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Hepatoprotective Effect of Carica papaya and Actinidia delicosa against Methotrexate Induced Liver Toxicity

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Abstract: Methotrexate is an effective drug for rheumatoid artheritis and cancer but has many harmful side effects. C. papaya and Actinidia delicosa (kiwi) fruits are hepatoprotective agents. This study explored the hepatoprotective role of C. papaya and Actinidia delicosa pretreatment against methotrexate (MTX) that induced hepatotoxicity in male rats. We prepared ethanolic extraction of C. papaya and Actinidia delicosa fruits then measured the total phenolics, total flavonoids and antioxidant activity of each extract. Forty rats were divided into four groups (10 rats each); control, MTX, (C. papaya + MTX) and (kiwi + MTX) groups. MTX group injected with (20mg/kg/Intra peritoneal twice weekly) at 5th week, (C. papaya + MTX group) pretreated with C. papaya fruit ethanolic extract (400 mg/kg/orally/day) for 8weeks + MTX (20mg/kg/Intra peritonial twice weekly) at 5th week and (Kiwi + MTX group) pretreated with Actinidia delicosafruit ethanolic extract (400 mg/kg/orally/day) for 8weeks before MTX injection (20mg/kg/Intra peritonial twice weekly) at 5th week. We measured (MDA) level, reduced Glutathione (GSH) and Superoxide Dismutase (SOD) in the liver homogenate. Moreover, Nitric Oxide (NO), Alanine Aminotransferease (ALT) and Aspartate Aminotransferase (AST) levels were measured in the serum. Histopathological examination of liver tissue. Biochemical analysis revealed significant increase in the liver MDA, serum NO and serum transaminases meanwhile liver GSH and SOD were significantly decreased in MTX group. C. papaya and Actinidia delicosa fruit ethanolic extract pretreatment significantly ameliorated histopathological and biochemical changes induced by MTX.

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Key words: MTX (methotrexate), Carica papaya fruit ethanolic extract, Actinidia delicosa (kiwi) fruit ethanolic extract

1. Introduction

Methotrexate (MTX) is the first drug in treatment of number of rheumatic and non-rheumatic diseases. It is effective in controlling disease activity and preventing disease-related damage, and significantly cheaper than many alternatives **(Conway & Carey, 2017).** MTX also, is an effective cytotoxic drug and has been widely used in chemotherapeutic based treatments for malignancies primarily in leukaemias **(Green, 2012)** but long-term methotrexate use, or its use in high doses, may cause hepatic steatosis, cholestasis, fibrosis and cirrhosis. Accordingly the dose of methotrexate should be lowered or the drug should be discontinued in case of hepatic toxicity which causes delay in the treatment of the disease. On the other hand much attention is now being paid to factors that may enhance the effectiveness of existing drugs while reducing their unwanted side effects **(Çakır et al.,2015)**.

Medicinal fruits played vital role in protecting human and animal body from harmful and toxic side effects of strong effective drugsas they containbioactive phytochemicals and natural antioxidants given their activity against free radicals, which contribute to chronic degenerative diseases and decreased risk for liver diseases **(Soni and Singhai, 2012)**. Caricaceae is a small family of angiosperms comprising six genera and 43 species. Carica papaya or papaw or papaya is the most popular and economically important species among the Caricacae family. Among the total tropical fruit production in the world (2012) **(Ezekiel Amri & Mamboya 2012).** *Carica papaya* (known in Ayurveda as Erand-karkati) is also well known for its medicinal properties **(Khare 2004)**. Traditionally, different parts of the papaya plant are used in the treatment of various ailments such as asthma, ulcers, eczema, diabetes, helminth infections and fever **(Nguyen et al. 2013)**. Research also demonstrated its beneficial traditional role in wound healing, and in the treatment of liver, cardiovascular diseases, dengue fever, cancer, malaria, hypoglycemia, hyperlipidemia, fungal diseases and as

