The PotentialEffect of Pentoxyfylline and Ambroxol on some organs in experimentally Induced hind limb ischemia reperfusion on top of typeIIDiabetes Mellitus in rats .

**Abstract**

**Background:** Ischemia reperfusion (I/R) islife-threatening condition.Reperfusion following ischemia can further exacerbate damage of different tissues,especially in the presence of Diabetes mellitus (DM). Metformin (MET) an antidiabetic medication has been reported to exert an anti-inflammatoryeffect.Pentoxyfylline (PTX)used in [intermittent claudication](https://en.wikipedia.org/wiki/Intermittent_claudication), has been reported to exert an anti-inflammatory and antioxidant effects .Ambroxol(AMB) an expectorant antitussive mucolytic drug has also demonstrated protective effects on ischemic damage. The rationale of this study is to test potential effects of PTX and AMB against remote organ injury induced by hind limb ischemia on top of STZ induced type 2 diabetes mellitus in rats and there efficacy compared with the standard hypoglycaemic drug MET. **Aim of the study:**The present study was designed to evaluate the potential effects of PTX and AMBon fasting blood glucose, liver function,kidney function, reduced glutathione andnuclear factor kappa B.**Materials and methods:** Rats were classified into: Group I: control normal. Group II: diabetic iscemic not treated (diseased group). Group III was pretreated with MET. Group IV: pretreated with PTX, Group V: pretreated with AMB,Group VI: pretreated with combination of MET with PTX, Group VII: pretreated with combination of MET with AMB.All treated groups received drugs for 7days prior to induction of hindlimb ischemia in diabetic rat. **Results:**All groups showed significant improvement in all parameters.**Conculsion:** All tested drugs alone or in combination showed improvement of parameters of IR on top of DM. It is found that all combinations show more efficacy than each drug alone.

**Key words:** Ischemia reperfusion, Diabetes mellitus, metformin,Pentoxyfylline, Ambroxol.

**1. INTRODUCTION**

Type 2 diabetes mellitus (T2DM) is a disorder characterized by insulin resistance and pancreatic β-cells dysfunction. The combination of glucotoxicity and lipotoxicity termed as “glucolipotoxicity” is said to be the cause for β-cell dysfunction in T2DM ***(1-2)* .**

High blood glucose level determines over production of reactive oxygen species (ROS) that virtually damages allcellular components including DNA and thus contributing in oxidative stress related diseases ***(3).***Evidence suggests that ROS overproduction may be the key starting event that results to the long term development of complications of diabetes ***(4).***

DM is a major cardiovascular risk factor, and it often leads to severe cardiovascular complications (peripheral arterial disease, myocardial infarction, and stroke). Coronary heart disease (CHD) is the main cause of death in patients with DM and accounts for more than 75% of deaths***(5).***Diabetes is a major risk factor for many surgical complications ***(6-7).*** It is particularly associated with poor prognosis of ischemia reperfusion (IR) injury. Many have reported that IR injury of heart, kidney, and neuron tissues tends to be more severe in diabetic patients ***(8-9).***

Metformin, an oral anti-diabetic drug in the biguanide class is a widely prescribed drug to treat high blood glucose in individuals with T2DM.Various investigations show that metformin decreases intracellular ROS***(4).***It modulates several oxidative stress markers and pro-inflammatory cytokines at the biochemical and gene expression levels [***(10***](http://www.ijp-online.com/searchresult.asp?search&amp;author=Rajesh%2BA%2BMaheshwari&amp;journal=Y&amp;but_search=Search&amp;entries=10&amp;pg=1&amp;s=0)***).***

Pentoxifylline is methylxanthine derivative used primarily to treat patients with peripheral vascular disease***(11).*** In addition to its hemorrhagic activity***(12),*** it has been experimentally shown to have potent anti-proliferative, anti-inflammatory***(13),***anti-fibrotic effects and anti-proteinuric agent in both human studies and animal models***(14),***so it could be symptomatic and in part, causal approaches to attenuate microvascular complications in diabetic patients.

