free radicals inside and outside cells. Vitamin C has beneficial metabolic roles in improving the metabolism of diabetic patients. It can restore the basal metabolic rate and improves the lipid profile[9, 10].

Few studies focused on the treatment of skeletal muscle injury in type2 DM. Therefore, in this study, we investigated the effect of combined vitamin C and metformin versus metformin in treating diabetic myopathy in the rat.

MATERIALS AND METHODS

2.1. Chemicals:

Streptozotocin (STZ) and nicotinamide (NA) were purchased from Sigma Aldrich Co-USA. Metformin was purchased from Chemical Industry Development Company (CID, Cairo, Egypt). Vitamin C was obtained in the form of cevarol 500 mg tablets from Memphis Pharmaceutical Company.

2.2. Experimental animals:

Forty adult male Wistar albino rats aged about 2 months and weighing between 230 - 280 g were used as experimental animals in the present investigation. They were obtained from the animal house of Faculty of Medicine, Zagazig University, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in metabolic cages with well-aerated covers at normal atmospheric temperature (25 ± 5 ºC) as well as under good ventilation and received water and a standard balanced diet. All animal procedures were following the recommendations for the proper care and use of laboratory animals stated by the Institutional Animal Ethical Committee.

2.3. Experimental Design:

Rats were divided into four groups designed as the following:

Group I (Control group): included 10 rats. They received ordinary water and food daily.

Group II (Diabetic group): included 10 rats. They were injected intraperitoneally with a single freshly prepared nicotinamide in PBS at a dose of 175 mg/kg body weight; then after 15 minutes, freshly prepared STZ in 50 mmol citrated buffer pH 4.5 was injected intraperitoneally at a dose of 65 mg/kg body weight. After 48 hr of NA-STZ injection, fasting blood glucose level was estimated using AccuChek glucometer (Roche, Germany). Blood glucose levels higher than 250 mg/dl were considered diabetic[11].

Group III (Diabetic group received metformin): included 10 rats. They were injected with the same dose of NA-STZ, and then they received metformin in a dose of 300 mg/kg/day orally for 4 weeks[12].

Group IV (Diabetic group received metformin and vitamin C) - included 10 rats. They were injected with the same dose of NA-STZ, and then they received metformin and vitamin C (50 mg / kg) for 4 weeks[13].

2.4. Body weights:

The body weight of each animal was measured before and at the end of the experiment.

2.5. Sampling:

2.5.1. Collection of blood samples:

At the end of the experiment period, (24 hours from the last dose), blood samples were collected using capillary tubes from the retro-orbital venous plexus for measurement of blood glucose level and serum CRP.

I. Blood glucose level:

Fasting blood glucose level was estimated using AccuChek glucometer (Roche, Germany).

II. Measurement of serum CRP:

Serum CRP was measured using a DuoSet ELISA kit (R&D systems, Inc., Minneapolis, Minnesota, USA) following manufacturer's instructions[14].

2.5.2. Collection of tissue samples for biochemical and histological analysis:

Rats from each group were anesthetized by ether. Skeletal muscles of the right thighs of rats from each group were assigned for analysis of oxidative stress markers. Skeletal muscles were rapidly dissected out, washed with saline and cut into small pieces for determination of malondialdehyde (MDA), and superoxide dismutase (SOD) activity.

Left thighs skeletal muscles were used for histological and immunohistochemical examination.

They were fixed in 10 % formalin, and then dehydrated with ascending grades of ethanol (70, 90 and 100 %). Dehydration was then followed by clearing the samples in two changes of xylene. Samples were then impregnated with two changes of molten paraffin wax, then embedded and blocked out to paraffin blocks. Sections of the skeletal muscle (4 µm thickness) were cut using a microtome and mounted on a glass slide to investigate the histological and immunohistochemical results.

2.6. Determination of oxidative stress biomarker and antioxidative enzymes:

2.6.1. Determination of Malondialdehyde level:

MDA was measured colorimetrically in skeletal muscle homogenates according to the method of Odukoya *et al*.[15].

2.6.2. Determination of SOD activity:

The SOD activity was measured in the skeletal muscle homogenates according to the methods of Weydert and Cullen[16].

2.7. Histological study[17]:

The sections were stained with:

1- Hematoxylin and eosin stains (H&E): for studying the general histological structure of the skeletal muscle.

2- Masson trichrome stain: for collagen fibers.

2.8. Immunohistochemical study:

Immunohistochemical expression of caspase-3 and iNOS was performed using streptavidin–biotin complex immune-peroxidase system according to Ramos-Vara *et al*.[18].

2.9. Morphometric analysis:

The diameter of the skeletal muscle, the area percentage of collagen fibers, and the area percentage of immune reaction to caspase 3 and iNOS were measured within 10 non-overlapping fields for each rat at a total magnification X 400 using Fiji image j analysis software (National Institute of Health; NIH, Bethesda, MD, USA)[19].