a male contraceptive **(Nunes et al. 2013**). Papaya fruit has its physicochemical properties, its phytochemicals and antioxidant capacity and so it has value in liver protection from damage **(Addai et al.,2013).** Papaya rich in phytochemicals that essentially split into two groups (primary and secondary metabolites). Primary metabolites include general sugar, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins etc., **(Dhongade and Chandewar, 2013**). Actinidia deliciosa which is famous as the king of fruits because of its remarkable highest vitamin C content and balanced nutritional composition of minerals, dietary fibre and different health - beneficial metabolites **(Huang et al., 2013).** Another name of Actinidia deliciosa as well known as green kiwi fruit is a member of the Actinidiaceae family. It is a member of the order of scaling woody vines. The genus Actinidia consist of kind with eating fruits **(Halimoon and Abdul Hasan, 2010).** Kiwi fruit contain isoflavones and flavonoids, as important phytochemical in kiwifruit extract have an important function as hepatoprotective, anti-carcinogenic, neuroprotective and cardio protective activity. Other researcher have indicated that kiwi fruit is a more potent antioxidant than vitamin C **(Vissers et al.,2011).** Recent studies have shown that kiwifruit has powerful antioxidant activity in vitro and in vivo in both experimental animal and humans **(Brevik et al., 2011).** Also kiwifruit is used for treatment of many different types of cancers, e.g. stomach, lung, and liver cancer in eastern medicine. In this present study we aimed to investigate whether the PEE and KEE had a prophylactic effect against MTX induced oxidative injury on the liver of male rats.

2. Materials and Methods

A) Materials

1) Animals:

The present study carried out on a total number of 40 healthy male Sprague Dawely rats, weighting130-160 gm were purchased from Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University. They were kept at the Animal House of, Faculty of Veterinary Medicine, Suez Canal University. They were housed in separate metal cages under controlled environmental (20-40ºC and 55-60% relative humidity) and nutritional conditions. They were maintained on standard balanced ration for 2weeks accommodation and they had free access of water and food.

2) **Drugs and chemicals**:

Methotrexate Sodium Vial 50mg/20ml (MTX) Mylan company Germany.

3) **Plants**:

Papaya and kiwi fruit:

Fresh and ripe fruits were purchased from local market in Ismailia.

4) Experimental design:

The duration of experiment was 8weeks from beginning of (papaya and kiwi) administration. Forty male albino rats weighting at the beginning of the experiment 130-160 gm were randomly divided into four groups (10 each) according to (**Kim et al., 2014)**:

Group (1): Normal control group: they kept on a standard diet and injected I/P with 0.2ml distilled water.

Group (2): Methotrexate (MTX) injected group: they kept on a standard diet and injected I/P with (20 mg/kg b.wt.MTX on the 5th week twice weekly)

(**Mehrzadi et al., 2018** and **Asci et al.,2017**).

Group (3): Carica papaya (CP) treated group: they kept on a standard diet and gavaged daily with ethanolic extract of CP at dose 400 mg/kg/day CP extract by oral route for 8weeks (**Ramesh et al.,2016** and **Mohamad et al.,2018**) and injected I/P with **(**20 mg/kg b.wt.on the 5th week twice weekly).

Group (4): kiwi treated group: they kept on a standard diet and gavaged daily with ethanolic extract of kiwi at dose 400 mg/kg/day by oral route for 8weeks and injected I/P with (20 mg/kg b.wt.MTX on the 5th week twice weekly) **(Jin et al.,2014**).

All groups were kept under the same condition for 8weeks under different treatments then one day after last treatment all rats were sacrified all samples were collected.

2- Phytochemical analysis

Chemical tests were organized on the ethanolic extracts (99.9%), of the fruits samples using standard methods.

2.1. Total Phenolic Content Determination

The total phenolic content of *C.papaya* and *Actinidia delicosa* fruit ethanolic extracts was determined by Folin-Ciocalteu spectrophotometric method (**Hamad et al.,2015**). Aliquate of 0.1 mL of Folin-Ciocalteu reagent was added to 2 mL of reconstituted extract. The mixture was allowed to stand for 15 min. Then, 3 mL of saturated sodium carbonate 2% (Na2CO3) was added. The mixture was allowed to stand for 30 min at room temperature and the total phenolic content was determined using spectrophotometer (Labo America, USA) at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of mg of gallic acid equivalent per gram of the sample using the linear regression equation obtained from the standard gallic acid calibration curve y = 0.014x - 0.168. All samples were analyzed in triplicates.

