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Ameliorative Morphological and Functional Effect of Metformin on Cyclophosphamide Induced Hepatotoxicity in Rat

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ABSTRACT

Background: *Cyclophosphamide is used as anticancer therapy and previous studies have been reported its hepatotoxic effect.*

Purpose: *This study was performed to inspect the antioxidant ameliorative influence of metformin as co-therapy on the hepatotoxicity caused by cyclophosphamide in the treatment of different malignancies.*

Methods: *Male Sprague Dawley rats were divided into four groups; Control group (C), Metformin (MET), Cyclophosphamide (CP), Protector group (CP.MET). At day 14 all rats were weighed and euthanized. For estimation of both hepatic structure and function as well as the oxidative stress, hepatic tissue and peripheral blood samples were collected.*

Results: *The results showed that CP produces many structural and functional damage of the liver. While, pretreatment of the rat with Metformin showed significant decrease in serum liver enzyme activity, significant enhancement of oxidative stress. Also, there are enhancements in liver tissue structure.*

Conclusion: *Depending on the previous results we recommend the routine usage of metformin with CP in the treatment of different diseases.*

Keywords: Cyclophosphamide; Metformin; Liver; Oxidative stress; BAX immunostaining.

Abbreviations: C: Control Group; CP: Group Received Cyclophosphamide only; CP.MET: Group Received Cyclophosphamide and Metformin; MET: Group Received Metformin only.

INTRODUCTION

Cancer diseases can affect different organs in the body [1]. There are about 200 different known types of cancers [2-4]. Cyclophosphamide (CP) is an alkylating agent which used for handling various malignancies as well as an immunosuppressive agent for organ transplantation, systemic lupus erythromatosus, multiple sclerosis and other benign diseases [5-9]. Cyclophosphamide have toxic effect on normal and malignant tissue [10,11]. Reported that the anticancer dose of CP causes acute inflammation in the bladder, kidney damage and liver damage as well as apoptosis.

The phosphoramide mustard and the acrolein are active ingredient of CP. Phosphoramide mustard responsible for the therapeutic role of the CP, where the toxic effect of CP caused by acrolein. The toxic effect of acrolein takes place through activation of cell death either by apoptosis or necrosis [12]. The oxidative stress of CP as a cause for hepatotoxicity was not studied well suggested that anticancer drugs toxic side effects could be diminished by certain antioxidant agents [13].

Metformin considered the broadest anti-diabetic insulin sensitizer drug. Most of its metabolic action applied on the liver reported that metformin activates antioxidant defense system and reduces the lipid peroxidation stated that metformin weakens the mitochondrial permeability transition pore preventing oxidative stress-induced apoptosis [14-17].

This work aimed to discover the possible ameliorative structural and functional effect of metformin in cases of hepatotoxicity caused by cyclophosphamide administration.

MATERIALS AND METHODS

Animals

Twenty male Sprague Dawley rats aged 2-3 months old and weigh 170g-200 g were acclimatized for a week before starting the treatment. They were held in reserve in polycarbonate cages at 22°C ± 2°C in a well-ventilated room, kept under standard laboratory conditions with 12 h light/ dark cycle and 50% ± 10% humidity. The animals were supplied with both diet and water as recommended. All the methods performed in this study were agreed by the ethical committee of the faculty of Vet. Med. University (Ethical committee number).

Experimental protocol

In random, the male rats were alienated into four groups. Each group have five animals. The control group received 0.2 ml saline I.P., Metformin group received 200mg/kg metformin by gavage daily for two weeks, Cyclophosphamide group received single dose of 200 mg/ kg CP I.P. as s single injection at day 7, Protector group received 200 mg/kg metformin by gavage daily for two weeks and 200 mg/ kg CP I.P. as s single injection at day 7. On day 14 the rats were weighed and euthanized.

Animal sampling

Male rats were euthanized on the 14th day of the treatment by injection of sodium pentobarbital (60 mg/kg) intraperitoneally. For estimation of both hepatic structure and function as well as the oxidative stress, hepatic tissue and peripheral blood samples were collected.

Clinical chemistry

Liver function estimated by measuring of ALT, AST in the serum. The protocol was according to the instruction of the commercial kits (Bio diagnostic Co.)

Oxidative stress markers

The level of reduced glutathione (GSH) as described [18], and the level of Lipid peroxidation, expressed by Malondialdehyde (MDA) formation, as described [19] were assessed in this study to evaluate the oxidative stress markers using commercial kits (Bio diagnostics).

Histological analysis

The livers of the rats were fixed with a 10% neutral formaldehyde solution and then were embedded in paraffin following routine histological preparations. Serial sections (5.0 μm thick) from the samples were stained with Hematoxylin-Eosin (H&E) for liver architecture. Also, sections were stained with Mallory Trichrome stain (MT) for the examination of the collagen fiber [20].

Immunohistochemistry

The sections were deparaffinized and rehydrated. Antigen recovery with citrate buffer (Ph-6.0) was achieved by heating the sections in a microwave at 700W for 10 min. After blocking with 3 mL/L H_2O_2 the sections were incubated. The primary antibodies directed against BAX (Thermo, Waltham, MA, USA) in dilution of the ultra-vision quanta detection system (Thermo Scientific) [21].