AMB hydrochloride is an active N-desmethyl metabolite of bromhexine hydrochloride. AMB has several new pharmacological properties as surfactant stimulatory, anti-inflammatory, anti-oxidant and local anesthetic effects is addition to the muco-kinetic and muco-ciliary effects of the parent compound***(15).***

These observations provided a rationale for testing potential effects of PTX and AMB against remote organ injury resulting fromischemia reperfusion injury on top fDM.Also in this backdrop, we conducted the present study to explore potential effects of PTX and AMB against remote organ injuryinduced by hind limb ischemia on top of STZ induced type 2 diabetes mellitus in rats and there efficacy compared with the standard hypoglycaemic drug MET.

**2.MATERIALS AND METHODS**

**2.1. Animals**

It is a prospective study carried out on 42 Adult male albino rats obtained from Experimental Animal Breeding Farm, Helwan-Cairo) weighing between 150-200 g (at the beginning of the study), were used for in-vivo experiments. They were acclimatized for one week and were caged (6 rat/ cage) in fully ventilated room at room temperature in the pharmacology department, Benha Faculty of Medicine. Rats were fed a standard chow with water. This study was approved from ethical committee of benha faculty of medicine.

* 1. **Drugs and chemicals**

Fructose powder (ADWIC) (Egypt), Streptozotocin (STZ) powder: (Sigma Chemicals Co., U.S.A), Metformin (ADWIC., Egypt), Pentoxyfylline powder (October pharmaEgypt),Ambroxolpowder(Octoberpharma,Egypt).Urethane,Ethylcarbamat,whitecrystals (Sigma Chemical Co., USA), Urea kits: Diamond diagnostic,Egypt,Creatinine kits: Diamond diagnostic,Egypt, ALT Liquicolor kit (Human, Egypt),AST Liquicolor kit (Human, Egypt),Glucose kits: Roche Diagnostic, Germany (test strips), Nuclear Factor Kappa B (NF-KB) kits (Biodiagnostic, EGYPT),Reduced Glutathione kits ( Biodiagnostic Co., Giza, Egypt).

* 1. **Induction of diabetes mellitus type II**

Rat were supplied with fructose 10% Solution for the initial 2 weeks only.After two weeks ,single injection (ip) of streptozotocin (STZ) (40 mg/kg b.w.). STZ was dissolved in citrate buffer (pH 4.5) with a concentration of 15 mg/ml***(16).***

* 1. **Induction of hind limb ischemia**

Adult rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg).A tourniquet was placed on both hind limbs at a site proximal to the trochanter major in anaesthetized rats. After 2 hours of ischemia, the tourniquet was released and reperfusion followed for 2 hours ***(17).***

**2.5Experimental design**:

 Rats were classified into 7 equal groups (6 rats in each group). **Group I:Control normal group:**Rats were received a standard chow and tap water with no medication.**Group II:Ischemic diabetic non treated group:**Diabetic rats continue only on standard chow and tap water with no medication 7 days prior to induction of hind limb ischemia.**GroupIII**:**Ischemic diabetic metformin (MET) treatedgroup:Diabetic**rats were continued on standard chow and tap water with Metformin 200mg/kg/day I.P ***(18)*** for 7 days prior to induction of hind limb ischemia **Group IV**: **Ischemic diabetic pentoxifylline (PTX) treated group:**Diabetic rats were continued on standard chow and tap water with Pentoxifylline 40 mg/kg P.O ***(14)***for 7 days prior to induction of hind limb ischemia.**Group V:Ischemic diabetic Ambroxol (AMB) treated:** Diabetic rats were continued on standard chow and tap water with Ambroxol 35 mg /kg ip***(19)*** for 7 days prior to induction of hind limb ischemia.**Group VI:Ischemic diabetic Metformin (MET) and pentoxifylline (PTX) treated group:**Diabetic rats were continued on standard chow and tap water with Metformin 200mg/kg/day I.P ***(18)***and pentoxifylline 40 mg/kg P.O ***(14)*** for 7 days prior to induction of hind limb ischemia.**Group VII:Ischemic diabetic metformin (MET) and Ambroxol(AMB) treated group:** Diabetic rats were continued on standard chow and tap water with Metformin 200mg/kg/day I.P ***(18)*** and Ambroxol 35 mg /kg ip***(19)***for 7 days prior to induction of hind limb ischemia. All treated groups received drugs for 7 days prior to induction of hind limb ischemia.Dose selection based on previously puplished studies and pilot experiments.