2.2. Total Flavonoid Content (TFC):

The TF content was determined by the colorimetric method as described by **Abu Bakar et al., (2009).** A total 0.5 mL of the extract was mixed

with 2.25 mL of distilled water in a test tube, followed by the addition of 0.15 mL of 5% (w/v) NaNO2solution. After 6 min, 0.3 mL of a 10% AlCl3·6H2O solution was added and the reaction was allowed to stand for another 5 min before 1.0 mL of 1 M NaOH was added. The mixture was mixed well by vortexing and the absorbance was measured immediately at 510 nm using a spectrophotometer (Epoch, Biotek, USA). The results were expressed as milligrams of Quercetin Equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW).

2.3. Antioxidant Activity Evaluation

The ability of the plant extracts to scavenge DPPH free radicals was assessed by the standard method of (**El Sohaimy et al.,2015**). Adopted with suitable modifications (**Hamad et al.,2017**). A stock solution of each extract was prepared in methanol to final concentration 1 mg/mL. Plant serial dilutions were made; about 1mL of each dilution was mixed with 1mL of methanolic solution of DPPH in a concentration of 1 mg/mL. After 30 min incubation in darkness, the absorbance was measured at 517 nm. IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm, after inhibition percentage was calculated using following equation;

Inhibition % = [(A of control - A of sample) /A of control] x100 =

2.4 HPLC Conditions for Phenolic Compounds (µg/ml), flavonoids (µg/ml) and vitamin C (µg/ml) Quantification

Quantification of phenolic, flavenoid compounds and vit.C of two fruit ethanolic extract were determined via High Performance Liquid Chromatography (HPLC) according to (**Sancho et al.,2011**). Nine phenolic standards of phenolic compounds, were used; gallic acid, caffeic acid, coumaric, syringic acid, vanillin, cinnamic acid and salicylic acid, freulic and chlorogenic, eight flavonoid standards of flavonoid compounds, were used; catechin, kaempferol, rutin, rosmarinic, quarcitin, hesperetin, apigenin and querctin and one vit.C standard for vit.C. Agilent 1260 infinity HPLC series (Agilent, USA), equipped with quaternary pump, a Zorbax Eclipse plus C18 column 100 mm x4.6 mm i.d., (Agilent technologies, USA) operated at 25°c was used for phenolic compound analysis. The injected volume was 20µ: VWD detector set at 284 nm. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade 0.2% H3PO4 (v/v), (B) methanol and (c) acetonitrile. The injection volume for 2 fruit ethanolic extracts were 1g/10 mL. All standards were dissolved in ethanol and injected with the different concentrations.

3- Sampling:

3.1Blood sampling:

At the end of experimental period rats from all groups were sacrificed and blood samples collected in a clean dry capped tubes. The blood samples were about 5ml collected without anticoagulant, left to clot at room temperature then centrifuged at 4000 r.p.m. for 5 min. to separate serum for biochemical analysis.

3.2 **Tissue samples**

3.2.1. Liver tissues from all rats were collected and divided into 2 parts:

1. first part was taken from all rats in all groups weighted 1gm, washed and kept on -20 till homogenized in distilled water using electrical homogenizer, centrifuged at 3000 r.p.m. for 15min., the resulting supernatant were collected and used for determination of lipid peroxidation (Malondialdehyde MDA), antioxidant levels (reduced glutathione GSH) and antioxidant enzyme activities superoxide dismutase (SOD).

2. The second part was collected from rats per group preserved in 10% neutral buffered formalin, processed and stained with haematoxylin and eosin (H & E) dyes for histopathological study using a light microscope according to **Bancroft et al., 2008.**

4. Biochemical analysis:

4.1. Determination of nitric oxide (NO):

Nitricoxide (NO) concentration was determined according to the method described by **Jabłonska et al. (2007).**

4.2. Determination of lipid peroxidation marker Malondaaldhyde (MDA) concentration according to the method of El Morsy et al., (2015).

4.3. Dtermination of reduced glutathione (GSH) in liver homogenate according to method described by Baeza et al., (2010).

4.4. Determination of superoxide dismutase (SOD) activity in liver homogenate according to method described by Kakkkar et al., (1984).

4.5. Determination of serum alanine aminotransferase (ALT):

Serum ALT level was determined according to **Maneerat et al., (1996).**

4.6. Determination of aspartate aminotransferase (AST):

The activity of serum AST was determined according to **(Maneerat et al., 1996)**.

