Arginine supplementation prevent diabetic mellitus complications on myopathy and visceral organs (liver & pancreas) in experimental model.

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Abstract

The study was designed to investigate the role of arginine on myopathy and some visceral organs like liver and pancreas in diabetic female rats at different period (15, 30, 45) days post diabetic induction.

In this study female rats (Rattus norvegicus) were used, total number (30) rats were divided into (non-diabetic and diabetic) groups, first group with (6) females as control group and diabetic group with (24) rats, this group subdivided into two groups (12) rats on each one, both of them was injected intraperitoneally with single dose about (60 mg/kg) B/W from streptozotocin (STZ) with (12) rats at each period (15, 30, 45) days post (DM) induction one of this two subgroup were given only tap water but the second subgroup (diabetic and treated with arginine) were given L-arginine dissolved in the drinking water as (10mg/L) after (3) days post (STZ) induction.

At (15) day post induction the skeletal muscles revealed to atrophied muscle fibers, infarction, irregular, crowded myonuclei, infiltration of inflammatory cells, tapers endings. Moreover at (30) days sever atrophied muscle fibers, myonuclei was detached from their normal location, thick, dark sarcolemma with zigzag shape, while post (45) day sever alterations included filamentous muscle fibers, some completely destructed, wavy fibers, bifurcated and other rounded or circular muscle fibers. The study determined the effect of arginine on diabetic skeletal muscles, post (15) days of induction, the muscle fibers more regular, clear striation, peripheral myonuclei and parallel muscle fibers. post (30) days, normal muscle fibers, number of satellite cells, normal capillaries, regenerated myotubes. At (45) days the muscle fibers with normal diameter, more intense, less inflammation, normal muscle spindles, less congested blood vessels and normal blood walls thickness.

Liver sections related to diabetes group showed post (15) days vacuolated hepatocytes with pyknotic nuclei, dilated sinusoids, increased in kupffer cells, mild fatty changes, bile ducts hyperplasia. Post (30) days sever
congestion, necrotic foci, hydropic changes, cloudy hepatocytes, swelling with pyknotic nuclei. At (45) days more severity included necrosis, kupffer cells hyperplasia, sever fibrosis, fatty changes. Role of arginine was determined and its effect on liver, after (15) day normal hepatocytes, more regular hepatic plates, mild inflammation, normal periportal area, post (30) days remarkable regeneration, foci of kupffer cells accumulated, proliferation of bile ductules and mild congestion, at (45) days liver structure resemble the control, most hepatocytes was binucleat, arrange in hepatic cords and normal sinusoid.

Harmful effect of (DM) on pancreatic tissue which showed after (15) day, shrinkage of Langerhans islets, atrophied pancreatic acini, destruction and necrosis of beta cells, reduced number and size of islets, changes more severity at (30,45) days, revealed to atrophy stroma replaced by fibrous and adipose connective tissue substance, vacuolated and degenerated islets. Whereas pancreas from diabetic rats treated with arginine after (15) day the changes less obvious, more numerous islet of Lankherhanse, regular acini and at (30,45) day normal pancreatic tissue, most islets with normal boundaries, organized pancreatic acini and the protective role on pancreas was noticed.

(DM) caused variable changes in glycogen content in both skeletal muscles and liver at each period (15, 30, 45) days. Moreover the results determined the role of arginine on accumulation and stored of glycogen content. The liver from diabetic rats showed the same results and there was mild reduction to moderate and then sever reduction in glycogen content at each period (15,30,45) days respectively whereas restored in glycogen content recorded on rats treated with arginine.

The study evaluated changes in cholesterol and Triglycerides (TG) level and the results showed a significant increase at (P<0.05) in their level in all diabetic rats compared to control while significant decrease at (P<0.05) in level of cholesterol and (TG) in rats with (DM) and treated with arginine. Moreover (HDL) showed a significant decrease at (P<0.05) of diabetic rats and there was increase in its concentration in all diabetes rats treated with arginine. Also insulin growth factor like-1 (IGF-1) in all rats related to control and treated group was estimated, the data clarified a significant decrease at (P<0.05) within diabetic group.
compared to control, while significant increase in rats related to treated group compared to diabetic group.

1- Introduction

Several pathogenic processes are implicated in the development of diabetes, serious hyperglycemia was detected under conditions of severe infective, traumatic circulatory or other stress may be transitory and should not in itself be considered as diagnostic of diabetes, there are many reports with (DM) effects on cells of muscles, also DM causes some metabolic disorder which may be leading to chronic irreversible harm to vital organs and systems (Stapleton ,2000). This disease was causes metabolic disorder in proteins carbohydrates and fats due to functional lack of insulin, which can be described by rising of blood glucose, this causes abnormal metabolism leading to hyperglycemia, resulting in an elevation risk of complications in kidney and neuropathy, it was described a condition characterized by chronic hyperglycaemia (Nathan, 2009).

(DM) is often called ‘The silent killer’, because it causes severe complications without acute symptoms and can affect many of main organs in the body, lead to serious complications which are categorized into acute, sub-acute and chronic (Gopi et al., 2012). (DM) was elevated concentrations of blood glucose which either due to absence of insulin-producing pancreatic B-cells as in (T1DM) or through absence of insulin responsiveness in its target tissues such as skeletal muscles and adipose tissue in (T2DM) (Schwarz et al.,2009). When liver tissue damage as complication of (DM), cellular enzymes may liberated into the serum and the increased of certain enzymes is often linked with damage to specified and experimentally elevation of these enzymes tissue or organs associated with liver dysfunction and injury (Bamidele et al., 2014).

In T2DM, two major pathological defects in it was impaired insulin excretion through a dysfunction of the pancreatic β-cell, and reduced insulin action through insulin resistance, T2DM has a major genetic association than T1DM (Ozougwu et al.,2013). Growth hormones (GH) increases insulin resistance by stimulating lipolysis and hepatic glucose production, while IGF-I, on the other hand, stimulates glucose uptake in muscles and down regulates the GH secretion through
negative feedback. So improving insulin sensitivity (Kaplan & Cohen, 2007). Samples from skeletal muscles related to TIDM showed structural and physiological changes that manifest into functional abnormalities and loss of force production (Cohen et al., 2015). Increasing arginine supply to diabetic rats ameliorate vascular reactivity, decrease blood pressure, normalized lipid peroxidation, and decrease concentration of malondialdehyde (MDA) (Ozcelikay et al., 2000).