Image analysis

The mean area percentage was determined randomly on ten non-overlapping microscopic fields from each slide and examined at 400x with Leica Quin 500 LTD using the software Quin 500 (England).

STATISTICAL ANALYSIS

The statistical analysis of the retrieved data was expressed as means ± error of the mean (SEM) with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The analysis established on one-way analysis of variance (ANOVA) tailed by Student's t-test comparison of means. A value of $P \leq 0.05$ was considered statistically significant [22].

RESULTS

Biochemical parameters

The liver function parameters of the control group exhibited levels of ALT and AST within range (35.33 ± 1.45 & 85.33 ± 2.03, respectively), while the CP group showed elevated levels of the ALT and AST (62 ± 2.52 &120 ± 2.89, respectively). The previous parameters indicated significant elevation in the group treated with CP compared with the control group ($P \leq 0.05$). On the other hand, significant improvement in ALT and AST levels (42 ± 1.73 & 100.67 ± 2.19, respectively) recorded in the protector group (Table 1, Figures 1& 2).

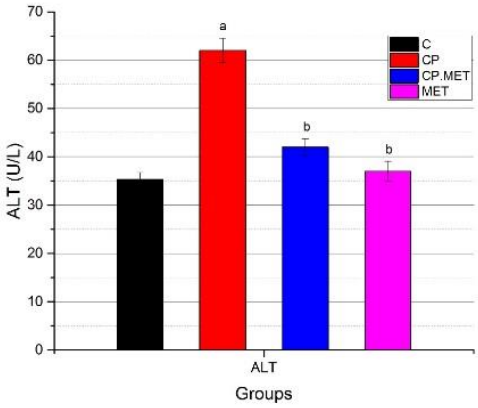

Figure 1: Serum levels of ALT.

Data represented as Mean ± SEM.

(a) Indicate significant difference from the corresponding control group at $P \leq 0.05$.

(b) Indicate significant difference from the corresponding CP group at $P \leq 0.05$.

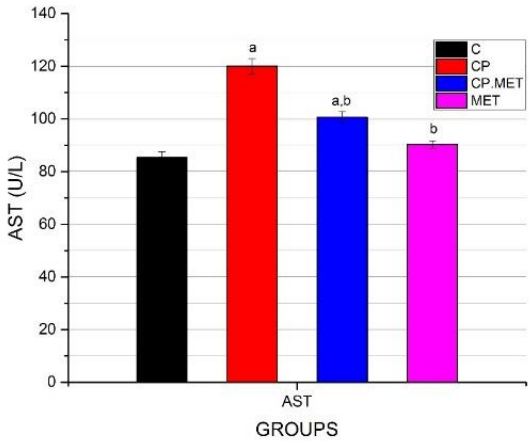

Figure 2: Serum levels of AST.

Data represented as Mean ± SEM.

a. Indicate significant difference from the corresponding control group at P ≤ 0.05.

b. Indicate significant difference from the corresponding CP group at P ≤ 0.05.

Table 1: Liver Biochemical parameters of different groups.

	Control (C)	CP	CP.MET	MET
ALT (U/L)	35.33 ± 1.45	62 ± 2.52 [a]	42 ± 1.73 [b]	37 ± 2.08 [b]
AST (U/L)	85.33 ± 2.03	120 ± 2.89 [a]	100.67 ± 2.19 [a,b]	90.33 ± 1.45 [b]
GSH (mmol/g.tissue)	2.34 ± 0.15	0.44 ± 0.08 [a]	1.44 ± 0.15 [a,b]	2.17 ± 0.15 [b]
MDA (nmol/g.tissue)	10.44 ± 1.22	19.14 ± 1.13 [a]	14.06 ± 1.16 [b]	11.09 ± 0.68 [b]

Data represented as Mean ± SE.

(a) Indicate significant difference from corresponding control group at P ≤ 0.05.

(b) Indicate significant difference from corresponding CP group at P ≤ 0.05.

Oxidative stress markers

Rat gave CP resulted in an increase in liver MDA level and a decrease in its GSH content. While rats treated by metformin showed a significant protective effect against oxidative stress induced by CP in the liver. There was significant enhancement in GSH content in the protector group contrast to the CP group (P ≤ 0.05) (Table 1, Figure 3). The level of the MDA exhibited significant decrease in the protector group compared to the CP group (P ≤ 0.05) (Table 1 and Figure 4).

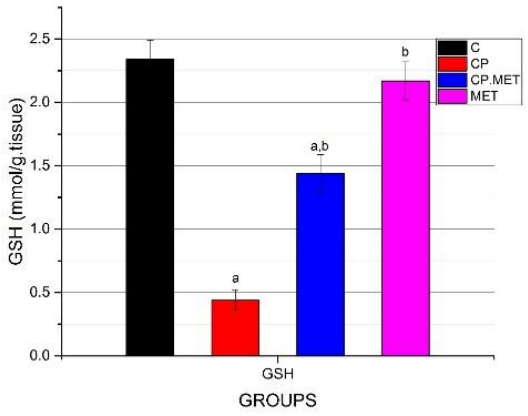

Figure 3: GSH level of the liver in different groups.

