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Pharmacological study of green tea extract effect against methotrexate-Induced liver injury in male rate

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Abstract

This study was performed to evaluate the influence of simultaneous administration of aqueous green tea extract (15%) with methotrexate (0.5 mg/Kg) on the status of glutathione (GSH) in liver tissue, with measuring the activity of serum liver enzymes (alanin aminotransferase ALT,aspartate aminotransferase AST) and serum bilirubin level.

Administration of aqueous green tea extract alone resulted in asignificant increase in liver tissue GSH level and when it was used with MTX it ameliorated MTX- induced change in level of this parameter.

Regarding enzymes ,administration of aqueous green tea extract resulted in an alleviating effect on MTX-induced elevation in serum ALT activity with non –significant reduction in serum AST activity : it also significantly reduced MTX-induced elevation in total serum bilirubin level, these effect may be related to direct or indirect antioxidant property of green tea extract.

INTRODUCTION:

A large number of drugs and chemicals can produce liver injury, drug - induced liver disease may present as a mild reaction or , much more seriously, as acute liver failure or chronic disease (1).Methotrexate(MTX)is one of the folic acid antagonists that is widely used in the therapy of various types of disease like psoriasis (2).psoriatic arthritis (3).One of the important long-term side effects includes liver fibrosis and cirrhosis because of MTX hepatotoxicity(4).

Green tea has been considered a medicine and a healthful beverage since ancient times (5).Several studies have shown that green tea component can act as an antioxidant by trapping proxyl radicals and inhibiting lipid peroxidation (6).The present study is designed to investigate the role of oxidative stress in M IX-induced hepatotoxicity, also to clarify the possible protective eeffect of orally-administered green tea, in experimental animal model against this toxicity.

MATERIALS AND METHODS:

Drugs:-

1- Methotrexate:

Methotrexate was used in a dose of (0.5mg/kg). Avial containing 50 mg/5 ml (Ebewe, Austria) was given to the rats according to the body weight twice weekly by intraperitonial injection.

2-Greenteaextract:

Green tea (lipton, UAE) was prepared according to maity and his team (7), by soaking 15g of instant green tea powder in 1L of boiling distilled water for 5minutes.The solution was filtered to make 1.5 % green tea extract . this solution was provided to rats as their sole source of drinking water.

Animal selection:

The study was carried out on 36 male adult Albino-Swiss rats weighing (200-250)gm .animal were housed in cages under controlled temperature around 25C and 12hours light-dark cycles.They were fed astandard commercial pellets and allowed free access to tap water.

Experimental design:

The animal were randomly divided into 4 groups, each containing nine animals as followed:

Group I (control group):

Rats were not receiving any drug during the period of the study(14weeks).

Group II (MTX group):

Rats were given an intraperitonal injection og methotrexate (MTX)
(0.5mg/Kg)and it was given two times weekly for14weeks.

Group III (green tea group):

Rats were received aqueous green tea extract (1.5%) (which was provided
to rats as their sole source of drinking water for 14 weeks).

Group IV(MTX-green tea group):

Rats were given an intraperitonial injection of methtrexate (0.3mg/kg)two times weekly for 14 weeks .They were also received aqueous green tea extract which was provided to rats as their sole source of drinking water for the same period of time.

Preparation of sample and analysis:

Preparation of serum samples:

At the end of 14 weeks the animals were anesthetized by ether , blood was
collect (3ml from each rat) by intracardiac puncture. Blood samples were centrifuged at 3000 rpm for 15minutes. Then, the serum was used for the estimation of ALT, AST activities and bilirubin levels.

Determination of serum alanine amino transferase (ALT):

Serum alanine aminotransferase (ALT) was determined according to the method of reitman and frankel in 1957 (8), using a readymade kit for this purpose. The principle of this method depends on colorimetric measurement of pyruvate hydrazone formed after the reaction of pyruvate with 2,4-dinitrophenyl hydrazine.

Determination of serum aspartate amino transferase (AST):

Serum aspartate aminotransferase (AST)was determined according to the method of reitman and frankle in1957 (8)using a readymade kit for this purpose . The principle of this method depends on the colorimetric measurement of oxaloacetate hydrazone formed from the reaction of oxaloacetate with 2,4-dinitropheny1-hydrazine.

Determination oftotal serum bilirubin:

Total serum bilirubin was determined according to sulfanilic acid method using a readymade kit for this purpose, the principle of this method depends on colorimetric measurement of azobilirubin that is formed from the reaction between bilirbin and diazotized sulfanilic acid (9).

The absorbance of azobilirubin is proportional to the concentration of bilirubin and was measured at 550 nm (10).

Preparation of tissue samples:

After the collection of blood samples has been completed, laparotomy was done and liver was quickly excised from each rat. The samples was placed in chilted phosphate buffer solution (ph7.4) at 4C for estimation of tissue GSH level.

After being placed in chilled phosphate buffer (a buffer for GSH estimation) (11), liver was blotted with filter paper and weighed .one gram from each organ was then taken to prepare 10%tissue homogenate using the same buffer solution. The homogenate was centrifuged at 3000rpm for

15 mins at 4 C and the supernatant eas used for the estimation of GSH(12),

Measurementoftissuereduced Glutathione (GSH):

Determination of GSH level depends on the action of sulfhydry l group.

GSH was determined by using a modified procedure utilizing Elman's method (11) which summarized as followed:

Reagent	Sample µL	Reagent blank µL	Standard µ L
Homogenate	200	----------	----------
Standard	----------	----------	200
D.W	1600	1800	1600
TCA	200	200	200

Tubes are mixed in vortex mixer intermittently for 10-15 minutes, and centrifuged for 15 minutes at 3000 rpm. then pipcucd into test tube.