**Aims of the study:** Since (DM) was metabolic disorder and its most complications were affect the visceral organs (liver & pancreas) and skeletal muscles, hence the study was designed to evaluate the histopathological changes of pancreatic, hepatic tissue, and muscle fibers in all diabetic rats at each period (15, 30, 45) days post (DM) induction and determined some biochemical parameters like (liver enzymes, lipids profile, and insulin growth factors), moreover to investigating the role of arginine in the pathogenesis of (DM) as protective agent.

**2- Material and Methods**

**2-1 Experimental animals**

Thirty healthy adult virgin females Wistar albino rats (Rattus norvegicus) age (10-12) weeks and the average weight (225±25) gm, which are breed at the animal house of the Science college, Al-Basarah University. The animals are housed under controlled standard conditions in a temperature (20-23 °C), controlled room on a (12:12) Light: Dark cycle, they are randomly isolated in plastic cages with hygienic bed and were fed on standard laboratory food. The animals divided into two groups (non-diabetic and diabetic group) with about (24) female in diabetic group and (6) female nondiabetic group (control group). The diabetic group are subdivided into two groups (12) of each one, both of them are single injected intraperitoneally with single dose of streptozotocin (STZ) (60 mg/kg of body weight), with mean (10) rats females for each period (15, 30, and 45) days post induction of DM. The first subdivided group (diabetic group) were given tap water but the second subdivided group (diabetic treated with
arginine) were given tap water containing L-arginine nitro-L-arginine methyl ester dissolved in the drinking water at 10 mg/L, 3 days after (STZ) injection (Ki chul choi et al., 1999).

2-2 Sacrificed the experimental rats
Fasting overnight experimental rats from each group (treated and control) were randomly sacrificed after being anaesthetized with overdose of chloroform and Sacrificed on (15 - 30 - 45) day post diabetic induction, then samples were collected included blood and tissues included fore and hind limbs skeletal muscle (triceps, gastrocnemius, biceps) in addition to pancreas and liver, all the samples collected as follow.

2-3 Collection of blood
After anesthesia about (5) ml of whole blood was collected from each animal by cardiac puncture and transferred to covered tubes, then, allowed the blood to clot by leaving it undisturbed at room temperature for (15-30) minutes, after that the clot removed by centrifuging at (1000-2000) rpm for 15 minutes in centrifuge, then the serum was separated and immediately transfer to clean polypropylene tube by a pipette, and stored at (-20) C° or lower until used for biochemical assay.

2-4 Tissues samples
Rats from all groups were anesthetized and Triceps, Biceps brachii skeletal muscles from fore limbs and Gastrocnemius muscles of hind limbs were dissected at each period (15, 30, 45) days, also specimens from pancreas and liver were excised, then all samples fixed with suitable fixative for histopathological study.

3- Results
3-1 Histological study
Liver sections related to control rats showed hepatic lobules composed of polygonal, regular hepatocytes with rounded vesicular
nuclei and normal cytoplasm, these cells formed hepatic cords and the liver sinusoids which found between each regular cords, some of kupffer cells distributed within the sinusoids, the hepatocyte with only single nucleus and others appeared binucleated arranged around the central vein (fig 1).

Microscopic observations in liver sections from diabetes rats at(15) days post STZ-induced showed distortion in normal architecture of hepatocytes around the central vein, focal necrosis of hepatocytes, degeneration, vacuolated cytoplasm with pyknotic nuclei, mild infiltration of inflammatory cells, accumulation of blood cells and inflammatory cells within the portal duct, hyperplasia of bile duct lining epithelial layer, and degeneration of endothelial lining layer of portal vein, edematous with mild haemorrhage within the vein lumen and detachment of surrounding hepatocytes (fig 2).

Results on liver sections at (30) days of diabetes induced referred to more, progressive alterations in liver tissue structure, sever congestion on portal ducts and portal vein, necrotic foci, hydropic changes, most hepatocytes cloudy, swelling with nuclei varied from pyknotic to karyolytic nuclei, extensive necrosis with increased in kupffer cells and mononuclear cells infiltration, moreover fibrosis around the portal duct and portal triad, also the portal triad reffered to thickness of hepatic artery with haemorrhage, loss of normal tissue stroma and thrombosis formed around the central vein (fig 3).

Sections on liver post (45) days of diabetes showed extensive hepatocytes degeneration with binucleated, pyknosis, necrosis regions, kupffer cells hyperplasia, inflammation, sever fibrosis around portal duct and portal triad and haemorrhage within the sinusoids. Moreover the hepatocytes loss their arrangement radially around the central vein, congested blood vessels with thrombosis formation and irregular dilated sinusoids hyperplasia of bile duct, fatty changes, cytoplasm vacuolation, changes may be shown as steatohepatitis, coaculative necrosis of the hepatocytes and lacerations extend to the all structures of liver (fig 4).

Histopathological observations on liver sections after (15) days post diabetes induction treated with arginine showed regeneration area, the hepatocytes appeared as sheath or plates within liver stroma, still there
was inflammatory cells, mild congestion of blood vessels, sinusoids and normal distribution of kupffer cells, the central vein with normal lining endothelial layer, and mild vacuoles shown peripherally. Normal periportal area, the cellular arrangement around the central vein, mild necrosis, the liver lobules with strains of hepatocytes compared to that with diabetic rat livers, the cell boundaries more clear, some hepatocytes was binucleate although the fatty changes was noticed (fig 5). After (30) days the liver sections showed with underwent regeneration, but inflammatory cells and kupffer cells proliferation were also seen, some hepatocytes are slightly vacuolated, the hepatic cords more regularity and separated with normal sinusoids, foci of kupffer cells accumulated around the portal triads, deposition of collagenous fibers was noticed and moderate lymphocytic lymphocytes showed more around the central vein and portal tract, also proliferation of bile ductules and portal veins with mild congestion (fig 6).