Data represented as Mean ± SEM.

(a) Indicate significant difference from the corresponding control group at P ≤ 0.05.

(b) Indicate significant difference from the corresponding CP group at P ≤ 0.05.

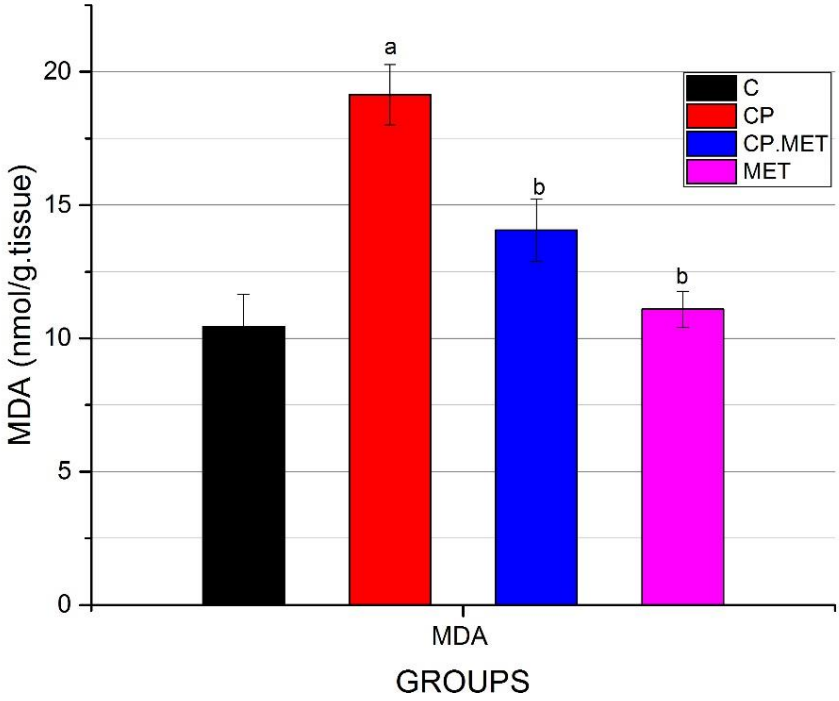

Figure 4: MDA level of the liver in different groups.

Data represented as Mean ± SEM.

(a) Indicate significant difference from the corresponding control group at $P \leq 0.05$.

(b) Indicate significant difference from the corresponding CP group at $P \leq 0.05$.

Histopathological results

H&E sections of the liver tissue displayed normal hepatocyte cell architecture around the central vein in the control group (Figure 5A). Besides, normal hepatocyte and Von Kupffer cells (Figure 6A). While in cyclophosphamide group, disorganization of vacuolated hepatocytes, congestion in central vein, dilatation and congestion in sinusoids, hyperplasia in von Kupffer cells (Figure 5B) and lymphocytic infiltration (Figure 5C), vacuolar degeneration in hepatocytes, apoptotic cells, Pyknotic hepatocytes, karyolitic hepatocytes (Figure 6B) and necrotic hepatocytes (Figure 6C) recorded. Meanwhile in protector group enhanced hepatocyte cell cords radiating around the central vein and mild congestion in medium-sized sinusoids (Figure 5D). Few vacuoles observed in hepatocytes and decrease the number of von Kupffer cells (Figure 6D). The sections of the metformin group were nearly like the control group (Figures 5E and 6E).