2.10. Statistical analysis:

Data were presented as means ± SD. SPSS .19 was used for all statistical analysis. Data were analyzed using One-way ANOVA followed by Tukey's post hoc multiple comparisons test for comparative analysis among the groups. Values for $p \leq 0•05$ were considered statistically significant[20].

2.11. Scaling of histological findings:

Grading of histological and immunohistochemical findings was performed using the data obtained from the morphometric analysis and histological examination.

RESULTS

3.1. Body weight:

A significant decrease in the body weight was detected in group II and group III as compared to normal rats. The body weight in group IV was significantly elevated as compared to the group II rats. There was no significant reduction in mean body weight between group IV and group I and between group IV with group III (Table 1).

Table 1: Statistical analysis of Initial body weight (g) and Final body weight (g) using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
Initial body weight (g)	261.8 ± 10.6	255.6 ± 12.4	255.9 ± 12.4	260.7 ± 11.3	0.74	> 0.05
Final body weight (g)	363.8 ± 39.4	287.1[a] ± 31.2	316.8[a] ± 30.8	344.5[b] ± 19.4	11.61	< 0.05 *

- Data represent mean ± SD
a: significant from group I
b: significant from group II
*: significant one-way ANOVA test

3.2. Blood glucose level:

Blood glucose level was highly elevated in group II and group III in comparison with group I. It decreased in group III and group IV in comparison with group II. There was significant difference between group III and group IV and no significant difference between group I and group IV (Table 2).

Table 2: Statistical analysis of blood glucose levels using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
Fasting Blood glucose (mg/dl)	92.2 ± 6	274.3^{a} ± 11	139.4^{ab} ± 27	109.7^{bc} ± 27.3	149.4	< 0.001*

- Data represent mean ± SD
a: significant from group I
b: significant from group II
c: significant from group III
**: highly significant one-way ANOVA test

3.3. Serum CRP:

Serum CRP of group II and group III was significantly higher than that of group I. Serum CRP of group III and group IV was significantly higher than that of group II. Serum CRP of group IV was significantly lower than that of group III. There was a significant difference between group IV and group I.

Table 3: Statistical analysis of serum CRP using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
CRP (pg/ml)	1.2 ± 0.12	5.3^{a} ± 1.04	2.9^{ab} ± 1.1	1.9^{bc} ± 0.4	51.03	< 0.001**

- Data represent mean ± SD
a: Significant from group I
b: Significant from group II
c: Significant from group III
**: Highly significant one-way ANOVA test

3.4. Tissue MDA and SOD activity:

Tissue MDA was significantly elevated in group II when compared to the control group. In group III, its level was markedly decreased, but still higher than that of the control group. Group IV showed a significant decline in MDA level from the level of group II. There was no significant difference between group III and group IV.

Tissue SOD activity in group II was significantly lower than that of the control rats and that of group III and group IV. The SOD activity was significantly increased in group III and group IV, but the increase was higher in the group IV where the level was nearly like that of the control group. There was no significant difference between group III and group IV.

Table 4: Statistical analysis of tissue MDA and SOD activity using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
MDA (mmol/g protein)	0.69 ± 0.1	4.4^{a} ± 1.14	1.51^{ab} ± 0.35	0.89^{b} ± 0.1336	81.44	< 0.001**
SOD activity (U/g protein)	912.22 ± 66.19	566.12^{a} ± 73.9	794.24^{ab} ± 99.76	884.24^{b} ± 91.87	34.92	< 0.001**

- Data represent mean ± SD
a: Significant from group I
b: Significant from group II
**: Highly significant one-way ANOVA test

3.5. Histological results:

H&E stained sections of group I showed that the skeletal muscle fibers with acidophilic cytoplasm and peripheral pale nuclei (Figure 1 A).Group II showed a decreased diameter of muscle fibers, fatty degeneration, separation of muscle fibers, and increased collagen deposition. Aggregation of nuclei and separation were also observed (Figure 1 B and C). In group III, there was aggregation of nuclei and separation of muscle fibers. Some nuclei were pale, while others were apoptotic (Figure 1 D). In group IV, there was a partial restoration of the normal histological structure. Aggregation of nuclei was evident. Some nuclei were pale, while others were apoptotic (Figure 1 E). The diameter of skeletal muscle fibers was measured in micrometer in all groups and statistically analyzed (Table 5, Figure 1 F).