5. Histopathological examination:

Following complete fixation of the collected specimens, the formalin-fixed samples were then preserved in 70% ethyl alcohol. The preserved specimens were dehydrated in an ascending, graded ethanol series (75%, 80%, 90%, 95% and three changes of absolute alcohol), subjected to three changes of xylene, and then routinely embedded in paraffin wax. The paraffin embedded specimens were serially sectioned at 5 – 7 µm thickness using a rotatory microtome. The obtained paraffin sections

were mounted on glass microscope slides then subjected to Harris hematoxylin and Eosin (H & E) for histopathological examination. The cover slips were mounted using Dpx.

Photomicrography

The obtained photomicrographic images for histopathological evaluations was taken using Olympus BX41 research optical photomicroscope fitted with an Olympus DP25 digital camera. The magnification scale bar was reported on the obtained photomicrographs.

6-Statisticalanalysis:

The obtained data of the present study were analyzed statistically using one way analysis of variances (ANOVA) for all tested groups. The present Data were analyzed using (SPSS, 20) for windows. Data were presented as mean ± stander error (SE) and results were considered significant at probability level of 0.05 ($P < 0.05$).

3. Results

3.1. Total phenolic and total flavenoide contents of kiwi and papaya fruit extract:

3.1.1 Total phenolic content

They were expressed as milligrams of gallic acid equivalents per gram of dry weight of extract of plant (DW) (mg GAE/g DW) in extract. There is significant increase in total phenolics of kiwi fruit extract than papaya fruit extract.

Table (1): Total phenolics content of Fruit extract:

Fruit extracts	Total phenolics (mg/g)
Papaya	136.60± 1.08[b]
Kiwi	138.96±0.5[a]

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different upper case letters are significantly different ($p<0.05$).

3.1.2. Total flavonoid contents of kiwi and papaya fruit extract:

The results were expressed as milligrams of Quercetin Equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW). We found that there is significant increase in total flavenoide contents in kiwi fruit extract than papaya fruit extract as showed on table (2).

Table2. Total flavonoids content of papaya and kiwi fruit extract:

Fruit extracts	Total flavonoids (mg/g)
Papaya	**27.50±1.19[b]**
Kiwi	**40.36±0.92[a]**

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different upper case letters are significantly different ($p<0.05$).

3.1.3. Antioxidant Activity Evaluation:

The inhibition radical scavenging assay by using DPPH shown in the table 4) by using ascorbic acid as standard showed that increase antioxidant activity in kiwi fruit extract than papaya fruit extract.

Table (4): The inhibition concentration values (IC50) value of three fruits extracts:

fruit extracts	DPPH (IC50) µg/ml
Ascorbic	4.38[a]
Papaya	47.09[b]
Kiwi	132.49c

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different upper case letters are significantly different ($p<0.05$).

3.1.4. Phenolic compounds analysis of fruit extract (papaya and kiwi) via HPLC:

The Kiwi extract showed high concentration levels of phenolic compounds than papaya extract exhibited a low concentration levels.

Table (5): Phenolic compounds analysis of fruit extract (papaya and kiwi) via HPLC

Phenolic compound	Concentration (µg/ml)	
	Papaya	Kiwi
A- Gallic Acid	15.54	29.13
B-Chlorogenic Acid	23.14	31.54
C-Salicylic acid	19.45	24.26
D-Syringic Acid	12.42	14.98
E-Coumaric Acid	19.65	25.29
F-Vanilic acid	24.31	16.24
G-Ferulic Acid	19.36	35.11
H-Cinnamic Acid	21.68	32.05

3.1.5 Flavonoid compounds analysis of plant extract (papaya and kiwi) via HPLC:

The Kiwi extract showed high content of flavonoid compounds than papaya extract exhibited a low concentration levels.

Table (6): Flavonoid compounds analysis of plant extract (papaya and kiwi) via HPLC

Flavonoid compound	Concentration (µg/ml)	
	papaya	Kiwi
Catechin	21.46	32.75
Kaempferol	35.10	47.57
Rutin	42.28	36.12
Rosmarinic	24.49	65.41
Hesperetin	25.35	34.65
Apigenin	19.45	26.48
Querctin	34.78	64.32
Narengnin	43.15	66.63

3.1.6 Vit C content analysis of fruit extract (papaya and kiwi) via HPLC:

Kiwi extract exhibited a concentration level of vitamin C content (1.52 µg/ml) much more higher than that in papaya extract detected (0.27 µg/ml).