Results referred to less changes at (45) days post diabetes induction and treated with arginine, rats livers resemble the architecture of control rats, there was normal hepatocytes, cellular arrangement radially around the central vein, mild leukocytes infiltration on periportal and perisinusoidal regions, hepatocytes was polygonal, normal nuclei less pyknotic, less sinusoids congestion, no necrosis so restoration tissue was clearly observed (fig 7).

Pancreas sections from control rats showed pancreatic acini formed the exocrine portion, rich supplied with blood vessels and consist of closely packed secretory acini, each acini made up of regular cluster of cells have pyramid shape, vesicular nuclei with basally position and apical zymogen granules, multiple, rounded, ovale groups of cells arranged and formed the endocrine portion known as Islets of Langerhans (fig 8).

Histological examination on pancreas sections related to diabetic rats post (15) days of diabetes induction showed reduced number, atrophy, shrinkage of most Langerhans islets, less number of cells within each islets and no distinct boundaries separated these islets from the pancreatic tissue, dilated of interlobular duct, Langerhans cells with eosinophil granules, deposition of fibrous and adipose tissue. Signs of endocrine cells death within atrophic islets regions with congested blood
vessels and collagen fibers deposited around the blood vessels and dilated interlobular ducts (fig 9). While pancreas sections post (30) days of STZ induction showed, irregular pancreatic acini, most cells degenerated, necrosis, congested blood vessels, extravasated RBC from vessels, degenerated most of islets of Langerhans and each islets appeared with irregular outline surface, vacuolated, some islets appeared segmented and most intralobular ducts showed dilation with inflammatory cells infiltration, the fibroid tissue noticed around interlobular ducts with signs of inflammation (fig 10).

Moreover the changes on pancreas sections from diabetes rats at (45) days showed more sever alterations either in pancreatic acini or the islets of Langerhanse, degenerated pancreatic acini, no nuclei obvious, disorganized of the endocrine structure, reduced number of Langerhanse cells which showed vacuolated and degenerated, there was fine fibers separated the cells within the islets and increased thickness of interlobular ducts walls and mild to moderate deposition of fatty tissue (fig 11).

The recent study clarified the role of arginine and results on pancreas sections related to diabetes rats post diabetes induction and treated with arginine at (15) days referred to less abnormal structural changes, the pancreatic tissue appeared nearest similar to the control, the acinar cells pyramid with basal dark nuclei and arranged as lobules, hypertrophied large islets of langerhanse and noticed more expansion increased number of endocrine cells compared to that in diabetic group, some islets showed with vacuolated cells, mild degeneration, deposition of collagen fibers, congested blood vessels surrounded with normal pancreatic tissue (fig 12).

Pancreas sections regarded to diabetic rats and treated with arginine post (30) days showed more regenerated islets of langerhanse with increased in number of normal endocrine cells, largest in size (hyperplasia), more regular, surrounded with organized pancreatic acini and normal blood vessels distributed within the connective tissue septa that separated between pancreas lobules, the sections revealed to normal pancreatic acini with basal nuclei, normal interlobular duct, mild fibrous tissue deposition on each islets (fig 13).
Pancreas tissue at (45) days post arginine treated showed variable changes concluded less degenerative changes in pancreatic acini, recovery of normal tissue, more organized with normal sections of interlobular ducts and more blood vessels with normal thickness wall, the endocrine portion revealed to regular surface, enlargement in size and healthy islets of Langerhans, increased in endocrine cells proliferation, some islets showed with sprouts and there was accumulation of endocrine cells cluster near the, interlobular ducts, other islets appeared with fenestrated capillaries and fibers deposition (fig 14).

The study showed changes with skeletal muscles post(15) days of (DM) induction included atrophied myofibers, infarction, irregular arranged of myonuclei, most fibers have wavy shaped, tapers endings, barely surface, the striation less obvious and dense collagenous fibers (Fig 15).

Observations post (30,45) days of (DM) induction revealed to sever atrophied muscle fibers, myonecrosis fibers, crowded nuclei, extensive necrosis, congested capillaries, nerve fibers loss their myelinated sheath and necrotic foci among the muscle fibers, also the changes more sever at (45) days like heavy infiltration of inflammatory cells, bifurcated and splitting of muscle fibers, extrusion of nuclei from their normal peripheral region and collagen fibers deposit around the intramuscular nerve trunk (Figs 16,17). All results compared with sections from control skeletal muscles showed the normal architecture (Fig 18). Findings on role of arginine on skeletal muscles related to rats with (DM) indicated to the protective action and concluded variable changes post (15,30,45) days, muscle fibers more regular, clear striations, dark sarcolemma and normal vesicular nuclei, at (30) days the muscle fibers supplied with normal motor nerves, condensed myofibrils, number of satellite cells noticed, the changes more developed post (45) days muscle fibers larger, no inflammation, numerous muscle spindles and normal nerve trunk (Figs 19,20,21). The histological observations on liver sections of control rats that stained with (PAS) stain for demonstration the glycogen content showed normal, homogenous distribution of fine glycogen granules within hepatocytes cytoplasm, area around the central vein, basement membrane of each cell and the cells boundaries, all the material stained pink and give (PAS) positive results or magenta stain (fig 22). While liver sections from
diabetes rats after (15) days of STZ injection showed mild reduction in glycogen content within hepatocytes and staining with PAS referred to reduction in glycogen granules accumulation (fig 23). Also changes was clear after (30) days represented to progressive reduction in glycogen content that mild coarse granules accumulated in the liver cells (fig 24). Moreover sections from diabetes rats post (45) days showed severe decreased in glycogen content and referred as pale staining with PAS stain and just fine granules deposit in hepatocytes cytoplasm and around portal area (fig 25).