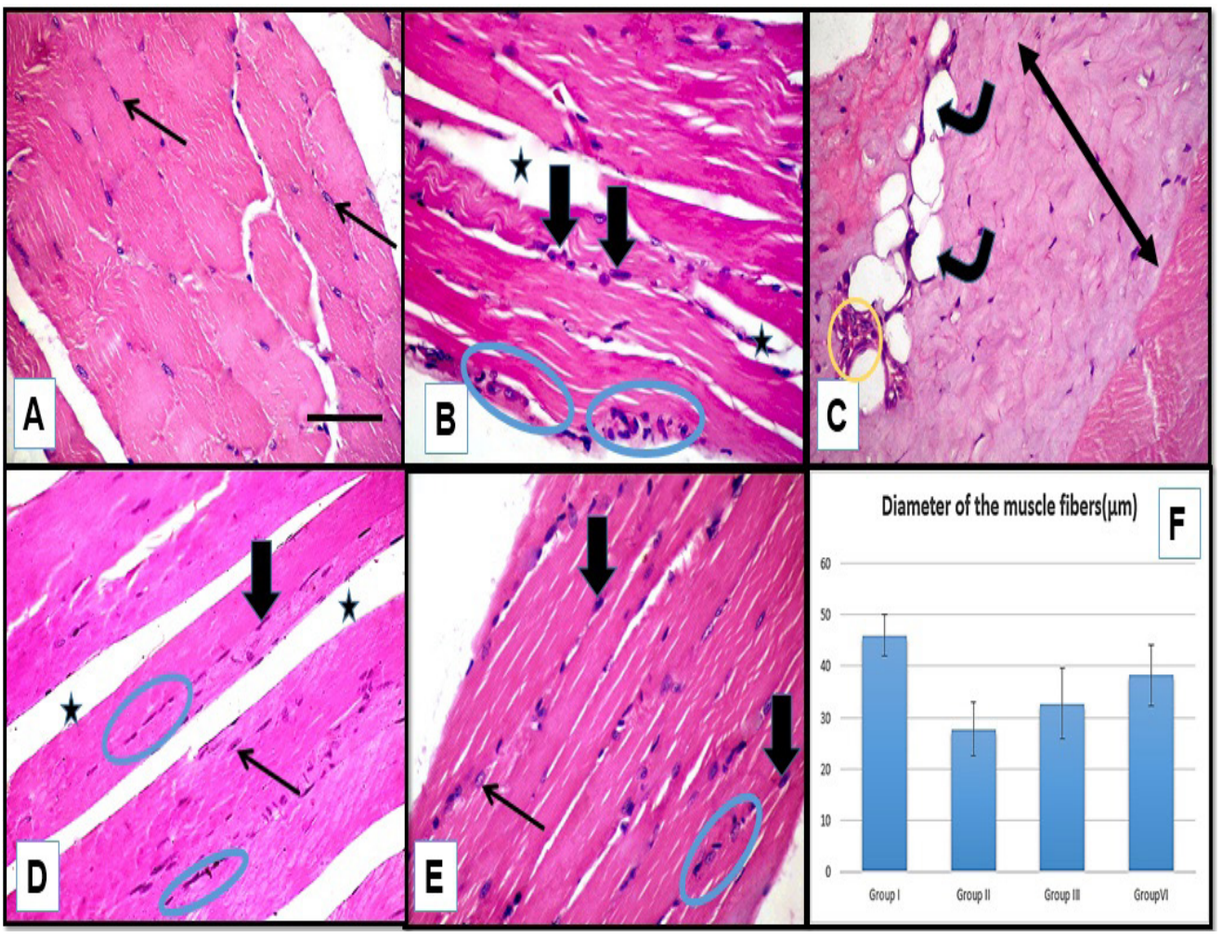

Figure 1: H&E-stained sections from skeletal muscles **A:** Group I. B, **C:** Group II. **D:** Group III **E:** Group. IV: (Thin arrow: peripheral pale nuclei, Thick arrow: pyknotic nuclei, Blue oval: aggregation of nuclei, Star: space between fibers, Curved arrow: fatty infiltration, Yellow circle: inflammatory cells, Double-ended arrow: collagen deposition). **F:** Bar chart of diameter of skeletal muscle [H&E X 400, scale bar = 50 µm].

Table 5: Statistical analysis of the diameter of skeletal muscle fibers (µm) using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
Diameter of skeletal muscle fibers (µm)	45.96 ± 4.03	27.77^{a} ± 5.29	32.74^{a} ± 6.83	38.25^{ab} ± 5.95	19.26	< 0.001**

- Data represent mean ± SD.
a: Significant from group I.
b: Significant from group II.
**: Highly significant one-way ANOVA test.

Masson trichrome stained sections of the control group revealed few collagen fibers between the muscle fibers (Figure 2 A).While in group II, increased deposition of collagen fibers were evident (Figure 2 B). A moderate amount of collagen fibers between the muscle fibers were detected in group III (Figure 2 C). Few collagen fibers were apparent in group IV (Figure 2 D). The area percentage of collagen fibers was measured in all groups and statistically analyzed (Table 6, Figure 2 E).