Table (6): Vit. C content detected via HPLC in papaya and kiwi fruit extracts.

Vit c	Concentration (µg/ml)	
	Papaya	Kiwi
Vit c	0.271	1.52

3.2. Biochemical analysis:

3.2.1 Prophylactic effect of kiwi and papaya fruit extract on serum parameters in MTX induce hepatotoxicity:

Our results showed a significant increase in NO level (µmol/l) and MDA **(mmol/g)** in MTX treated groups when control healthy group. Also we found significant reduction in **SOD (U/g tissue)** and **GSH (ng/g tissue)** in MTX treated groups when compared with control healthy group. Meanwhile the papaya and kiwi pretreated groups showed a significant increase when compared with the MTX treated group as saw in table (9) Meanwhile the papaya and kiwi pretreated groups showed a significant reduction when compared with the MTX treated group.

Table (7): Effect of papaya and kiwi fruit extract on some antioxidant biochemical parameters of male rats (means ± SE) on different groups.

	NO (µmol/l)	MDA (mmol/g tissue)	SOD (U/g tissue)	GSH (ng/g tissue)
Control	45.83±1.17 [c]	37.44±0.98 [c]	60.46±2.27 [a]	30.76±0.97 [a]
Methotrexate	65.28±1.01 [a]	83.7±0.63 [a]	38.59±2.85 [b]	14.22±0.59 [b]
Papaya + methotrexate	51.12±2.62 [b]	48.88±3.85 [b]	63.79±0.5 [a]	28.34±2.58 [a]
kiwi + methotrexate	43.81±0.82 [c]	36.2±0.96 [c]	57.65±1.64 [a]	26.95±0.62 [a]

Means within the same column carrying different superscripts are sig. different at $P < 0.05$ based on Tukey's Honestly Significant Difference (Tukey's HSD) test.

3.2.2 Prophylactic effect of kiwi and papaya fruit extract on tissue (liver) parameters in MTX induce hepatotoxicity:

Our results showed a significant increase in CRP (ng/ml), ALT (IU/L) and AST (IU/L) in MTX treated groups when compared with control healthy group. Meanwhile the papaya and kiwi pretreated groups showed a significant reduction when compared with the MTX treated group...

Table (8): Effect of kiwi and papaya fruit extract on antioxidant biochemical parameters of male rats (means ± SE) on different groups

parameters Groups	ALT (IU/L)	AST (IU/L)
Control	44.1±0.37 [b]	44.35±0.42 [b]
Methotrexate	66.8±2.1 [a]	72.15±1.82 [a]
Papaya + methotrexate	49.62±3.47 [b]	47.73±1.52 [b]
kiwi + methotrexate	45.13±1.43 [b]	44.18±0.72 [b]

Means within the same column carrying different superscripts are sig. different at $P < 0.05$ based on Tukey's Honestly Significant Difference (Tukey's HSD) test.

4. Histopathological findings:

The untreated control group showed normal hepatic architecture where the hepatic lobular structure seemed quite normally arranged. The hepatic lobule contains a central vein from which radiating cords of hepatocytes with hepatic sinusoids in-between (Fig. 1A). The liver of methotrexate-treated group exhibited sever hepatic alterations. Signs of sinusoidal dilatation and mild congestion of liver parenchyma; numerous intense inflammatory cells infiltrate, mild bile ductal proliferation and vacuolar degenerative changes of some pyknotic hepatocytes (Fig. 1B). Animals treated with papaya then MTX injection at 58 day showed less hepatic vascular congestion; pyknosis of some hepatocytes, mild inflammatory cells infiltrate and less vacuolar degenerative changes of hepatocytes (Fig. 1C). Meanwhile, animals treated with kiwi then MTX injection at 58 day showed no morphological changes in liver (Fig. 1D). In kiwifruit-treated rats, the liver structures were not different from the untreated control.