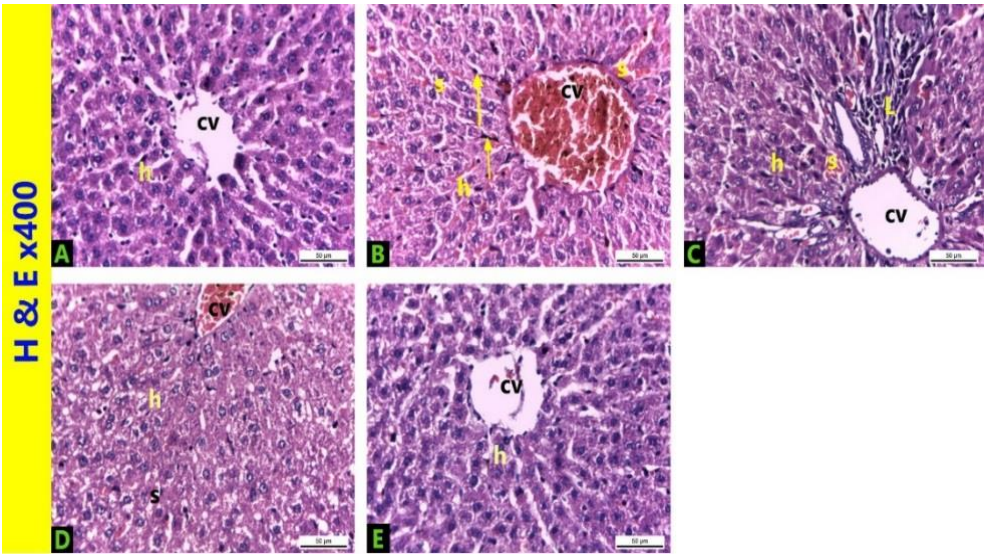

Figure 5: Light microphotographs of sections from male Sprague Dawley rat's liver showing A: normal hepatocyte cell architecture (h) around the central vein (CV) in the control group. B: disorganization of vacuolated hepatocytes (h), congestion in the central vein (CV), dilatation and congestion in sinusoids (s) and hyperplasia in von Kupffer cells (arrows) in the cyclophosphamide exposed group. C: Lymphocytic infiltration and dilated and congested sinusoids (s) in the cyclophosphamide exposed group. D: Enhanced hepatocytes cell (h) cord radiating from the (CV) and mild congestion in medium-sized sinusoids (s) in the protector group. E: Normal hepatocyte cell architecture (h) around the central vein (CV) in the metformin group. (H&E 400x).

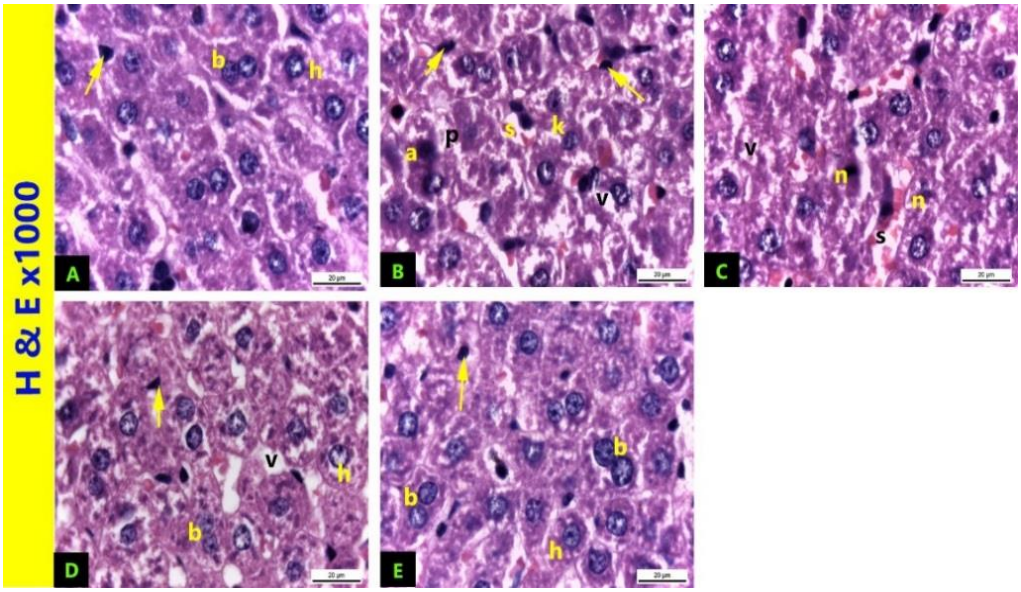

Figure 6: Light microphotographs of sections from male Sprague Dawley rat's liver showing A: normal hepatocyte (h), binucleated hepatocytes (b) and von Kupffer cells (arrow) in thecontrol group. B: vacuolar degeneration in hepatocytes (v), dilatation and congestion in sinusoids (s) and hyperplasia in von Kupffer cells (arrows) apoptotic cells (a), Pyknotic hepatocytes

(p) and karyolitic hepatocytes (k) in thecyclophosphamide exposed group. C: necrotic hepatocytes (n) and dilated and congested sinusoids (s) in thecyclophosphamide exposed group. D: Enhanced hepatocytes cell (h), few vacuoles (v), binucleated hepatocytes (b) decrease number of von Kupffer cell (arrow) in theprotector treated group. E: Normal hepatocyte cell architecture (h), binucleated hepatocytes (b) normal von Kupffer cell number in the metformin group. (H&E 1000x).

The control group exhibited minimal collagen fibers deposition together with the metformin group (Figures 7A & 7D). The collagen fibers deposition presented a marked increase in the CP group (Figure 7B) while, the protector group showed moderate collagen fibers deposition (Figure 7C).