Findings on liver sections from diabetes rats and treated with arginine showed variable degree of glycogen granules content at different periods of induction, post (15) days there was mild accumulation of glycogen granules in hepatocytes but lower when compare with control sections (fig 26), after (30) day of arginine treatment there was moderate reaction with PAS that indicate to glycogen granules deposite within hepatocytes cytoplasm and some hepatocytes showed strong positive reaction with (PAS) and appeared red to magenta colour specially around the portal vein with stained granules around branches of bile ducts compared to control and diabetes rats (fig 27), while the liver sections from rats treated with arginine on (45)days showed restore liver paranchyma with more amount of coarse glycogen content deposition appeared as granules stained pink to magenta compared to the liver sections of diabetes rats (fig 28).

The study determined the effect of DM on pancreatic (B and α-cells) compared to control so sections from normal rats stained with aldehyde fuchsin showed normal Islets of Langerhans usually circular shape, embedded within pancreas tissue and separated from acini by thin collagenous capsule, the alpha (α-cells) arranged as single layer of cells distributed around the islet periphery, Beta (B-cells) were at the core of islets (fig 29). While pancreas sections related to diabetic rats after (15, 30, 45) days showed variable changes either in exocrine acini or the islets of Langerhans, these alteration became more obvious as the period of diabetic increased, the tissue appeared with degeneration and necrosis of most B-cells and may be completely degenerated, few surviving B-cells in the islet of langerhans may appeared with small, scattered granules at (15,30) days, nuclear shrinkage and pyknosis with
vacuolated cytoplasm were evident at different periods post diabetes in the centre and peripheral region of islet of Langerhans, at (45) days, focal acinar damage, vacuolation and only apoptotic bodies from (B) cells noticed (fig 30,31,32).

Active role of arginine was assessed and pancreas sections stained with aldehyde fuchsin regarded to diabetes rats and treated with arginine after (15) days showed the islets were larger in size and more abundant B-cells located in different regions with more regularity, most islets looked intact except few vacuoles, the damage partially repaired and there was mild collagen deposition and fine clear distinct area between endocrine and exocrine portion, post (30) days the islets of Langerhans appeared expanded with obvious endocrine cells and few vacuolated cells and the sections at (45) days from rat pancreas related to diabetic group and treated with (Arg) showed larger islets of Langerhans, clear proliferative endocrine cells surrounded with normal pancreatic acini (fig 33, 34, 35)

3- Biochemical study

3-1 Evaluation of Insulin like growth factor(1) (IGF-1) level

Results clarified a significant decreased in concentration of (IGF) of diabetic rats at (P<0.05) at all periods (15, 30, 45) days, the value was (2623±20.55, 2074.5±45.64, 1174.75±155.87) pg/ml compared to control (3875±35.35, 3776.4 ±41.4782, 3891.2 ±43.34) pg/ml (table 3-6).

Moreover significant increase at (P<0.05) in the level of (IGF) on rats from diabetic and arginine group at periods (30, 45) days, the mean value was (2963.5±49.99, 3083.25±49.87) pg/ml compared to the diabetes group (2074.5±45.64, 1174.75±155.87) pg/ml, while on (15) day no significant differences was recorded (table 3-6). Data also showed significant difference at (P<0.05) in the mean level of IGF-1 in (diabetic + arginine) group at period (15, 30, 45) day the mean value (2741.5±34.6266, 2963.5±49.99, 3083.25±49.87) pg/ml compared to control group (3875±35.35, 3776.4 ±41.4782, 3891.2 ±43.34) pg/ml (table 3-6).
Table (3-6) :Effect of arginine on Insulin like growth factor 1 (IGF-1) in diabetic rats compared to control at (15,30, 45) days of diabetes induced, values expressed as ( mean ± SD) .

| IGF-1 Concentration (pg/ml) | Day s | Control group Mean ±SD (pg/ml) | Diabetic group Mean ±SD (pg/ml) | Diabetic & Arginine group Mean ±SD (pg/ml) |
|-----------------------------|-------|--------------------------------|---------------------------------|--------------------------------------------|
|                             | 15    | 3875±35.35<sup>c</sup>         | 2623±20.5589<sup>a</sup>        | 2741.5±34.6266<sup>ad</sup>             |
|                             | 30    | 3776.4 ±41.4782<sup>c</sup>    | 2074.5±45.6472<sup>a</sup>      | 2963.5±49.99<sup>bd</sup>              |
|                             | 45    | 3891.2 ±43.34<sup>c</sup>      | 1174.75±155.8747<sup>a</sup>    | 3083.25±49.8757<sup>bd</sup>           |

* Represents a significant difference in comparison between diabetic group and control (P< 0.05)

<sup>ab</sup> The same letter means no significant different between groups while different letter means there was significant different (P<0.05) between diabetic group and (diabetic +arginine) group.

<sup>cd</sup> The same letter means no significant between groups while different letter means there was significant different (P<0.05) between (diabetic +arginine) group and control.
Fig (1) Section in the normal liver showed hepatic cord (-----) separated by smaller sinusoids { }, the hepatocytes (-----) around central vein (-----), some hepatocyte binucleate (-----) and kupffer cells (-----) was obvious. H&E stain (10X).

Fig (2) Section on liver after (15) day of diabetes induction showed congested portal tract (-----) with accumulation of red blood cells (-----) and inflammatory cells (-----) extend through the degenerated hepatocytes (-----) with mild fatty changes (-----). H&E stain (40X).
Fig (3) Section on liver after (30) day of diabetes induction showed extensive necrosis (---). Kupffer cells (---), monocyte infiltration (---), and portal vein (☆) with degenerated lining layer (---). H&E stain (10X).

Fig (4) Section on liver after (45) day of diabetes induction showed hypertrophied hepatocytes with dark stain (→), mild area of fibrosis (☆), dilated septa (---) between hepatic lobules, laceration (---) with inflammatory cells, congested blood vessels (→) and the liver stroma with multiple necrosis foci (---). H&E stain (10X).
Fig (5) Section on liver after (15) day of diabetes induction and treated with arginine showed normal portal tract ( ), mild kupffer cells and inflammatory ( ), some hepatocytes with vacuolated cytoplasm ( ) and mild haemorrhage ( ) within connective tissue surrounded the portal tract, hepatic artery ( ) and hepatic venule ( ). H&E stain (10X).