At the end of experiment a blood sample of about 2ml was withdrawn by unheparinized cannula from right carotid artery ***(20).***The blood samples (each=2ml) were allowed to clot at room temperature, centrifuged at 3000 rotation/minute and the sera were separated. Samples were stored at -20 Ć in dark containers for measurement of nuclear factor kappa B (NFKB) and reduced glutathione (GSH).

* 1. **Assessment of DM**

One week after the STZ injection, animals with non-fasting blood glucose levels > 300 mg/dl were considered as diabetic ***(16).*** Blood glucose level was measured by using a portable glucometer in the blood collected from tail vein. Animals with blood glucose concentration more than 300 mg/dl were used for the study.

* 1. **Assessment of hindlimb ischemia**

 Ischemia was observed by skin color and coldness of the limbs ***(17).***

**2.6 Biochemical assays**

 a- Serum fasting blood glucose was determined using glucometer***( 21).***

b- Serum Aspartate aminotransferase (AST) &Serum Alanine aminotransferase (ALT) were measured by ELISA **(*22).***

c- Serum level of urea was determined using an enzyme-linked immunosorbent assay ***(23).***

d-Serum level of creatinine was determined using an enzyme-linked immunosorbent assay ***(24)***

e-Serum level of CPK-MB. was determined using an enzyme-linked immunosorbent assay ***(25)***

f- Estimation of level of Nunclear factor kappa B (NF-KB ) in the brain, hepatic ,renal and cardiac tissues were determined using an enzyme-linked immunosorbent assay ***( 26)***

g-Estimation of level of GSH in in the brain, hepatic ,renal and cardiac tissue tissueswere determined using an enzyme-linked immunosorbent assay ***(27).***

**3. Statistical analysis:**

 Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 23.0 to obtain descriptive data. Descriptive statistics were calculated in the form of Mean ±Standard deviation (SD).

In the statistical comparison between the different groups, the significance of difference was tested using one way ANOVA (analysis of variance) to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey. A P value <0.05 was considered statistically significant.

**4. RESULTS:**Induction of hind limb ischemia for 2 hrs and reperfusion for 2 hrson top ofexperimentally fructose fed-STZ type 2 diabetic rats resulted in significant elevation of fasting blood glucose level, serum level of CPK-MB,serum ALT, serum AST,serum urea and serum creatinine ( as shown in table 1).Also,there was significant decrease of levels of GSH in the cardiac, hepatic , renal and brain tissueindicating the presence of I/R-induced oxidative damage in diabetic rats ( as shown in table 2)..Moreover,there was significant elevation of levels of NFKB in the cardiac, hepatic , renal and brain tissue in ischemic diabetic non treated group compared to control normal group( as shown in table 3) .

 Regarding, monotherapy treated groups leaded to significant improvement in serum fasting blood glucose level, serum ALT, serum AST,serum urea and serum creatinine compared to diseased groups (as shown in table 1).Monotherapy with metformin and ambroxol leaded to significant improvement in serum CPK-MB compared to diseased groups. Meanwhile,Monotherapy with pentoxyfylline showed non significant change in serum CPK-MB compared to diseased groups (as shown in table 1) .

Also, monotherapy treated groups leaded to significant increase of levels of GSH in the cardiac, hepatic , renal and brain tissue compared to diseased groups (as shown in table 2) , and significant reduction of levels of NFKB in the cardiac, hepatic , renal and brain tissuecompared to diseased groups(as shown in table 3) .

In addition, combination between metformin with pentoxyfylline and combination between metformin with ambroxol leaded to significant improvement in serum AST,ALT,urea,creatinine& tissue GSH and NFKB compared to diseased groups(as shown in table 1).They showed better result than monotherapy treated groups.Combination between metformin with pentoxyfylline showed the best result.