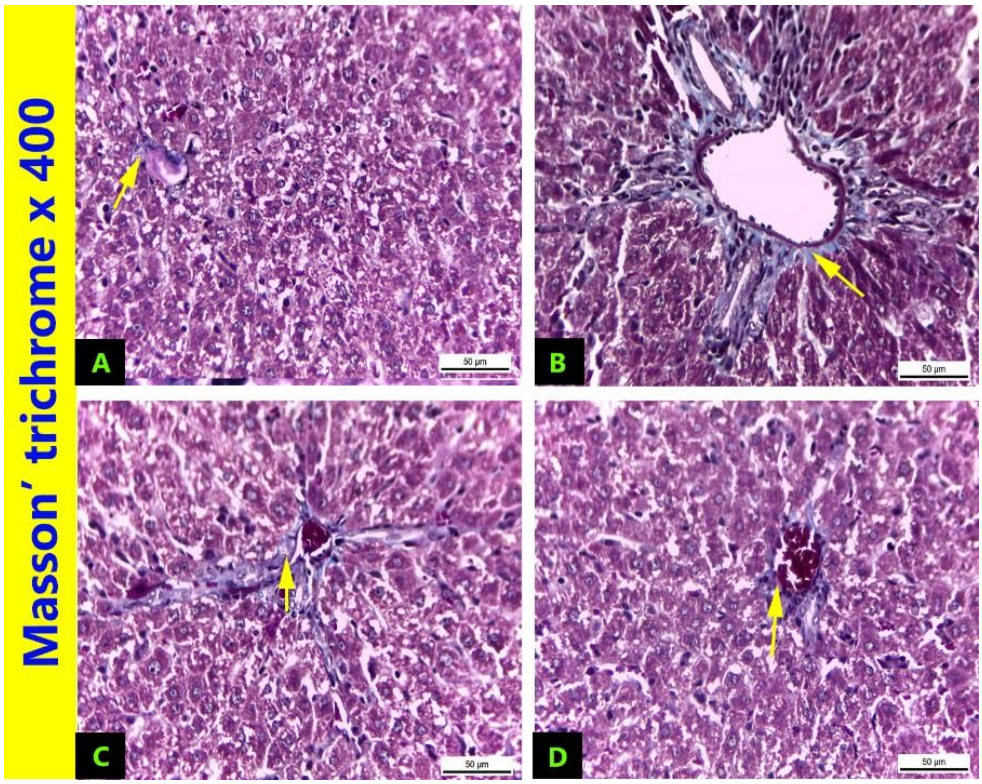

Figure 7: Light micrograph of sections from male Sprague Dawley rat's liver showing A: minimal collagen fibers (arrow) in the control group). B: marked increase of collagen fibers deposition (arrow) in the CP group. C: moderate collagen fibers deposition (arrow) in the protector group. D: mild collagen deposition (arrow) in the metformin group. (Masson' trichrome x 400).

Immunohistochemistry for detection of Apoptosis

The hepatocyte apoptosis was determined by BAX immunostaining in the different groups. The CP group showed strong positive Bax immunostaining (Figure 8B), while the control group and metformin group showed few immunostainings of BAX (Figures 8A & 8D). The protector group showed mild BAX immunostaining (Figure 8C).

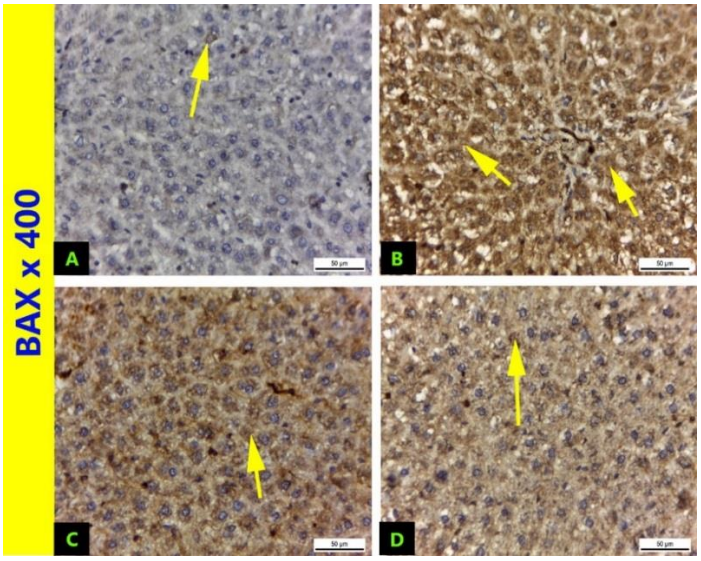

Figure 8: Light micrograph of BAX Immunohistochemistry of liver sections from male Sprague Dawley rat showing: A: Few immunostaining in the control group. B: Marked increase immunostaining of Bax in the CP group. C: Marked decrease in immunostaining of Bax in the protector group. D: Few immunostaining of Bax in the metformin group. (BAX 400x).

Image analysis

The stained liver tissue examined exhibited marked rise in the mean area percentage of BAX immunostaining in CP group (2 ± 0.23) which was significant to the control group (0.33 ± 0.05) and metformin group (0.5 ± 0.08), while the protector group showed significant decrease in the area percentage of Bax immunostaining (1 ± 0.34) in comparison to CP group at ($P \leq 0.05$) (Table 2 and Figure 9).

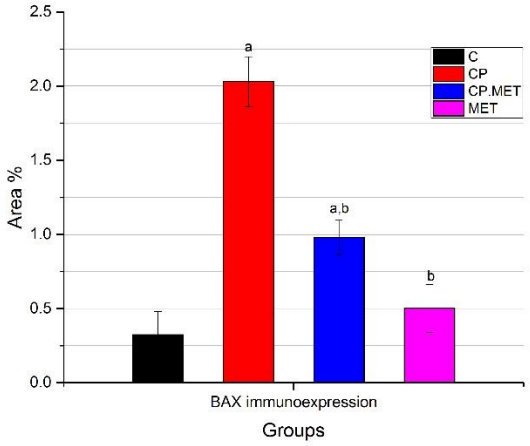

Figure 9: Percentage of BAX immunostaining in the different groups.