Reagent	Sample µL	Reagent blank µL	Standard µ L
supernatant	800	800	800
Tris-EDTA buffer	1600	1600	1600
DTNB	40	40	40

Tube are mixed in vortex mixer. The spectrophotometer was adjusted with reagent blank to read zero absorbance at 412nm and the absorbance of standards and sample were read within 5 minutes of the addition of DTNB.

Statistical Analysis:

All data were expressed as mean $\pm$S.D, statistical analysis have been done by using LSD and ANOVA by using computer program SPSS version 17, P-V value less than 0.05 were considered significant for all data showed in our results.

RESULTS

In this study the use of green tea extract resulted in a significant increase in serum ALT activity, serum bilirubin level with a non-significant increase in serum AST activity as compared with NTX treated group, as in table (1), it also resulted in a significant increase in tissue GSH level as in table (2):

Group	Serum ALT activity (U/I)	Total serum bilirubin level (mg/dl)	Serum ALT activity (U/I)
Control group	15.5± 3.8	0.12± 0.06	35.8± 7.9
Green tea group	16.1± 5.8	0.10± 0.07	38.7± 8.4
Green tea and MTX group	20.5± 5.6	0.13± 0.07	58.9± 7.1
MTX group	27.7± 9.4 *	0.35± 0.18 *	66.0± 12.1 *

The value is mean ± *S.D, * P< 0.05*

Table (2) : the effect of 14 week of treatment with methotrexate (MTX) and green tea on GSH level in liver tissue homogenate in male rats.

Group	Tissue GSH level (micro molar/gm tissue)
Control group	679.1± 240.95
Green tea group	981.5± 200.11*
Green tea and MTX group	796.6± 275.740
MTX group	263.3± 156.91 *

The values expressed as mean ± *S.D, ⁻P< 0.05*

DISCUSSION:

Increasing in ALT and AST activities are considered to be a conventional indicators of liver injury [13],The present study revealed a significant increase in the activities of ALT,AST on exposure to MTX, indicating considerable hepatocellular injury,this might be resulted from the binding of MTX to enzyme dihydrofolic reductase ,thus preventing the conversion of folic acid to its active form ,folinic acid.

This in turn blocks the synthesis of nucleic acids, certain amino acids and indirectly proteins. This might lead to damage of organelles and plasma membranes of hepatic parenchymal cells interfering with their function and allowing leakage of enzymes these findings were in agreement with some workers [14].

Administration of aqueous green tea extract with MTX in this study resulted in a non-significant decrease in AST activity and a significant reduction in ALT activity as compared to MTX group this indicate the ability of aqueous green tea extract for stabilizing plasma membrane as well as repalring of hepatic tissue damage that caused by oxidative sress but it might need longer time for AST to restore its normal activity , these finding were in coherent with a previous[15].

Administration of MTX in this study produced a significant increase in total serum bilirubin level. Since the liver is responsible for clearing the blood of bilirubin [16], so increasing the total serum bilirubin level indicated a reduction in the excretory capability of the liver as a consequence of liver injury .Analysis of our data showed

that administration of aqueous green tea extract with MTX resulted in a significant decrease in total serum bilirubin level as compared with the MTX group indicating the improvement effects of green tea on liver function these finding were in a agreement tea with some studies [17] and regarding GSH levels, administration of MTX resulted in a significant decrease in GSH level in liver tissue homogenate by about (60%) as compared with the control group ,similar results were previously reported [18]. Actually the reduction in liver GSH content promoted by MTX represents an alteration in the cellular red ox stare; some reports indicate that cytosolic nicotine amide adenosine diphosohate (NADP)- dependent dehydrogenises and NADP malic enzyme are inhibited by MTX, suggesting that the drug could decrease the availability of NADPH in cells [19].

.Under normal conditions. NADPH is used by glutathione reductase to naintain the reduced state of cellular glutathione ,an important cytosolic antioxidant, which protects against reactive oxygen species (ROS). Thus, the significant reduction in glutathione (GSH) levels induced by MTX leads to reduction in the level of the antioxidant enzyme defense system , sensitizing the cells to ROS(20).

The administration of aqueous green tea extract in this study resulted in a significant increase in tissue GSH level as compared with the control group

Moreover ,the same result was observed when aqueous green tea extract was given together with MTX ,in significantly increased GSH level as, compared with MTX-treated group ,the protective effect of green tea was attributed to the fact that it is rich in polyphenolic compounds (particularly catechins and gallic acid) which exhbit antioxidant activity by scavenging reactive oxygen and nitrogen species and chelating redoxactive transition metal ions; also it can chelate metal ions like iron and copper to prevent their participation in fenton and Heber-Weiss reactions. In addition to that ,it green tea contains other components such as cerotenoids ,tocopherols, ascorbic acid (vitamin C) ,minerals such as Cr , Mn , Se or Zn ,and certain phytochemical compounds. These compounds could increase the antoxidant potential of polvphenolic compounds(21).

CONCLUSIONS:

It seems that the hepatotoxice effect of MIX might be attributd to oxidative stress as MlTX treatment decrease GSH *level* in liver tissue homogenate.

Aqueous green tea extract administration reduce the oxidative stress that was induced by MTX by increasing GSH level in liver tissue ,it also improves liver function.

The protective mechanism of aqueous green tea extract may involve its antioxidant activity against the production of free radical and ROS.

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