**Table (1): Effect of administration of Metformin , Pentoxyfylline and Ambroxol on Fasting blood glucose (FBG) level,CPK-MB,AST,ALT,urea,creatinine on model of experimentally induced hind limb ischemia on top of type ӀӀ DM in male adult albino rats (n=6).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  **Parameter**  **Group**  | **Fasting Blood Glucose** **(mg/dl)** | **CPK-MB****( U/L)** | **AST****( U/L)** | **ALT****( U/L)** | **Urea****(mg/dl)** | **Creatinine****(mg/dl)** |
| **Control group** | 96.5 ± 5.12 | 483.77 ± 20 | 46.16 ± 3.31 | 31.16 ± 2.78 | 41.63 ± 1.02 | 0.86 ± 0.04 |
| **Ischemic diabetic non treated group**  | 353.5± 8.45\* | 2610.4± 50**\*** | 117.83±4.85\* | 104.5± 5.95**\*** | 154.3± 3.74**\*** | 3.34± 0.26**\*** |
| **Ischemic diabetic metformin treated group**  | 173.5 ± 5.64\*+ | 976.33 ± 39**\*+** | 53.33 ± 2.38\*+ | 45.2 ± 2.69**\*+** | 75.08 ±3.19**\*+** | 1.5 ± 0.06**\*+** |
| **Ischemic diabetic pentoxifylline treated**  | 185.33±4.88\*+$ | 2585.66 ±45**\*$** | 57.66 ±3.72\*+$ | 48.5 ±2.58**\*+** | 79.81±3.10**\*+$** | 1.8±0.07**\*+$** |
| **Ischemic diabetic Ambroxol treated**  | 195.33±3.72\*+$# | 1110.02±41**\*+$#** | 62.5 ±2.88\*+$# | 52±2.16**\*+$#** | 83.3±1.64**\*+$#** | 2 .1±0.13**\*+$#** |
| **Ischemic diabetic Metformin and pentoxifylline treated group**  | 103 ± 5.7+$#  | 954 ± 32**\*+#** | 47.16 ± 2.2 +$# | 34.16 ±3.02**+$#** | 61.1 ± 3.02**\*+$#** | 0.9 ± 0.02**+$#** |
| **Ischemic diabetic metformin and Ambroxol treated**  | 125.1±4.2\*+$&Δ | 899.33±29.8**\*+$**&**Δ** | 50.36±2.21\*+$&Δ | 41.16±2.2**\*+$**&**Δ** | 48.99±1.27\*+$&Δ | 1.1 ±0.02**\*+$&Δ** |

Data represented as Mean ± SD (n = 6)

\* significant ( p < 0. 05) compared to control group

+ significant ( p < 0. 05) compared to diabetic ischemic non treated group

 $ significant ( p < 0. 05 ) compared to ischemic diabetic MET. treated group

 # significant ( p < 0. 05 ) compared to ischemic diabetic PTX treated group.

& significant with ischemic diabetic AMB treated group(P <0.05)

Δ significant with ischemic diabetic MET + PTX treated group (P <0.05)

**Table (2): Effect of administration of Metformin , Pentoxyfylline and Ambroxol on GSH concentration in different tissue homogenate on model of experimentally induced hind limb ischemia on top of type ӀӀ DM in male adult albino rats among the studied groups (n=6).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter**  **Group**  | **GSH concentration in cardiac tissue homogenate****(mmol/g. tissue)** | **GSH concentration in renal tissue homogenate****(mmol/g. tissue)** | **GSH concentration in hepatic tissue homogenate****(mmol/g. tissue)** | **GSH concentration in brain tissue homogenate****( mmol/****g. tissue)** |
| **Control group** | 52.99± 1.5 | 39.49 ± 1.06 | 53.74 ± 2.7 | 23.36 ± 1.38 |
| **Ischemic diabetic non treated group**  | 24.94± 2.01**\*** | 16.7± 1.52**\*** | 20.64± 1.94**\*** | 9.15± 0.94**\*** |
| **Ischemic diabetic metformin treated group**  | 47.2 ± 1.36**\*+** | 34.99 ± 1.21**\*+** | 47.13 ± 2.5**\*+** | 19.76 ± 1.26\*+ |
| **Ischemic diabetic pentoxifylline treated**  | 44.96±2.1**\***+**$** | 32.74±2.10**\***+**$** | 43.51±2.81**\***+**$** | 17.83± 1.58**\***+**$** |
| **Ischemic diabetic Ambroxol treated**  | 40.7±1.57**\***+**$#** | 29.39±1.71**\***+**$#** | 39.97±2.54**\***+**$#** | 15.85±1. 46**\*+$#** |
| **Ischemic diabetic Metformin and pentoxifylline treated group**  | 50.69 ± 1.36**\***+**$#** | 39.99± 1.3+**$#** | 53.99 ± 2.16+**$#** | 21.66 ± 1.66+**$#** |
| **Ischemic diabetic metformin and Ambroxol treated**  | 48.99±1.27\*+$&Δ | 38.2±1.18**+$&Δ** | 50.89±2.26+**$&Δ** | 18.66±1.66\*+**&** Δ |