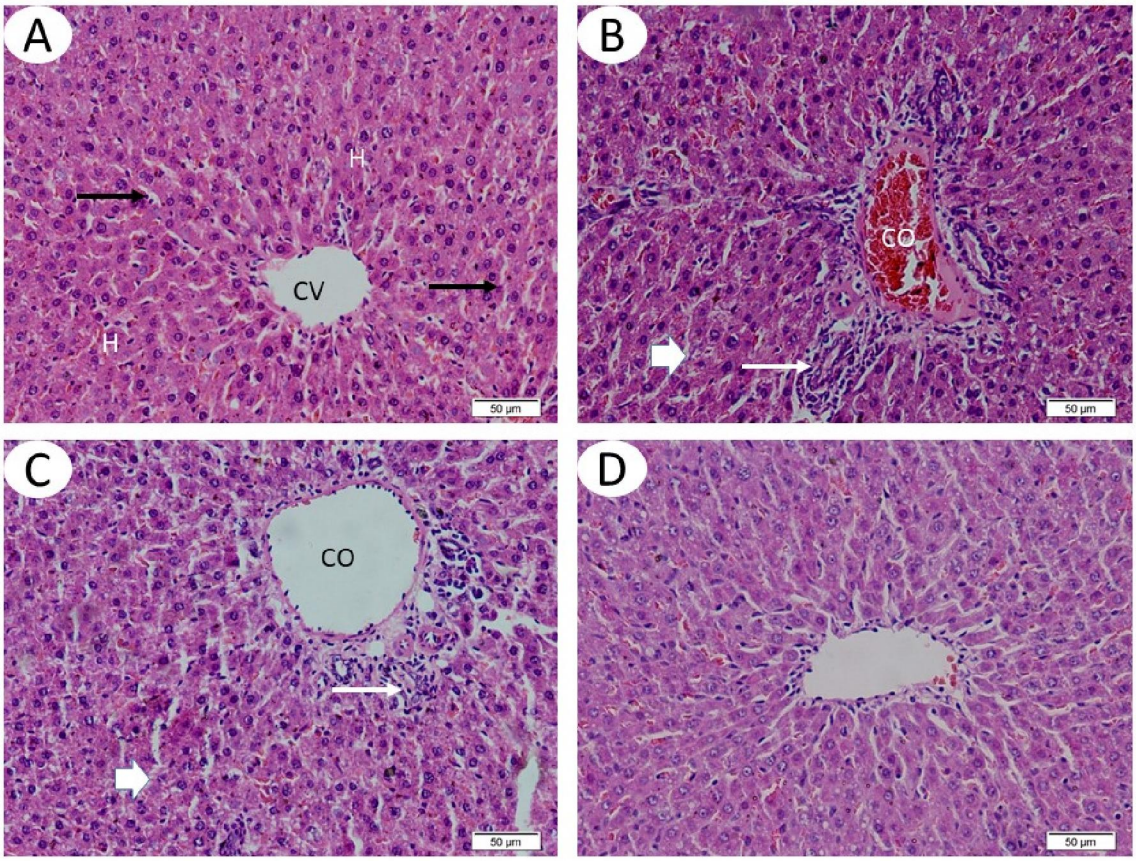

Fig. 1: A representative photomicrograph of liver sections, untreated control group (A), methotrexate-treated group (B), papaya-treated group (C), kiwifruit-treated group (D). Central hepatic vein (CV), hepatocytes (H), hepatic sinusoids (black thin arrows), inflammatory cells infiltrate (white thin arrows), degenerated hepatocytes (whit thick arrows), and congested and dilated blood vessels (CO). H & E

4. Discussion

Although methotrexate is effective in treatment many diseases it cause hepatotoxic effect. Our results increased MDA, NO and CRP suggested that oxidative stress and excessive generation of reactive oxygen and nitrogen species (ROS/RNS), along with reduced antioxidant defense mechanism promote the development and progression of hepatotoxicity and increasing liver enzymes (ALT and AST) also, liver histopathology confirmed our results.