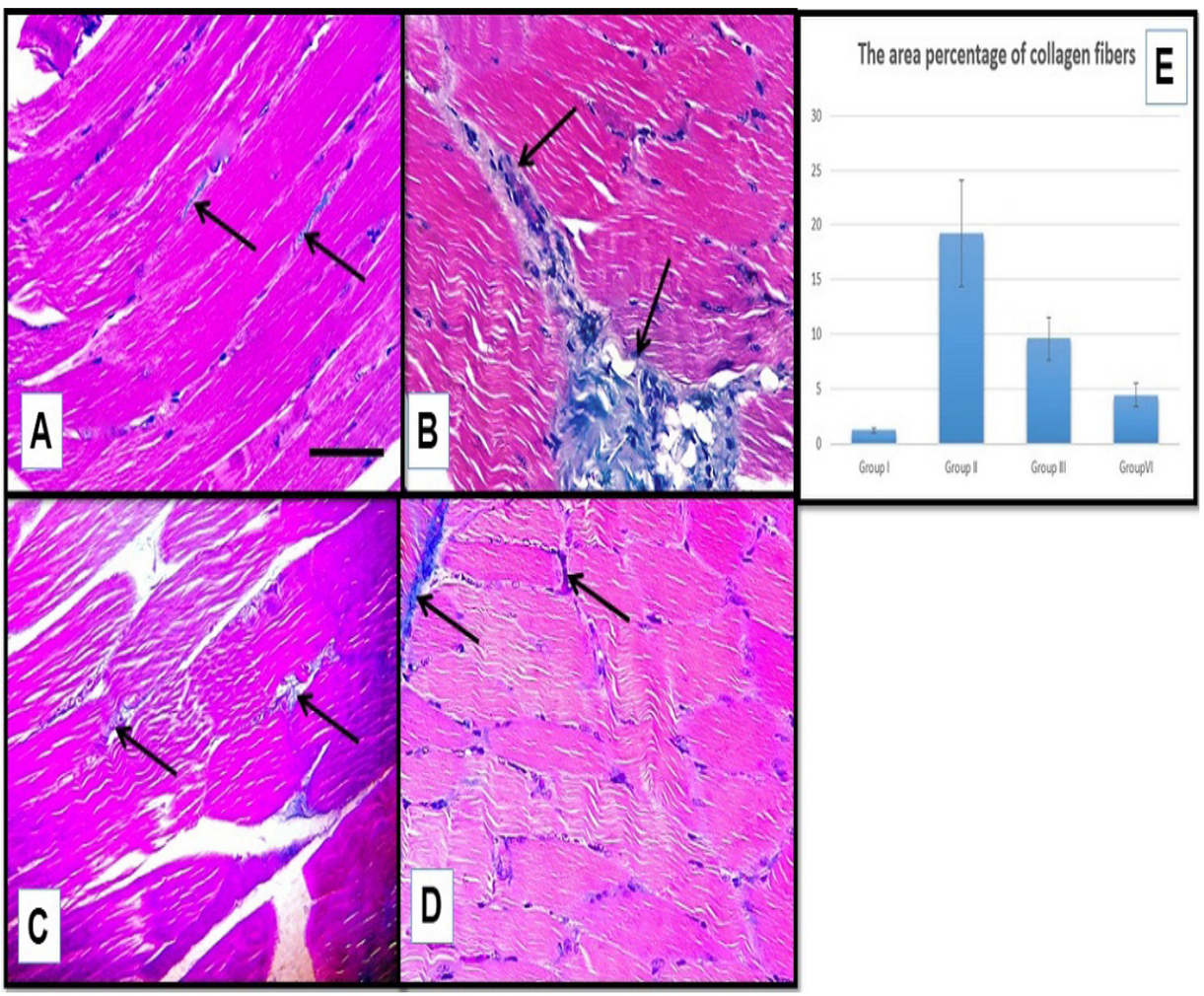

Figure 2: Masson trichrome stained sections from skeletal muscles, **A:** Group I showing few collagen fibers between the muscle fibers (Thin arrows). **B:** Group II showing increased deposition of collagen fibers between the muscle fibers (Thin arrows). **C:** Group III showing a moderate amount of collagen fibers between the muscle fibers (Thin arrows). **D:** Group VI showing few collagen fibers between the muscle fibers (Thin arrows). [Masson trichrome X 400, scale bar = 50 µm] **E:** Bar chart for area percentage of collagen fibers: collagen fibers between the muscle fibers).

Table 6: Statistical analysis of the area percentage of collagen fibers using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
The area percentage of collagen fibers	1.2 ± 0.2	19.25a ± 4.84	9.6 ab ± 2	4.46abc ± 1.05	86.82	< 0.001**

- Data represent mean ± SD.
a: Significant from group I.
b: Significant from group II.
c: Significant from group III.
**: Highly significant one-way ANOVA test.

Examination of caspase-3 immunostained sections revealed very weak caspase-3 immunoreaction in the cytoplasm of skeletal muscle fibers in group I (Figure 3 A). A strong positive immunoreaction for caspase-3 was detected in group II (Figure 3 B). A moderate immunoreaction for caspase-3 was detected in group III (Figure 3 C) and a weak reaction was detected in group IV (Figure 3 D). The area percentage of the immune reaction to caspase 3 was measured in all groups and statistically analyzed (Table 7, Figure 3 E).