Data represented as Mean ± SD (n = 6)

\* significant ( p < 0. 05) compared to control group

+ significant ( p < 0. 05) compared to diabetic ischemic non treated group

 $ significant ( p < 0. 05 ) compared to ischemic diabetic MET. treated group

 # significant ( p < 0. 05 ) compared to ischemic diabetic PTX treated group.

& significant with ischemic diabetic AMB treated group(P <0.05)

Δ significant with ischemic diabetic MET + PTX treated group (P <0.05)

**Table (3): Effect of administration of Metformin , Pentoxyfylline and Ambroxol on NF-KB level in different tissue homogenate on model of experimentally induced hind limb ischemia on top of type ӀӀ DM in male adult albino rats among the studied groups (n=6).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Parameter****Group** | **NF-KB concentration in cardiac tissue homogenate** **( ng/100 mg wet tissue)** | **NF-KB concentration in renal tissue homogenate** **( ng/100 mg wet tissue)** | **NF-KB concentration in hepatic tissue homogenate** **( ng/100 mg wet tissue)** | **NF-KB concentration in brain tissue homogenate** **( ng/100 mg wet tissue)** |
| **Control group** | 0.98 ± 0. 04 | 0.35 ±0 .02 | 0.63 ±0 .03 | 0.86± 0.02 |
| **Ischemic diabetic non treated group**  | 2.01±0 .07**\*** | 0.95±0 .02**\*** | 1.62± 0.03**\*** | 1.83± 0.03**\*** |
| **Ischemic diabetic metformin treated group** | 1.3 ± 0.03**\***+ | 0.44 ± 0.02**\***+ | 0.75 ± 0.03**\***+ | 0.97 ±0 .02**\***+ |
| **Ischemic diabetic pentoxifylline treated** | 1.4±0.03**\***+**$** | 0.48±0.03**\***+**$** | 0.79±0.02**\***+**$** | 1.01±0.02**\***+**$** |
| **Ischemic diabetic Ambroxol treated** | 1.5±0.03**\***+**$#** | 0.52±0.02**\***+**$#** | 0.83±0.01**\***+**$#** | 1.1±0.02**\*+$#** |
| **Ischemic diabetic Metformin and pentoxifylline treated group**  | 1 ±0 .02+**$#** | 0.35± 0.01+**$#** | 0.64±0 .01**\***+**$#** | 0.93± 0.02**\***+**$#** |
| **Ischemic diabetic metformin and Ambroxol treated**  | 1.21±0.04**\***+**$&Δ** | 0.38±0.03+**$&Δ** | 0.66±0.01**\***+**$&Δ** | 0.96±0.02**\***+**&Δ** |

Data represented as Mean ± SD (n = 6)

\* significant ( p < 0. 05) compared to control group

+ significant ( p < 0. 05) compared to diabetic ischemic non treated group

 $ significant ( p < 0. 05 ) compared to ischemic diabetic MET. treated group

 # significant ( p < 0. 05 ) compared to ischemic diabetic PTX treated group.

& significant with ischemic diabetic AMB treated group(P <0.05)

Δ significant with ischemic diabetic MET + PTX treated group (P <0.05)