Fig (6) Section on liver section related to diabetes rat at (30) day of induction and treated with arginine showed dilated central vein ( ), normal regenerated hepatocytes ( ), distribution of kupffer cells ( ) and mild lymphocytes infiltration ( ). H&E stain (10X).
Fig (7) Section on liver section related to diabetes rat at (45) day of induction and treated with arginine showed normal regeneration of hepatic cells (→), normal central vein (→) normal sinusoids (→) and no inflammatory signs. H&E stain (10X).

Fig (8) Transverse section on pancreas from control rats showing normal architecture of rounded islets of Langerhans (→) surrounded with normal pancreatic acini (→) which arranged as lobules (→), normal interlobular ducts (⊙) and normal septa (⊙). H&E stain (10X).
Fig (9) Section on pancreas from diabetes rats after (15) of diabetes induction showed disorganized pancreatic tissue ( ), necrosis ( ), degeneration of some islets of Langerhans ( ), and other appeared with vacuolated, degenerated cells ( ) and dilated interlobular duct ( ). H&E stain (10X).

Fig (10) Section on pancreas from diabetes rats after (30) day of diabetes induction showed complete disorganized of islet of Langerhans ( ), abnormal pancreatic acini ( ), degeneration around pancreatic duct ( ) and distribution of cellular remnant through pancreatic tissue ( ). H&E stain (10X).
Fig (11)  Section on pancreas from diabetes rats after (45)day of diabetes induction showed pancreas lobules completely destruction ( ), accumulation of adipocytes ( ), deposition of collagenous fibers ( ),and heavy infiltration of inflammatory cells ( ) and fine fibers within pancreatic septa ( ). H&E stain (10X)

Fig (12) Transverse section of pancreas section from diabetes rats after (15)day of diabetes induction and arginine treatment showed enlargement of islets of Langerhans ( ), with increased cell population, some cells with darkly stained nuclei ( ), sections of mild congested interlobular ducts ( ), the endocrine cells separated by fenestrated capillaries ( ) and foci of degeneration within pancreatic tissue ( ). H&E stain (10X)
Fig (13) Section on pancreas from diabetes rats after (30) day of diabetes induction and arginine treatment showed the normal structure of islet of Langerhans ( ), increased number of endocrine cells ( ), boundary ( ), separated the endocrine and exocrine portions, normal acinar cells ( ). H&E stain (40X).

Fig (14) Transverse section of pancreas section from diabetes rats after (45) day of diabetes induction and arginine treatment showed normal regenerated pancreas tissue ( ), healthy, regular islet of Langerhans ( ), proliferation of endocrine cells ( ), normal interlobular septa ( ) and regular intralobular ducts ( ) and fenestrated capillaries ( ) within the islet. H&E stain (10X).
Fig (15) Section on gastrocnemius after (15) day of diabetes induction showed irregular degenerated muscle fibers ( ), extend among normal muscle fiber ( ), with strands of connective tissue ( ), normal blood vessels ( ), fragments of muscle fibers ( ) also noticed, and nerve trunk ( ) showed. H&E stain (10X).

Fig (16) Section on biceps muscle after (30) day of diabetes induction showed sever atrophied of muscle fibers ( ), pale region of degeneration ( ), some fibers were filamentous ( ) and irregular accumulation of myonuclei ( ). H&E stain (10X).
Fig (17) Section on biceps muscle after (45)day showed severe atrophied muscle fibers, myonecrosis, bifurcate muscle fibers. H&E stain (10X).

Fig (18) Transverse section on control triceps brachii showed regular sections of muscle fibers separated by normal endomysium and fine strands of collagen fibers. H&E stain (10X).
Fig (19) Section on triceps muscle after (15) day of diabetes induction and treated with arginine showed normal muscle fibers ( ), large nerve trunk ( ), strand of epineurium ( ), large congested artery ( ), perimysium ( ), and bundles of collagenous fibers ( ). H&E stain (10X).

Fig (20) Section on triceps muscle after (30) day of diabetes induction and treated with arginine showed normal muscle fibers ( ) with cross striation, satellite cells ( ), normal sarcolemma ( ), mild congested blood vessels ( ). H&E stain (40X).
Fig (21) Transverse section on gastrocnemius muscle after 45 days of diabetes induction and treated with arginine showed structure of normal intramuscular nerve trunk, composed of large nerve ( ), more normal nerve fibers ( ), surrounded with epineurium ( ), strands of perineurium ( ), large artery ( ), vein ( ) and number of blood capillaries ( ), normal muscle fibers ( ). H&E stain (10X).

Fig (22) Section on liver from control rats showing homogenous distribution of glycogen granules ( ), around faint stained nuclei ( ). PAS stain (40X).
Fig (23) Section on liver from diabetes rats after 15 day of diabetes induction showing fine glycogen granules ( ) and hyperplasia ( ) in bile duct wall and inflammatory cells ( ), mild fatty change and nucleus vacuolation ( ). PAS stain (10X).

Fig (24) Section on liver from diabetes rats after 30 day of diabetes induction showing mild coarse glycogen granules ( ) in hepatocytes cytoplasm, moderate positive reaction with (PAS) ( ). PAS stain (10X).
Fig (25) Section on liver from diabetes rats after (45) day of diabetes induction showed weak reaction with (PAS) stain indicate to mild glycogen content [→] in liver cells and moderate amount of lipid droplets [→]. PAS stain (10X).

Fig (26) Section on liver from diabetes rats after (15) day of diabetes induction and treated with arginine showed fine glycogen granules [→] deposition, regenerated hepatocytes [→]. PAS stain (10X).
Fig (27) Section on liver from diabetes rats after (30) day of diabetes induction and treated with arginine showed moderate distribution of glycogen granules ( ), inflammatory cells ( ) around normal central vein ( ). PAS stain (10X)

Fig (28) Section on liver from diabetes rats after (45) day of diabetes induction and treated with arginine showing restore cells with mitotic figures ( ), strong positive reaction with (PAS) indicate to red or pink granules ( ) in hepatocytes cytoplasm. PAS stain (10X)
Fig (29) Section on pancreas from control rats showing cluster of B-cells which are centrally placed ( ), surrounded with acinar cells ( ). Aldehyde fuchsin stain (40X).