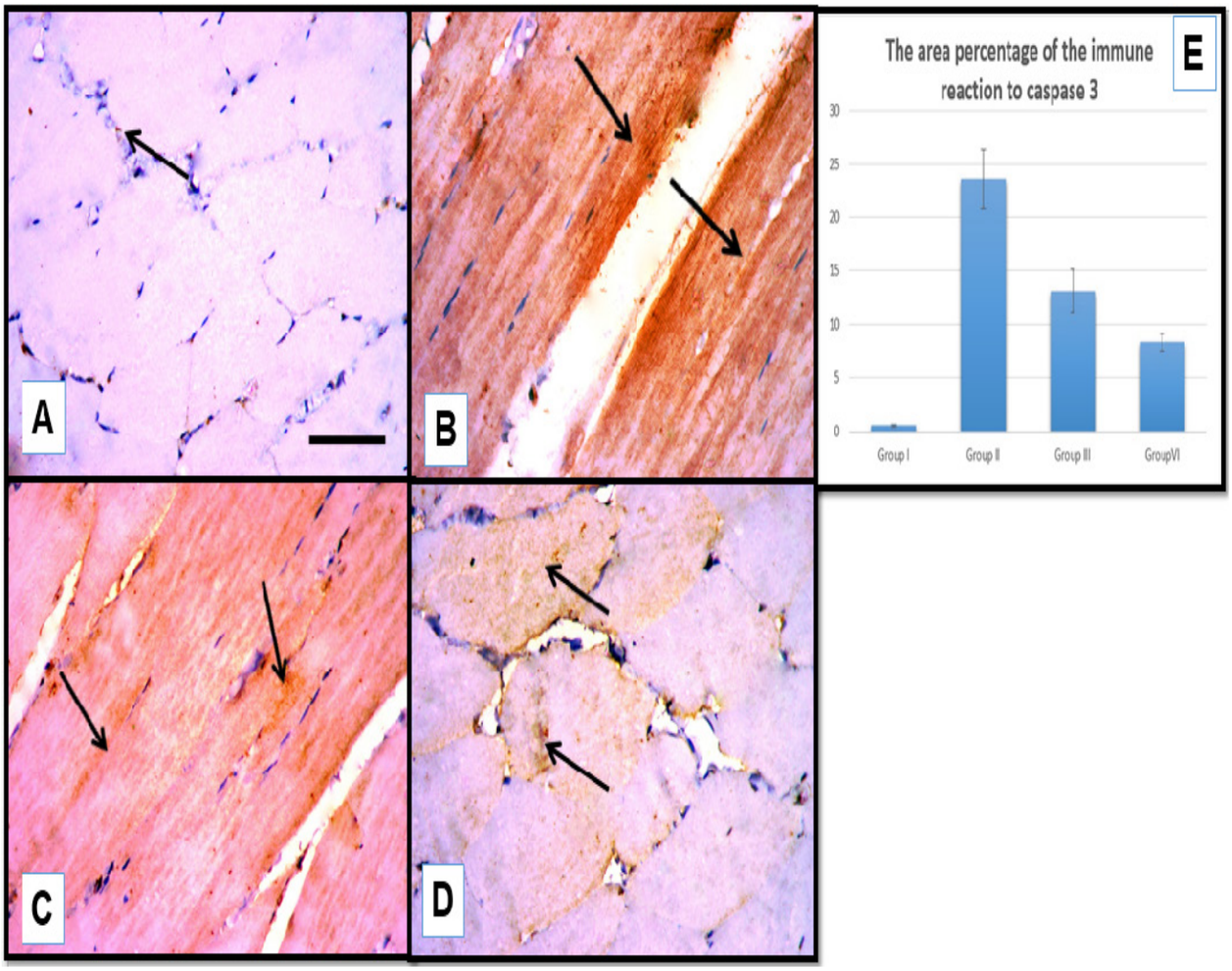

Figure 3: Caspase-3 stained sections from skeletal muscles, **A:** Group I showing a minimal caspase-3 immunoreaction (Thin arrows) in the cytoplasm of skeletal muscle fibers. **B:** Group II showing a strong positive immunoreaction for caspase-3 (Thin arrows) **C:** Group III showing A moderate immunoreaction for caspase-3 (Thin arrows)**. D:** Group IV showing a weak immunoreaction for caspase-3 [Immune reaction to caspase 3X 400, scale bar = 50µm]. E: Bar chart for area percentage of immune reaction.

Table 7: Statistical analysis of the area percentage of the immune reaction to caspase 3 using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
The area percentage of the immune reaction to caspase 3	0.57 ± 0.13	23.59^{a} ± 2.78	13.13^{ab} ± 2	8.36^{abc} ± 0.83	296.7	< 0.001**

- Data represent mean ± SD.
a: Significant from group I.
b: Significant from group II.
c: Significant from group III.
**: Highly significant one-way ANOVA test.