**5. Discussion:**

 Type 2 DM induced experimentally in rat by fructose ingestion for the initial 2 weeks only, then single injection (ip) of streptozotocin (STZ) (40 mg/kg b.w.) ***(28).*** It has been reported that only fructose feeding for a long period of time can lead to nutritional tolerance without developing classical signs and symptoms of T2DM and impaired glucose tolerance ***(29).***Hence, this hypothesized that the combination of fructose-feeding for a shorter period of time and a lower dose of STZ injection may induce all major pathogeneses of T2D in rat***(16),***then rats were subjected to bilateral hind limb ischemia for 2 hour followed by reperfusion for 2 hours which induced by tourniquet (a non loosening nylon cable tie) placed on both hind limbs at a site proximal to the trochanter major in anaesthetized rats ***(17).***

The data of the present work revealed that Induction of hind limb ischemia for 2 hrs and reperfusion for 2 hrs on top of experimentally fructose fed-STZ type 2 diabetic rats resulted in significant elevation of fasting blood glucose level, serum level of CPK-MB, serum ALT, serum AST,serum urea and serum creatinine.Also,there was significant decrease of levels of GSH in the cardiac, hepatic , renal and brain tissue indicating the presence of I/R-induced oxidative damage in diabetic rats.Moreover, there was significant elevation of levels of NFKB in the cardiac, hepatic , renal and brain tissue in ischemic diabetic non treated group compared to control normal group.

These data are in agreement with previous study ***(30)*** who reported that Hind limb I/R resulted in a significant elevation of circulating levels of CPK, ALT, AST.Also, These data are in line with ***31***who reported that renal I/R caused a marked increase in serum Cr as well as BUN after I/R in diabetic rats. These data are also in agreement with ***32*** who reported that renal I/R injury caused significant increase in serum kidney markers urea and creatinine when compared to control rats.

Studies (***33-34)*** showed that the inflammatory response induced by I/R injury was further exacerbated in diabetic rats, in which TNF-a and IL-1β levels in serum or tissues increased significantly. These data are in agreement with (***35)***who reported that I/R was accompanied by a significant decrease in GSH content in the rat heart. These findings imply that I/R play a causal role in heart injury due to overproduction of oxygen radicals or insufficient antioxidant.

Metformin significantly improved all tested parameters of ischemia reperfusion on top of DM.The result of this study is in agreement with ***36***who found that metformin administration daily for 6 days as mono-therapy in acute study in experimentallySTZ type 2 diabetic rats resulted in significant reduction of FBG level. It can be explained that metformin is an oral antidiabetic agents via reducing hepatic glucose production, delaying digestion and absorption of intestinal carbohydrate or improving insulin action***(37).***It has been reported that metformin has multiple activities, including suppression of hepatic glucose, production and improvement of peripheral insulin sensitivity,in type 2 diabetic patients.Thus,it reduces hyperglycemia ***(38).***

Also, these data are in agreement with (39 who reported that administration of MET as monotherapy in the experimental induced ischemia reperfusion rats resulted in significant reduction of CK-MB release in comparison with IR group.The possible cardio-protective mechanisms of MET have been suggested to be due to its ability to activate adenosine monophosphate- activated protein kinase (AMPK) which is considered to be a common cellular “energy-sensor” activating the energy-sparing metabolic processes resultant in increased tolerance to hypoxia and ischemia ***(18).***

In addition, our results are in agreement of with the study of ***(10***)who reported that administration of METorally daily to diabetic rats just after developmentof STZ type 2 DMleads tosignificant decreasein FBGlevel, serumurea leveland serum creatinine level.These results are in agreement with ***(40)*** whorevealed that METreversing liver function testes (LFT) aminotransferase (AST &ALT) abnormalities in mice.These data are is in agreement with(41) who reported that metformin protected against the Adriamycin-induced cardiac oxidative stress via increasing cardiac GSH. These data are is in agreement with(42) who reported that MET inhibited the cardiac expression of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) in endotoxin-challenged mice via activating AMPK. Also, these data are in agreement with(43) who proved that the intrinsic activation of AMPK attenuates the cardiac inflammation which occurred during ischemia/ reperfusion through the modulation of the JNK mediated NF-κB signaling pathway.