Data represented as Mean ± SEM (N=10).

(a) Indicate significant difference from the corresponding control group at $P \leq 0.05$.

(b) Indicate significant difference from the corresponding CP group at $P \leq 0.05$.

Table 2: Mean values ± SE of area percentage of BAX immunostaining.

Variable	Control (C)	CP	CP.MET	MET
Mean area (%)	0.33 ± 0.05	2 ± 0.23[a]	1 ± 0.34[a,b]	0.5 ± 0.08[b]

Data represented as Mean ± SE.

(a) Indicate significant difference from corresponding control group at $P \leq 0.05$.

(b) Indicate significant difference from corresponding CP group at $P \leq 0.05$.

DISCUSSION

The protective role played by the liver against various toxins in the body is preserved by a healthy liver. The drawbacks of liver injury include the reduction of the detoxification capability of the liver [23]. The present study attentions on the ameliorative effect of metformin to the hepatotoxicity induced by cyclophosphamide. Cyclophosphamide is anticancer alkylating agent intended for the treatment of different types of malignancies [24,25].

Several toxic effects of CP were reported in addition to its pharmacological action [26]. Hepatoxicity is one of CP's main toxic effects [27]. Cyclophosphamide plays a crucial role in the production of ROS and MDA and depletes the antioxidant defense mechanism of the liver [28]. The inflammatory response and increases the production of inflammatory cytokines are the main consequence of oxidative stress induced by cyclophosphamide in liver tissue [29].

Metformin is insulin sensitizer intended for the dealing with hyperglycemia of type II diabetes mellitus [30]. It has several clinical trials also for the treatment of cardiovascular complications and hepatotoxicity through enhancement of mitochondrial redox state [31]. Authors in [32-34] stated that, the reduction of the GSH level and concurrent increase in the production of malondialdehyde represented oxidative stress which guides the cytotoxic of the substance. This may be one of the anticancer drugs mechanistic pathways for its toxicity. In current study, liver function enzymes (ALT & AST) and redox status were improved in the protector group with metformin in comparison to the cyclophosphamide group. The increase in the serum ALT and AST levels in the CP group are compatible with the report [21]. This investigation is following *in vivo* [31,35,36] they showed the hepatoprotective effects of metformin against several types of toxicity including anticancer drugs such as cisplatin, methotrexate, ethanol, and arsenic trioxide toxicity.

The metformin surges PPAR-γ levels as well as triggers AMPK which may refer to its defensive action against the anticancer drugs. Where PPAR-γ activation could be diminished inflammation, oxidative stress and which will reflect on prevention of apoptosis induced by xenobiotic as mentioned [37,38]. AMPK activation prevent TNF-α-induced apoptosis and liver injury [39], with control on the growth of cancer as well as cellular death, oxidative stress and inflammatory injury [40,41]. Metformin treatment could decrease hepatic damage induced by CP through modulating oxidative stress and apoptosis.

In agreement with [29,42-46], the rats exposed to CP showed marked liver structural and functional injuries revealed by histopathology and elevation of liver enzymes. These results could be referred to as the membrane damage caused by CP

metabolites [42,47,48]. Histopathological evaluation of the CP treated group revealed massive degenerative changes include disorganization of vacuolated hepatocytes, congestion in a central vein, hyperplasia in von Kupffer cells. These findings were under that of [42,43,45]. There was extensive sinusoidal swelling and narrowing of the liver tissues in the CP treated group. The evaluated sectioned of CP group showed multiple inflammatory cellular infiltrations in the hepatic tissue, this could be as a result of oxidative stress induced by CP as mentioned [42,49].

In accordance with [50], the collagen tissue deposition was highly observed in the CP group around the central vein which reflects fibrosis of hepatic tissue. The strong positive BAX reaction of the CP treated group indicates the apoptotic effect of CP on the hepatocytes, which matched the results [43,51]. The antioxidant ameliorative effect of the metformin against the CP was reported [35,52,53].

CONCLUSION

The hepatic injuries induced by CP were indicated by hepatic degenerative changes, elevated liver enzymes and increased BAX immunostaining. The antioxidant hepatoprotective result of metformin refers to the inhibition of GSH depletion and diminution of marked inflammatory response and cell apoptosis. Depending on the previous results we recommend the routine usage of metformin with CP in the treatment of different diseases.

REFERENCES

[1]. Pearson, S.J., et al., Worldwide burden of cancer attributable to diabetes and high body-mass index: A comparative risk assessment. *Lancet Diabetes Endocrinology*. **2018**. 6: e6-e15.

[2]. De La Puente-Yagüe, M., et al. Vitamin D and its role in breast cancer. *Kaohsiung J Med Sci*. **2018**. 34: 423-427.