Sections stained with iNOS staining showed a very weak reaction in the control group (Figure 4 A). Group II sections showed a strong positive immunoreaction (Figure 4 B). A moderate immunoreaction for iNOS was detected in group III (Figure 4 C) and a very weak reaction was detected in group IV (Figure 4 D). The area percentage of the immune reaction to iNOS was measured in all groups and statistically analyzed (Table 8, Figure 4 E).

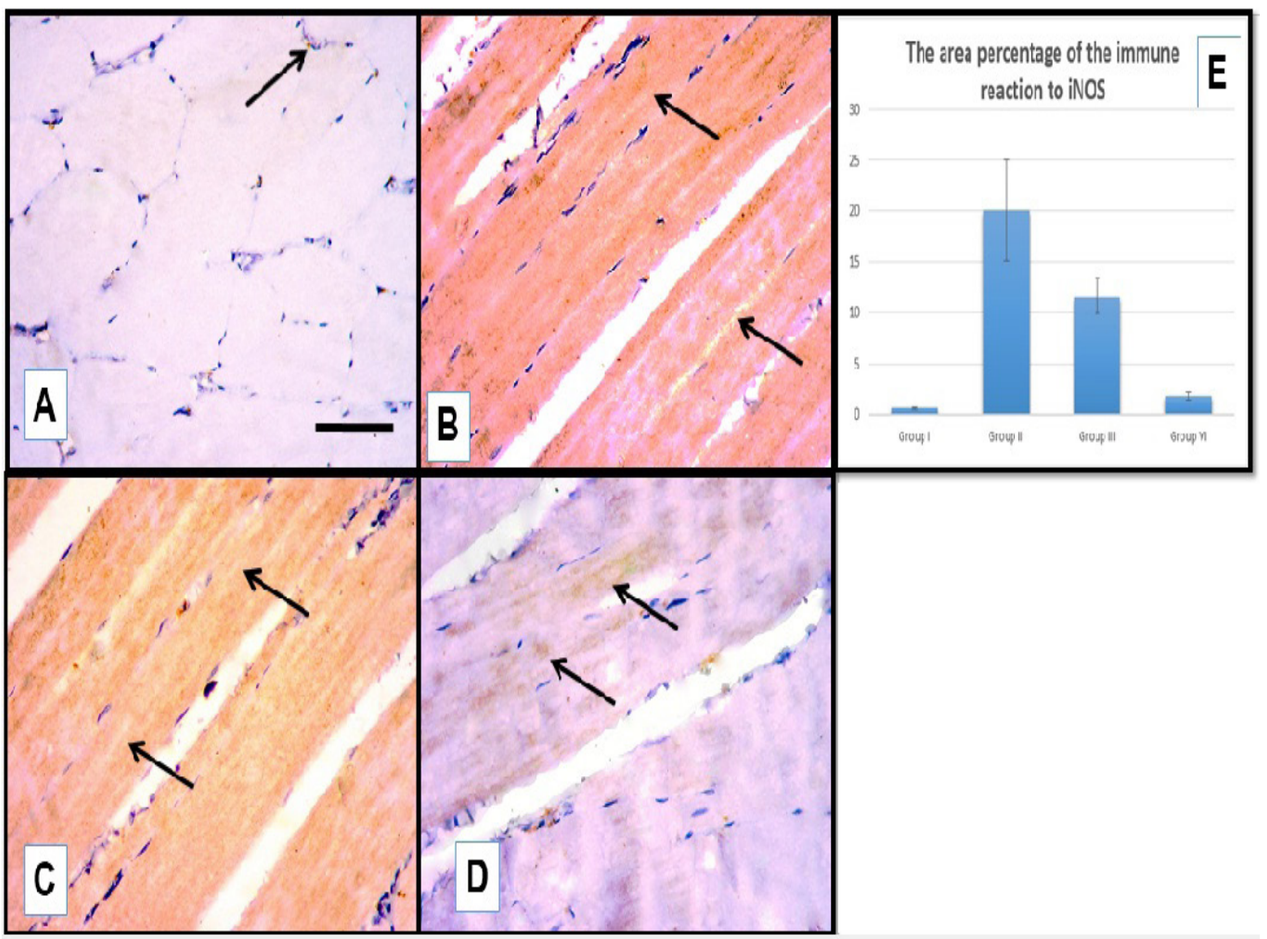

Figure 4: iNOS immunostained sections from skeletal muscles, A: Group I showing a very weak reaction to iNOS (Thin arrows) in the cytoplasm of skeletal muscle fibers. B: Group II showing a strong positive immunoreaction to iNOS (Thin arrows). C: Group III showing a moderate immunoreaction (Thin arrows) in the cytoplasm of skeletal muscle fibers. D: Group IV showing a very weak reaction to iNOS (Thin arrows). [Immune reaction to iNOS X 400, scale bar = 50μm]. E: Bar chart for area percentage of immune reaction.