Fig (30) Section on pancreas from diabetes rats after (15) day of diabetes induction showed pathological changes of both exocrine and endocrine portion revealed by reduced (B) cells, degranulation of most endocrine cells ( ) and vacuolation, degeneration of acinar cells ( ). Aldehyde fuchsin stain (40X).
Fig (31) Section on pancreas from diabetes rats after (30) day of diabetes induction showed apoptotic B-cells ( ) and absence of secretory granules ( ). Aldehyde fuchsin stain (40X).

Fig (32) Section on pancreas from diabetes rats after (45) day of diabetes induction showed complete degenerative of endocrine cells ( ), deposition of collagen fibers ( ) within the islet of Langerhans, and around ( ), severe vacuolation ( ). Aldehyde fuchsin stain (40X).
Fig (33) Section on pancreas after (15) day of diabetes induction and treated with arginine showed remarkable increase in B-cells, still vacuolation within the islet of Langerhans, slightly reduction in the histological alterations of the pancreatic acini and few acinar cells with basal nuclei. aldehyde fuchsin stain (40X).

Fig (34) Section on pancreas after (30) day of diabetes induction and treated with arginine showed regenerated B-cells, few α-cells, vacuolation within islet of Langerhans and heavy deposition of collagen fibers. aldehyde fuchsin stain (40X).
Fig (35) Section on pancreas after (45) day of diabetes induction and treated with arginine showed islet of Langerhans nearly similar to control, variable number of B-cells ( ), distinct boundary ( ) between endocrine and exocrine portions, mild collagen fibers ( ), aldehyde fuchsin stain (40X).
4- Discussion

Results showed that liver sections from normal rats exposed to normal saline referred to normal architecture, the liver lobule has the classical features and have no changes and this result indicated that normal saline have not caused any injury, this agreement with (Farokhi et al., 2012; Anahita Aboonabi et al., 2014).

Liver sections from diabetic rats showed changes at each period (15, 30, 45) days included degenerated hepatocytes, pyknotic nuclei, vacuolated cytoplasm, congested blood vessels and inflammatory cells infiltration these alteration more obvious at (30) days post diabetic induction, progressive changes in liver tissue, sever congestion, necrotic foci, hydropic changes and karyolytic nuclei while at (45) days post (DM) induction showed severe alterations included extensive degeneration, kupffer cells hyperplasia, dilated sinusoids, irregular arrangement of hepatocytes and fatty changes, these changes may be related to the toxicity of (STZ) and its metabolic compounds, deficiency in insulin secretion that lead to an increased in glucose level, this imbalance caused generation of free radicals and (Ros) which lead to destruction of cells membranes and lipid peroxidation and all these caused liver damage, dysfunction, histological alterations and associated with variable histopathological changes like congestion, necrosis and extensive degeneration, these results was established by (Ragavan & Krishnakumari, 2006; Farokhi et al., 2012) who reported that liver sections from diabetic rats showed marked structural alterations as a results of absence of insulin secretion and imbalance homeostasis between cellular oxidation and reduction especially in liver tissue. Moreover, liver has a major role for maintaining normal glucose concentration and it is the main site of insulin clearance and its associated with specific diabetic complications and disturbances in various tissues, also the main function of this big organ was the managing and controlling carbohydrates, lipids, proteins metabolism, maintaining normal glucose by storing the glucose in form of glycogen (glycogenesis) and break down glycogen into glucose (glycogenolysis), also forming glucose from no carbohydrate sources such as the amino acids (gluconeogenesis) (Farokhi et al., 2012).
Furthermore in (STZ) treated rats the studies found that organelles degeneration, hepatocytes vacuolization and congestion resulted by insulin deficiency and not only by (STZ) toxicity and also due to restricted synthesis capacity of the liver and has been described as degranulation and fragmentation of hepatocytes cell membranes (Bothaina et al., 2017).

Changes on liver tissue at (30, 45) days was the fibrosis, heavy infiltration of inflammatory cells, increased of blood vessels congestion and fatty changes, these results were considered as indices for the pathological events and may be an increases in oxidative stress or the tissue responses against injury which included formation of collagenous fibers and changing with permeability of cell membranes which lead to disturbances with ions changing. These findings were also recorded by other researchers who also concluded that oxidative stress associated with multiple changes in (DM) patients such as glycation of some products and to hypoxia resulting from hyperglycaemia which lead to imbalance and result in the consumption of antioxidant defenses which cause disruption of cellular function (Des-corbeth & Anard-srivastava, 2010).

It has also been shown that fatty infiltration as a precursor of cirrhosis in diabetic patients and hepatic fatty steatosis and pericentral fibrosis, the workers recognized markedly swollen hepatocytes and suggested that this case may be intermediate lesion between fatty steatosis and cirrhosis (Noman et al., 2009; Amanda et al., 2013).

The study established the important role of (arg) in the pathogenesis of (DM) and liver sections related to diabetic rats treated with (arg) at different periods (15, 30, 45) days were shown since (15) days included regeneration and restore normal hepatocytes within liver stroma, mild blood vessels congestion, there results may be explained that the (arg) act as preservation agent, enhanced the immune response, decreased the (STZ) toxicity or may increase the hepatocytes regeneration and it was necessary for protein synthesis.