Pentoxyflline significantly improved all tested parameters of ischemia reperfusion on top of DM,but it showed non significant change regarding CPK-MB.These results are in agreement with (44) who reported that Pentox has anti diabetic effect &(45) who observe that Pentox administration to diabetic rats for 8 weeks study resulted in significant reduction in HbA1c leading to improvement of glycemic control. These data are in agreement with (46)who reported that administration of PTX as mono-therapy resulted in non significant reduction of CK-MB release in comparison with non treated group. In contrast, (47) reported that PTX (50 mg/kg) administration for concutive 21 days to Doxorubicin (DOX)- induced oxidative stress in the heart (cardiotoxicity) rat model resulted in significant reduction in CPK-MB level in comparison with untreated group.

This is in agreement with (48) who reported that administration of PTX orally daily to diabetic rats just after development of STZ type 2 DM resulted in significant decrease in serum urea level and serum creatinine level. In contrast, (49) reported that PTX administrated in the beginning of ischemia do not reduced significantly the plasmatic level of urea, creatinine,in a model of unilateral hindlimb ischemia/reperfusion injury. The PTX results is in agreement with (50) who reported that administration of PTX orally daily to ischemic rats resulted in significant decrease in serum AST level and serum ALT level.

 These data are is also in agreement with(51) who reported that Prophylactic treatment of rats with PTX protected against the doxorubicin-induced cardiac oxidative stress via diminishing doxorubicin -induced alteration in cardiac GSH. Also, these data are is in line with (52) who proved that pretreatment by PTX restored malathion-induced oxidative stress as it restore changes in brain GSH/GSSG ratio.These data are is also in agreement with(53) who reported the cardioprotective effects of pentoxyfylline against heart I/R injury may be due to reductions in the activation of NF-kappa B and production of TNF-alpha content.The findings of this work are is in agreement with(54) who demonstrating the simultaneous nephroprotective and hepatoprotective effects of PTX after renal I/R.

 As regard to AMB ,it significantly improved all tested parameters of ischemia reperfusion on top of DM., This is in agreement with **(55)** who found that ambroxol administration daily for 5 days as monotherapy in diabetic animals resulted in significant reduction of FBG level and Ambroxol treated animals showed better glucose tolerance in Impaired Glucose Tolerance Test (IGTT).

 Also the result of this study is in linewith **(56)**who reported that AMB is effective in reducing cardiopulmonary bypass (CPB)-induced renal injury in children undergoing repair of ventricular septal defect (VSD) through normalization of abnormal kidney functions( serumurea leveland serum creatininelevel) .These data are is in agreement with **(57)** who proved AMB attenuated rat hepatic I/R through up regulation of intracellular antioxidant and anti-apoptotic signaling pathways and significantly decreased serum AST and ALT level.

 Also, these data are is in line with **(58)** who stated that AMB ameliorate cisplatin induced liver and kidney oxidative stress as it inhibited oxidative damage indicated by increase of GSH by replenished the store of reduced glutathione likely by up-regulating glutathione.Moreover, these data are in agreement with**(59)** who demonstrated that AMB injected once per day for three consecutive days decreased the lipopolysacharrides induced lipid peroxidation in murine organs. AMB reveal protective efficacy for lung and heart lipids protective effects of AMB may result from its ability to scavenge -OH and the inhibition of IL-1 and TNFα release. Also, these data are is in line with **(58)** who stated that AMB ameliorate cisplatin induced liver and kidney inflammatory damage by inhibition of tumor necrosis factor-α, interleukin-1β, and nuclear factor kappa-B.

 In conclusion, pentoxyfyllineand/orambroxol alone or in combination with metformin improves ischemia reperfusion on top of diabetes mellitus.When a comparison between combinations and each drug alone is carried out, it is found that all combinations showed more efficacy than each drug alone.Pentoxyfylline and/or Ambroxol can be used alone or in combination with metformin in prevention of remote organ injury resulting from ischemia reperfusion and considered a new line of treatment of ischemia reperfusionThis pave a new path forthe treatment of ischemia reperfusion injury on top of DM and opens a new window for the use of pentoxyfylline&ambroxol in clinical practice.

 It is recommended to elucidate other mechanisms of action of pentoxyfylline,ambroxol in prevention and treatment of of remote organ injury resulting from ischemia reperfusion. Also, further investigations are needed to confirm the mechanism of action of pentoxyfylline and ambroxol in ischemia reperfusion injury to confirm results of this study.

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