Table 8: Statistical analysis of the area percentage of the immune reaction to iNOS using one-way ANOVA test:

Group / Parameter	Group I	Group II	Group III	Group IV	F	P
The area percentage of the immune reaction to iNOS	0.55 ± 0.12	20.11^{a} ± 4.98	11.65^{ab} ± 1.71	1.9^{bc} ± 0.4	120.3	< 0.001**

- Data represent mean ± SD.
a: Significant from group I.
b: Significant from group II.
**: Highly significant one-way ANOVA test.

3.6. Scaling of Histological Findings:

Table 7: Scaling is based on data obtained from histological examination and morphometric analysis.

	Group I	Group II	Group III	Group IV
Apoptosis	-	+++	++	+
Collagen deposition	-	+++	++	+
Aggregation of nuclei	-	+++	++	+
Diameter of muscle fiber	-	↓↓	↓	↓
Fatty Degeneration	-	+++	-	-
inflammation	-	+++	++	+

DISCUSSION

Diabetic myopathy is a complication of type 2 DM. Researches showed that older chronic type 2 DM patients suffer from more loss of muscle strength and mass than their healthy peers[21]. In this study, the effect of combining vitamin C with metformin versus metformin alone in treating diabetic myopathy of the adult male albino rat was investigated.

Streptozotocin (STZ) induces DM through inhibition of the insulin secretion fragmentation of DNA, and induction of β-cell death by inhibiting the enzyme O-GlcNAc-selective N-acetyl-β-d-glucosaminidase (O-GlcNAcase)[22, 23]. The underlying mechanisms of diabetic myopathy include oxidative stress, abnormal function of satellite cells, polyol pathway flux via aldose reductase activity, protein glycosylation, and abnormal Ca2+ signaling [4, 21].

Diabetic rats showed significant weight loss due to the accelerated catabolism of proteins and wasting of muscle. This agreed with Haidari *et al.*[24], who detected a marked decrease in the body weight of STZ-induced diabetic rats.

The imbalance between the generation of the reactive oxygen species (ROS) and the ability of the body to get rid of the reactive substances results in oxidative stress. Oxidative stress induces lipid peroxidation and the formation of malondialdehyde (MDA).Therefore, measuring MDA levels in tissues is used as a marker for lipid peroxidation for in vitro and in vivo studies. Superoxide dismutase (SOD) is a part of the antioxidant system in body tissues. It removes the superoxide radicles by converting them to hydrogen peroxide (H2O2), which is then converted to water and oxygen by catalases[25].

Oxidative stress in type 2 DM causes lipid peroxidation with subsequent production of malondialdehyde (MDA) that induces defects in the cell membrane ion transportation and enzyme activity. The histological changes observed in group II are due to the lipid peroxidation. Oxidative stress also stimulates inflammation in the skeletal muscles; this stimulates the NF-KB pathway leading to the formation of iNOS. Therefore, the immune reaction to iNOS was increased in group II. The inflammatory state in group II also increased serum CRP and caused the infiltration of muscle tissue by inflammatory cells[26 - 29]. These results agreed with Hameed et al.[30] who concluded that type 2 DM results in inflammation and increased level of CRP and increased expression of iNOS.

The increased level of muscle MDA and the low SOD activity was also detected in group II; these results agreed with Yin *et al.*[31] who detected a high level of MDA and a lowered level of SOD activity in the pancreas of type 2 diabetic rats. Fatty degeneration of muscle fibers and the infiltration by inflammatory cells occurred in group II; this agrees with Hashemi *et al.*[32] who detected similar findings in the skeletal muscles of the diabetic rats.

Increased collagen deposition was apparent in group II. This is explained by the diminished activity of AMP activated protein kinase (AMPK), a cellular bioenergetic sensor and metabolic regulator in diabetes mellitus which leads to accelerated collagen deposition in different organs[33].This agrees with Zhou *et al.*[34] who detected increased fibrosis in the cardiac muscle of diabetic rats. Immune reaction to caspase 3 was highly elevated in group II due to the accelerated apoptosis which is considered as one of the major mechanisms of cell death in diabetic myopathy. Diabetes induces oxidative stress that leads to the destruction of the mitochondrial membrane resulting in the translocation of BAX from the cytosol to mitochondria and the release the cytochrome c[35].

In group III, body weight showed a significant decrease compared to the control group and a non-significant change with diabetic group. This agreed with other authors who detected that there was no significant change in the body weight of diabetic group with metformin when compared with the diabetic group[36]. Metformin decreased the blood glucose levels significantly in comparison to group II. This agreed with Pournaghi *et al.*[37] who detected a marked decrease in the fasting blood glucose level in streptozotocin- induced diabetic rats.

There was a significant decrease in MDA level and increase in SOD activity. This was attributed to the metformin effect in reducing the oxidative stress and the mitochondrial production of ROS[38]. In addition, Chakraborty *et al.*[39] proved that metformin was effective in restoring the antioxidant status in a clinical study. The reduction of the oxidative stress resulted in regression of the histological alterations induced by diabetes. The diameter of skeletal muscle was increased in group III in comparison to that of group II; this agreed with Hassan *et al.*[40] who detected that metformin partially repaired the skeletal muscles of rats received high-fat diet.