[3]. Moses, C., et al. Hallmarks of cancer: The CRISPR generation. *Eur J Cancer*. **2018.** 93: 10-18.

[4]. Brown, M., et al. Chapter 29 - Hormone-Responsive Cancers. In: Strauss, J. F. & Barbieri, R. L. (eds.) Yen and Jaffe's Reproductive Endocrinology. **2019**. Philadelphia.

[5]. Abdul Razak, R.N.H., et al. Ameliorative effects of Aquilaria malaccensis leaves aqueous extract on reproductive toxicity induced by cyclophosphamide in male rat. *Malays J Med Sci.* **2019**. 26: 44-57.

[6]. Mielcarek, M., et al., Post transplantation cyclophosphamide for prevention of graft-versus-host disease after HLA-matched mobilized blood cell transplantation. *Blood*. **2016**. 127: 1502-1508.

[7]. Kim, Y.H., et al., Mechanisms involved in synergistic anticancer effects of anti-4-1BB and cyclophosphamide therapy. *Mol Cancer Ther*. **2009**. 8 :469-478.

[8]. Awad, A., Stuve, O. Cyclophosphamide in multiple sclerosis: Scientific rationale, history and novel treatment paradigms. *Ther Adv Neurol Disord.* **2009**. 2:50-61.

[9]. Petri, M. Cyclophosphamide: New approaches for systemic lupus erythematosus. *Lupus*. **2004**. 13: 366-371.

[10]. Ayhanci, A., et al. Hepatoprotective effect of Seleno-L- methionine on cyclophosphamide toxicity in rats. *Drug Chem Toxicol*. **2009.** 32: 424-428.

[11]. Sharma, N., et al., Inhibitory effect of *Emblica officinalis* on the *in vivo* clastogenicity of benzo[a]pyrene and cyclophosphamide in mice. *Hum Exp Toxicol*. **2000**. 19: 377-384.

[12]. Kern, J.C., Kehrer, J.P. Acrolein-induced cell death: A caspase-influenced decision between apoptosis and Oncosis/Necrosis. *Chem Biol Interact*. **2002**. 139: 79-95.

[13]. Manda, K., Bhatia, A.L. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biol Toxicol*. **2003**; 19: 367-372.

[14]. Palomba, S., et al. Evidence based and potential benefits of metformin in the polycystic ovary syndrome: A comprehensive review. Endocr Rev. **2009**. 30:1-50.

[15]. Faure, P., et al., An insulin sensitizer improves the free radical defense system potential and insulin sensitivity in high fructose-fed rats. *Diabetes*. **1999**. 48 :353-357.

[16]. Khouri, H., et al., Radical-induced oxidation of metformin. *Eur J Biochem*. **2004.** 271: 4745-4752.

[17]. Jacotot E, et al., Mitochondrial membrane permeabilization during the apoptotic process. *Ann N Y Acad Sci.* **1999**. 887: 18-30.

[18]. Beutler, E., et al. Improved method for the determination of blood glutathione. *J lab clin Med*. **1963**. 61: 882-890.

[19]. Ohkawa, H., et al. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. **1979.** 95: 351-358.

[20]. Lamar, J.M. Mastering the Trichrome stain in: Special stains education guide, Second Edition, edited by G. L. Kumar and J. A. Kiernan. Chapter 10. **2011**:93-97. California, USA.

[21]. Ayhanci, A., et al. Protective effects of ellagic acid in D-galactosamine-induced kidney damage in rats. *Cytotechnology*. **2016.** 68: 1763-1770.

[22]. Armitage, P., Berry, C. Statistical methods in medical research, 3rd edition. Blackwell Scientific Publications, London.**1994**: 40-48.

[23]. Ahmed, A., Alkreathy, H.M. Comparative biochemical and histopathological studies on the efficacy of metformin and Nigella sativa oil against thioacetamide-induced acute hepatorenal damage in rats. *Biomed Res*. **2018**. 29: 3106-3116.

[24]. Moignet, A., et al. Cyclophosphamide as a first-line therapy in LGL leukemia. *Leukemia*. **2014.** 28: 1134-1136.

[25]. Lawson, M., et al. Urological implications of cyclophosphamide and Ifosfamide. *Scand J Urol Nephrol***. 2008.**42: 309-317.

[26]. Papaldo, P., et al., Impact of five prophylactic filgrastim schedules on hematologic toxicity in early breast cancer patients treated with epirubicin and cyclophosphamide. *J Cli Onco*. **2005**. 23: 6908-6918.

[27]. Basu, A., et al. Vanadium (III)-l-cysteine enhances the sensitivity of murine breast adenocarcinoma cells to cyclophosphamide by promoting apoptosis and blocking angiogenesis. *Tumor Biol.* **2017.** 39.

[28]. Cuce, G., et al. Chemoprotective effect of vitamin E in cyclophosphamide-inducedhepatotoxicity in rats. *Chemico-Biological Interactions.* **2015**. 232: 7-11.

[29]. Germoush, M.O, Mahmoud, A.M. Berberine mitigates cyclophosphamide-induced hepatotoxicity by modulating antioxidant status and inflammatory cytokines. *J Cancer Res Clin Oncol*. **2014**. 140: 1103-1109.