These results also suggested by others that (arg) amino acid, in certain situation such as trauma and stress, it is a precursor for synthesis proteins.
and polyamines, linked with release of human growth hormones and rapid healing from injury and reported to have immuno-supportive effects specially under catabolic conditions (Al-DAlAEN et al., 2016). Also the results showed that the sections of liver from the same group (diabetic + Arg) rats revealed the liver nearest to the normal liver structure at (45) days, normal cellular arrangement, mild necrosis, most blood vessels with very mild congestion and slightly lipid droplets deposition. All these observations may be considered that the (Arg) has the ability to ameliorate the oxidative stress and metabolic changes in liver after diabetic induction and it act as hepatic protective agent. These results in agreement with (EL-Missiry et al., 2004; Nitin & Sudhir, 2009) who reported that exogenously administrated of (Arg) decrease the oxidative stress in the liver and to the direct effect of (Arg)on nitric oxide (No) dependent antioxidant capacity or No-independent pathways.

The study focuses on the pancreas pathogenicity through (DM) induction in rats compared to control, sections on control pancreas revealed to normal pancreatic acinus and endocrine pancreatic islets formed of endocrine cells embedded within the pancreas stroma, this result showed control rats injected with normal saline which it is normal substance have no effect on pancreas architecture so results associated with many studies (Danish et al., 2014; Sara et al., 2016). Pancreas sections stained with (H&E) stain related to diabetic group rats after (15) days showed mild degeneration in pancreatic acini, less number and atrophied, shrunken pancreatic islets and congested blood vessels with dilation of interlobular ducts, these results related to the toxicity of (STZ) which used to induced diabetes, its effect directly on (B) cells damaged and its responsibility on sever hypo insulinaemia which lead to hyperglycemia, this findings are similar to those previously described (Omer et al., 2004; Ku et al., 2009; Amr et al., 2011) who mentioned that diabetic experimental model exhibit depletion with the activity of antioxidative defense system and promote free radicals generation, so the oxidative stress was responsible for the B-cells dysfunction caused by glucose toxicity, moreover the reactive oxygen species that produced under hyperglycemia caused tissue damage.
Moreover the (STZ) contain anitroso moiety and can released (NO) which may be responsible for the (STZ)-induced B-cells destruction of rodents, in addition DNA strands break and generation of free radicals appear to be a common factors in B-cells death and destruction of islets of Langerhans (Klaidman et al., 2001; Faris, 2009).

At (30) days post (DM) induction the pancreas sections showed degeneration, necrosis and irregular pancreatic acini, congested blood vessels, degeneration of most islets of Langerhans, some of segmented islets appeared and inflammatory cells with collagenous fibers deposited, most endocrine cells with pyknotic nuclei, atrophied and with vacuolated cytoplasm, this result regarded to the toxic effect of (STZ) and its metabolic compounds, hyperglycemia and the role of glucose on lipids and proteins or it may affect on cells membranes since the (STZ) have the ability to enter the pancreatic cells, our results in agreement with other studies indicated that the destruction of B-cells starts after (3) days of (STZ) administration and reaches its peak at (3-4) weeks in most experimental rats, also (STZ) was apotent DNA methylating agent and act as (NO) donor in pancreatic islet cells and thought to be mediated by the inhibition of free radicals scavenger enzyme there by enhancing the production of superoxide and this implicated in lipids oxidations and DNA damage (Adeghate & Ponery, 2002; Adeyemi et al., 2007; Hanaa & Seham, 2012).

The histological findings in this study in agreement with others (Danish et al., 2014; Sara et al., 2016; Hussein, 2017) who reported histological changes in pancreatic tissue of diabetic rats after different periods of (STZ) injection like swelling, degeneration, necrosis, destruction and atrophy of islets of Langerhans in addition to the degeneration and necrosis resulting from the toxicity effect of (STZ).

Sever changes was shown in pancreas sections of diabetic rats after (45) days like disorganized, abnormal exocrine portion, no obvious nuclei, heavy deposition of collagenous fibers around the congested blood vessels and interlobular ducts, the islets of Langerhans was completely destruction, reduced in size and each islet with irregular surface, moreover accumulation of lipid or fatty tissue with pancreatic acini, these
results clarified that changes in pancreas associated with diabetes, there was hormones disturbances and changes with metabolic rate, these results was confirmed by others whose reported the oxidative stress as implicated in the clinical disorders to the mismatch between production of the free radicals and the capability of cells to defense against them this lead to accumulation of oxidative macromolecules specially superoxide (O$_2^-$), hydroxyl (OH$^-$) action and hydrogen peroxide (H$_2$O$_2$) (Stephen et al., 2007; Jamshid & Prakash, 2012; Hanaa & Seham, 2012).

Furthermore (L-arg) is reported to have beneficial effect on several complications including (T1D), B-cells neogenesis insulin sensivity and improvement of endothelial function and reduction of fat accumulation in tissues of diabetic rats (Ramprasath et al., 2012).

Histologically observations on triceps, biceps and gastrocnemius skeletal muscles sections from control rats showed normal structure of muscle fibers which appeared parallel, striated with peripheral myonuclei, the perimysium connective tissue fill the intercellular space that separated muscle fibers and the strands of collagenous fibers (Dalia & Salwa, 2012) who describe the normal structure of skeletal muscles.

Skeletal muscles in diabetic group showed obvious changes at (15) days post diabetes induction such as distoration, mild atrophy, infarction, irregular arranged of myonuclei, most muscle fibers wavy, barely surface, the striaion less clear, these changes will be more sever when the period increased and sections from diabetes skeletal muscles at (30, 45) days revealed to degenerated myofibrils, hypercontracted fibers, internal myonuclei more than peripheral location, dense collagenous fibers extend within dilated intercellular spaces in addition to the inflammatory cells and atrophied, less number of muscle spindles, these changes at (15, 30, 45) days post (STZ) induction revealed to the toxicity of (STZ), the hyperglycaemia, hypoinsulineamia, disturbances in metabolism of proteins, carbohydrates and lipids increased with cholesterol, increased in myofibrils destruction, also the imbalance with glucose level may be caused damaged and destruction in skeletal muscles organelles and all caused by hyperglycaemia for long time, These results in agreement with (He et al., 2001; Adib et al., 2006; Hany, 2008) who reported that changes in skeletal muscles to be due to reduced vascular supply the effect of insulin.
deficiency and lead to decreased with protein synthesis, the low serum insulin have direct effect on motor end plates and synthesis of contractile proteins, abnormal microangiopathies and increased in ribosomal degradation that associated with level of circulating amino acids.