Metformin decreased fibrosis significantly. This agrees with Rangarajan *et al.*[41] who reported that metformin reversed the lung fibrosis induced by bleomycin by activating AMPK enzyme. It decreased immune reaction to caspase3; this agreed with other investigators who detected that metformin-decreased apoptosis in testes of diabetic rats[42]. Serum CRP, immune reaction to iNOS, and inflammatory cells were diminished in group III when compared with group II. This agreed with Oliveira *et al.*[43] that reported that metformin decreased inflammation in the diabetic mice.

Body weight in group IV showed a significant increase compared to the diabetic group. This disagreed with Antunes *et al.*[44] who concluded that different doses of vitamin C didn't restore the body weight of rats treated with cisplatin. Vitamin C lowered blood glucose levels in group IV. These results are similar to other authors who detected that vitamin C lowers blood glucose levels in patients with type 2 DM[45]. Vitamin C eliminates the free radicals. It up-regulates the endogenous antioxidant defenses and guards against the DNA damage[10]. Therefore, in group IV, MDA level decreased and SOD was increased. Vitamin C partially restored the normal histological structure of the skeletal muscles, and decreased fibrosis and apoptosis significantly. This agreed with Abdo *et al.*[46] who reported that vitamin C induced similar effects in the adrenal glands of MSG-treated rats. Serum CRP, immune reaction to iNOS, and inflammatory cells were further reduced in group IV when compared with group II. This agreed with other investigators, who found that vitamin C decreased serum CRP and other inflammatory mediators in diabetic obese patients[47].

CONCLUSION AND RECOMMENDATION

Combing vitamin C with metformin significantly reduced the blood glucose and ameliorated the oxidative stress, inflammation, and histopathological changes induced by type 2 DM in the skeletal muscle of rats. So, it is recommended to perform clinical studies about the value of the intake of vitamin C for diabetic patients on metformin therapy.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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الملخص العربى

دراسة نسيجية وهستوكيميائية مناعية عن تأثير مزيج فيتامين ج والميتفورمين مقابل الميتفورمين وحده في علاج الاعتلال العضلي السكري في الجرذان

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الخلفية: الميتفورمين هو عقار الخط الأول للنوع الثاني من مرض السكري وفيتامين سي هو فيتامين قابل للذوبان في الماء وله خصائص مضادة للأكسدة ومضادة للالتهابات لذلك، تم في هذا البحث دراسة تأثير الجمع بين الميتفورمين وفيتامين ج مقابل الميتفورمين وحده في علاج الاعتلال العضلي السكري.

المواد والطرق: تم استخدام أربعين فأر مقسمة إلى 4 مجموعات. المجموعة الضابطة (المجموعة الأولى)، مجموعة مرضى السكر (المجموعة الثانية)، مجموعة الميتفورمين (المجموعة الثالثة)، ومجموعة فيتامين ج ومجموعة الميتفورمين (المجموعة الرابعة) قيست اوزان الجرذان قبل وبعد انتهاء التجربة. تم قياس مستوى المالوندهايد ونشاط سوبر أوكسيد ميوتاز في الأنسجة. تم قياس مستويات السكر في الدم والبروتين التفاعلي C في الدم (CRP). تم صبغ مقاطع العضلات الهيكلية بـالهيموكسيلين والإيوسين الماسون ثلاثية الألوان ورد الفعل المناعي للكاسباز 3 وال iNOS ، تم إجراء التحليلات المورفومترية والإحصائية للنتائج.

النتائج: قل وزن الجسم في المجموعة الثانية والثالثة بينما كان وزن المجموعة الرابعة مقارب للمجموعة الظابطة. ازدادت مستويات السكر في الدم في المجموعة الثانية. ثم انخفضت في المجموعة الثالثة والمجموعة الرابعة. وزاد مستوى المالوندهايد في المجموعة الثانية، ثم انخفض في كل من المجموعة الثالثة والمجموعة الرابعة، وانخفض نشاط السوبر أوكسيد ميوتاز في المجموعة الثانية، ثم زاد في المجموعة الثالثة والمجموعة الرابعة نحو المستوى الطبيعي، زاد ترسيب الكولاجين والتفاعل المناعي تجاه الكاسباس 3 مرتفعين المجموعة الثانية، والمجموعة الثالثة والمجموعة الرابعة كما ارتفع رد الفعل المناعي لـ iNOS في المجموعة الثانية والمجموعة الثالثة، وكان تقريباً مستواه في المجموعة الرابعة مقارب للمجموعة الأولى.

الخلاصة: أظهر الجمع بين فيتامين ج والميتفورمين نتائج أفضل في علاج الاعتلال العضلي السكري من الميتفورمين وحده.