[30]. Bailey, C.J. Biguanides and NIDDM. *Diabetes Care*. **1992**.15: 755-772.

[31]. Ling, S., et al. Metformin ameliorates arsenic trioxide hepatotoxicity via inhibiting mitochondrial complex I. *Cell Death & Disease*. **2017**. 8: e3159.

[32]. Hamdy, R., et al., Ameliorative effects of bone marrow derived pancreatic progenitor cells on hyperglycemia and oxidative stress in diabetic rats. *Acta Histochem*. **2018**.120: 412-419.

[33]. Hashem, M.M., et al., Metabolic profile and hepatoprotective effect of Aeschynomene elaphroxylon (Guill. & Perr.). *PLoS One*. **2019**.14: e0210576.

[34]. Hassanen, E.I., et al., Toxicopathological and immunological studies on different concentrations of chitosan-coated silver nanoparticles in rats. *Int J Nanomedicine*. **2019**. 14: 4723-4739.

[35]. Borole, K.D., et al. Assessment of antioxidant activity of metformin in ethanol induced liver damage in Sprague Dawley rats. *Int J Basic Clin Pharmacol*. **2016**. 5: 324-328.

[36]. Mansour, H.H., et al. Metformin and low dose radiation modulates cisplatin-induced oxidative injury in rat via PPAR-gamma and MAPK pathways. *Arch Biochem Biophys.* **2017**. 616: 13-19.

[37]. Razavi, A.K., et al. The protective role of phenolic compounds against doxorubicin-induced cardiotoxicity: A comprehensive review. *Nutrition and Cancer*. **2016.** 68: 892-917.

[38]. Roohbakhsh, A., et al. Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. *Biomed Pharmacother*. **2017**. 91: 31-42.

[39]. Saeedi, S.S., et al. The protective potential of metformin against acetaminophen-induced hepatotoxicity in BALB/C mice. *Pharm. Biol*. **2016**. 54: 2830-2837.

[40]. Salminen, A., Kaarniranta, K. AMP-Activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Research Reviews***. 2012**. 11: 230-241.

[41]. Green, D.R., et al. Metabolic control of cell death. *Science*. **2014**. 345: 1250256.

[42]. Mustafa, C., et al. Hepato-preventive and anti-apoptotic role of boric acid against liver injury induced by cyclophosphamide. *J Trace Elem Med Bio*. **2019**. 53:1-7.

[43]. Alqahtani, S. Mahmoud A. Gamma-glutamyl cysteine ethyl ester protects against cyclophosphamide-induced liver injury and hematologic alterations via upregulation of PPAR and attenuation of oxidative stress, inflammation, and apoptosis. *Oxidative Medicine and Cellular Longevity*. **2016**. 14: 4016209.

[44]. Mahmoud, AM. Hesperidin protects against cyclophosphamide-induced hepatotoxicity by upregulation of pparγ and abrogation of oxidative stress and inflammation. *Can J Physiol Pharm*. **2014.** 92: 717- 724.

[45]. Mahmoud, AM., Al-Dera, H.S. 18β-Glycyrrhetinic acid exerts protective effects against cyclophosphamide-induced hepatotoxicity: Potential role of PPARγ and Nrf2 upregulation. *Genes & Nutrition*. **2015**.10: 41.

[46]. Kamel, E.M., et al. A phytochemical and computational study on flavonoids isolated from Trifoliumresupinatum L. and their novel hepatoprotective activity. *Food & Function***. 2016**. 7: 2094-2106.

[47]. Cengiz, M., et al. Kekik Bitkisinin Temel Bileşeni Olan Karvakrolün Sıçanlarda Siklofosfamid Nedenli Üreme Sistemi Hasarı Üzerine Koruyucu Etkileri. *Turk J Agric For*. **2017**; 4: 171-175.

[48]. Cengiz, M., et al. Comparative studies on the therapeutic effects of silymarin and silymarin-loaded solid lipid nanoparticles on D- GaIN/TNF-α-induced liver damage in Balb/cmice. *Food Chem Toxicol*. **2015**. 77: 93-100.

[49]. Senthilkumar, S., et al. Effect of squalene on cyclophosphamide-induced toxicity. *Clin Chim Acta*. **2006**. 364:335-342.

[50]. Abd El-Hady, S.H., et al. Metformin attenuates thioacetamide-induced hepatotoxic effects in rats. *Arizona J Pharmaceut Sci*. **2015**.52.

[51]. Germoush, M.O. Diosmin protects against cyclophosphamide-induced liver injury through attenuation of oxidative stress, inflammation and apoptosis. *Int J Pharm Pharm Sci*. **2016**. 12: 644-654.

[52]. Iranshahya, M., et al. Hepatoprotective activity of metformin: A new mission for an old drug. *Eur J Pharmacol*. **2019**. 850:1-7.

[53]. Woudenberg, T.E, et al. Metformin protects rat hepatocytes against bile acid- Induced apoptosis. *PLoS ONE*. **2013**. 8: e71773.