The beneficial effect of arginine in the recent study was proved histopathologically on diabetes skeletal muscles at different periods (15, 30, 45) days post diabetes induced, observations clarified the changes on sections from muscles with (DM) and treated (Arg) post (15) day, the muscle fibers more regular, parallel to each other, peripheral myonuclei while still some fibers with taper endings, moreover figures from diabetes skeletal muscles and treated with (Arg) at (30) days post (DM) induction, clear striations, condensed myofibrils, normal blood vessels and capillaries, large nerve trunk with branches of nerves active satellite cells located beneath sarcolemma, also the changes more obvious at (45) days, that the muscle fibers arranged as bundles, normal muscle spindles, no inflammatory signs, narrow interstitial spaces separated the muscle fibers and formed of new myotubes, these results may regarded to the effect of (Arg) on insulin secretion, its role in homeostasis of glucose, inhibit the (ROS) that form which thought to contribute to lipid peroxidation, or may increased the protein synthesis, increased the blood vascular supply to skeletal muscles and lead to regenerated muscle fibers and its contractile proteins, increased insulin synthesis and activate the antioxidant enzymes.

Our results in agreement with other study reported the beneficial effect of (Arg) in diabetic animals, that rats treated with (Arg) recorded normal glucose level and all other markers tend to be normal and this reflect that the (Arg) has antioxidant activity (Mendez&Dettaro-Hernandez, 2005).

Liver sections from diabetes rats at (15) days showed mild reduction in glycogen content and mild positive reaction with (PAS) stain, more progressive changes and glycogen depletion was associated with (DM) after (30, 45) days and the figures revealed to weak, pale staining with (PAS) stain at each period, these results may be due to the effect of (STZ) which caused damage to B-cells, decrease in insulin secretion, disturbances with glucose metabolism and this results lead to inactivation of glycogen synthesis. These findings was explained by investigators and
regarded was a metabolic disorder in addition to fatty changes and markedly decreased in glycogen content and related to the displacement of glycogen as a consequence of lipid droplets accumulation (Sara et al., 2016; Imad et al., 2017).

Recent study showed that disturbances with total protein, lipids profiles, changes with level of (IGF) and glucose level associated strongly with changes in liver and its metabolic activity, these results were confirmed by Timothy et al., (2009) who reported that high level of glucose converted to glycogen stored in liver, when liver too saturated, the glucose instead used to synthesized fatty acids that released to bloodstream, were used to form triglycerides (TG) which build in fat cells and caused obesity.

Results also clarified the role of arginine on liver glycogen deposition in all rats with diabetes at each period (15, 30, 45) days, the observations at (15) days showed mild accumulation of glycogen granules within hepatocytes cytoplasm, then moderate degree of positive reaction with (PAS) stain indicates to coarse glycogen granules with pink colour deposit in liver cells at (30) days while strong positive reaction with (PAS) stain appeared in all liver sections related to diabetes rats at (45) days, this result may be related to the beneficial role of (Arg) that may caused regeneration of B-cells and this lead to secretion of insulin which mean normal glucose level and stored some of blood glucose as glycogen within liver, or the (Arg) have important role in liver regeneration, normal enzymes that affect on glucose homeostasis (Fatima & Eman, 2011; Omnia et al., 2014).

The findings mentioned that pancreas sections stained with aldehyde fuchsin showed degeneration and necrosis of B-cells within the islet of Langerhans with focal acinar damage, apoptotic B-cells at (15, 30) days and completely B-cells degeneration was noticed at (45) days post diabetes these results may be caused by the (STZ) toxicity, its metabolites, cytotoxic action of (STZ) on B-cells, persistent of hyperglycemia and inhibition of important enzymes these results also suggested by other researchers who revealed that pancreas sections...
stained with aldehyde fuchsin showed extensive B-cells destruction, less cellular density and no distinct boundary between endocrine and exocrine portions (Stephen et al., 2007; Faris, 2009). Hyperglycemia, low level of plasma insulin, lead to atrophic pancreatic islets, reduced B-cells mass, proliferation and then depletion of islet insulin content (Ahmadi et al., 2010).

Moreover arginine has protective effects due to it direct chemical interaction with oxygen radicals, in other hand induced intensive formation of new B-cells with regularity since it was a companied by apoptosis (Lass et al., 2002; Rooman et al., 2002).

Moreover (arg) was engaged with several metabolic pathways within human body, its associated with signal molecule (NO) which play critical roles in diverse physiological processes (Gad et al., 2010).

Study showed significant decrease with (IGF-1) level in diabetes rats compared with the control value, the lower level was at (45) days with significant difference at (P <0.05), this result related to the cytotoxic effect of (STZ) and its metabolites compounds which altered the level of glucose and insulin secretion, this result was obtained by others investigators who related the changes with (IGF-1) to diabetes either in human or experimental model, concentration of (IGF-1) tend to be lower in untreated independent diabetes mellitus (IDDM) children, and this concentration normalized after the start of insulin treatment also the free (IGF-1) was more depressed than the total (IGF-1) in most diabetes rats (Graubert et al., 1991; Berket et al., 1996). So (IGF-1) was important growth factor and has major metabolic effects in glucose homeostasis and its disturbance are associated with the risk of diabetes (Min Sun Kim & Dae-Yeol Lee, 2015).

The results referred that diabetic rats which treated with (Arg) showed increased with (IGF-1) level at each period (15, 30, 45) days post (DM) induction and the level restore to normal compared to diabetic group with significant difference, this may be related to the protective role of (Arg) and appear to protect the pancreas from damaged caused by (STZ) and then maintain glucose levels in diabetic rats, increasing insulin sensitivity and all this effect on regulation of (GH), particularly (IGF-1).
These findings also discussed by other investigators who showed that (Arg) stimulated (GH) secretion, production of (IGF-1) which acts as anabolic hormone by raising protein synthesis (Martha et al., 2009; Davi et al., 2014).

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