Our results agreed with **Vardi et al.,2009 who** demonstrated the role of ROS/RNS in the pathogenesis of MTX-induced hepatotoxicity. These highly reactive species react with biological macromolecules producing lipid peroxides, inactivating proteins and mutating DNA. MTX significantly altered the oxidant/antioxidant balance. MTX increased MDA level accompanied with decreased GSH content and SOD activity. Also, **Cetin et al., 2008** supports the hypothesis that oxidative

cellular damage with profound lipid peroxidation are hallmarks of MTX toxicity. Methotrexate is an antimetabolite that competitively inhibite the folic acid metabolism thus impairs the DNA synthesis. 7-hydroxymethotrexate (7-OH-MTX) is the major extracellular metabolite of MTX that is metabolized in the liver by an enzymatic system. In the cell MTX store in a polyglutamate form. With the use of MTX intracellular amount of polyglutamate increases on the other hand folic acid levels decreased, that leads to necrosis of hepatocyte. Hepatotoxic effect of methotrexate was caused by an increase of its polyglutamate form intracellularly. The hepatotoxic effects of MTX have been reported in many studies as **Sener et al.,2006.**

Lipid peroxidation by free oxygen radicals is an important causes of destruction and oxidative damage to cell membranes these containing unsaturated fatty acids, nucleic acids and proteins. It has contribute to develop methotrexate associated tissue damage. With the attack of free oxygen radicals lipid peroxidation increase and fail the Na+/K+-ATPase activity. Na+/K+-ATPase is the other target of cellular oxidative tissue damage. In this present study, MTX administration caused to a significant liver tissue damage since MDA which was the end product of lipid peroxidation is increased while Na+/K+-ATPase activity is depressed due to damage of cell membrane. Liver tissue injury was also observed microscopically. On the other hand, following MTX administration, treatment with ALA was significantly reduce the MDA levels and increased the Na+/K+-ATPase enzyme activity, while normal histological appearance was observed in liver tissue. Glutathione (GSH) plays a particularly important role in the maintenance and regulation of the thiol-redox status of the cell. Tissue GSH depletion is one of the primary factors permitting liver tissue damage is associated with oxidative stress caused by MTX (**Çakır et al., 2015**)

Our study showed that treatment with MTX caused dilatation and congestion of portal vein epatic necrosis, inflammatory cell infiltration especially in the periportal area and widespread intracellular vacuolization in hepatocytes and dark eosinophilic cytoplasm and heterochromatic, fragmented nuclei in hepatocytes and apoptotic bodies and vascular-sinusoidal congestion.

The nutritional and biochemical results were in accordance with the fact that that antioxidant micronutrients may account for the beneficial effects of fruits on human health. Consumption of fruit decreases oxidative DNA damage in human cells. The antioxidant status of papaya ethanolic extract could prevent the biochemical and histological changes of methotrexate as Papaya mediating its protective effects either by decreasing the metabolic activation of methotrexate, or by acting as a chain-breaking antioxidant for scavenging free radicals or by a combination of these effects and the presence of high aphenolics and flavonoids in *CPE* that increased its antioxidant activity. our results agreed with (**Adeneye and Olagunju, 2009**) who studied the protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities of *CPE* and could be via antioxidant and/or free radicals scavenging activities. *C. papaya*. treat the elevated serum levels of ALT and AST in a high dose through protection of liver tissue from toxic effect of MTX as it contain high phenolics and flavonoid contents (**Awodele et al.,2016)**.

Kiwi fruits are rich in many flavonoids and minerals vitamin C, folate, and vitamin E. In particular, they contain a high amount of vitamin C (more than oranges), as much potassium as bananas and a good amount of beta-carotene (**Amer et al., 2014)**. Vitamin C is a water-soluble antioxidant that has been proven to protect body from free radicals, dramatically improving the health of individuals who consumed it regularly against all kinds of disease, so can protect rats treated group from methotrexate hepatotoxicity and this agreed with **(Hunter et al., 2012)** who studied kiwi antioxidant activity as The body absorbs the antioxidants found in kiwi fruit more effectively than other antioxidant-rich fruits. Pretreatment with KFE prior Indo administration resulted in marked ameliorations of the hepatic lesions induced by Indo. We can conclude that kiwi fruit extract is useful in combating tissue injury caused by indomethacin toxicity and protect hepatic tissues from toxicity of indomethacin **(Amer et al.,2014).**

Conclusions

The result of this study revealed that ethanol extract of *Carica papaya and kiwi fruit extract* both had protective effect against MTX hepatotoxicity when they administered at the single, daily oral dose of 400 mg/kg body weight for 4 weeks before MTX injection.

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