

Effect Of Some Antioxidants On Some Liver Growth Factors

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ABSTRACT

According to most researchers all types of liver affections are associated with oxidative brunt that affects proteins, nucleic acids, and lipid bi-layer plasma membranes. In this work experimental animals (male albino rats) were fed thermally treated oil and ethanol alcohol as inducers of liver affections. Vitamin E and Curcumin were administered to those animals in a trial to investigate their ability to combat with this oxidative attack. Malondialdehyde (MDA) was assayed as a measure of the oxidative affections. Since liver regeneration needs the participation and cooperation of other factors, growth factors (HGF, TGF α and TGF β) were assayed in liver homogenates of the sacrificed rats. Results showed that. Antioxidant enzymes levels were significantly low in animals treated with oil or ethanol as compared to control. These levels were improved on giving either vitamin E or Curcumin as exogenous antioxidants

Cucuminn was more powerful than vitamin E in this respect

The elevated growth factors in animals with oxidative attack were declined on administration of curcumin or vitamin E

The liver functions measured were just a mirror or reflection of liver pathology induced by oxidant agents

More work is needed to add other natural antioxidants, investigate their mechanism of action and to ascertain the optimum period of their use, and mode of supplementing them- if present.

INTRODUCTION

Liver affections are now Egyptian national problem that require research joint effort focusing upon hepatic pathological and biochemical changes and different ways that might end with complete eradication of the disease. The liver is well-known by its ability to regenerate lost tissues. This regeneration can be induced by specific stimuli such as toxic damage, partial hepatectomy that may amount to 70% of the liver mass. In rats it was documented that the organ rebuilding can be accomplished within 10 days (1).

Whatever the cause of liver damage i.e. toxic, Viral, chemical, or parasitic infection, the degenerative process requires the activation of multiple pathways with particularly complex interactions that may occur in different cell

types and may be present only at certain stages of the liver regeneration (2).

All types of liver damage are known to be associated with remarkable oxidative stress as reported by the vast majority of interested authors (3, 4).

Since the effects of such oxidative stress are our main concern in this work, it should be firstly stated that in hepatic injury whatever sub-clinical or overt, there is perturbation of normal liver homeostasis with extra-cellular release of free radicals, intra-cellular constituents and signaling molecules. The oxidative stress-mediated necrosis, leading to stellate cell activation may underlie varieties of liver diseases e.g. hemochromatosis, alcoholic or viral diseases (5)

The free radicals -as we all know - can be produced by different biochemical processes within the body including reduction of molecular oxygen during aerobic respiration yielding superoxide and hydroxyl radicals. These ROS (Reactive oxygen species) attack the hepatic tissues with unmistakable oxidative damage of proteins (6)

Oxidative damage also happens to DNA where the sugar and base moieties are prone to oxidation (7).

In the peroxidation of lipid bio-membranes, where damaged lipids are constituents of biological membranes -the cohesive lipid bi-layer arrangements and structural organization are disrupted (8).

On the other hand -thanks God- most biological systems and organisms are protected against free radicals damage.

The anti-oxidant defense mechanism include anti-oxidant enzymes (Superoxide dismutase SOD, glutathione peroxidase and GPX and catalase) vitamin antioxidants like tocopherols (vitamin E) ascorbic acid (vitamin C) and flavenoids (9). Besides there is also other category of oxidants scavengers like Glutathione, selenium, taurine, bilirubin, uric acid melatonin ...etc (10)

Oxidative stress occurs when the production of ROS exceeds the level the natural defense mechanisms that can cope with, causing damage to the macro molecules (11). This is often characterized by high level of malondialdehyde (MDA). High MDA level is a marker of lipid per oxidation which is a fallout of oxidative damage (12).

In addition to the antioxidant vitamins, enzymes and other compounds naturally present in the body, efforts has been made to distinguish and characterize other materials that can be supplied normally in diet in order to combat the heavy oxidative stress e.g. curcuminoids. The antioxidant supply of curcuminoids -which is isolated from Turmeric (C.Long L.) that belong to the Zingiberaceae plant family ,like Ginger was considered. It belongs to the genus curcima that consists of

hundreds of species of plants which possess rhizomes and underground root-like stem (13).

The coloring principle of Turmeric was isolated and named "curcumin" Generally the term curcuminoids refers to a group of compounds -which are chemically related to curcumin like demethoxycurcumin and bisdemethoxy curcumin

The fine chemical structure of curcumin was elucidated in 1970s and 1980s Apart from its well-known activities -like anti-protozoal , anti-microbial, antivenom and antitumor activities, curcumin is almost known for its anti-oxidant power that may however underlie all these above listed activities .It is good antioxidant inhibiting lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenate . Curcumin also protects hemoglobin from being taken to the way of methemoglobin.

In this respect (14) reported that curcumin was more active than tocopherols , as can be anticipated from the chemical structure where the methoxy and phenolic hydroxyl group and 1,3 diketone system seem to be responsible for the antioxidant power. Apart from the oxidative stress underlying liver diseases, the liver regeneration process must be considered, and the role of different growth factors has to be investigated in this issue. Regeneration of the damaged liver might also be influenced by many factors such as increased metabolic demands after loss of functioning liver mass.

According to (15) the critical event in initiating hepatocyte regeneration seems to entail growth factors interacting with specific receptors on the cell wall surfaces. A lot of them are now characterized and studied like:

- a- The Epidermal Growth Factor (EGF) would emerge as a major stimulator of hepatic regeneration (16). It is a peptide with a molecular weight of 6000 having growth activities on many epithelial tissues
- b- Hepetocyte growth factor (HGF) was described in serum of rats by (17), 24 hours after partial hepatectomy. Its effect on hepatic fibroin was reported by (18), who

administered a deletion variant of HGF to rats with dimethyl nitrosamine (DMN)-induced liver fibrosis. The liver histology was improved and collagen content-measured as hydroxyl proline- was however decreased.

c-Transforming growth factor-alpha (TGF - α) has 30- 40 % sequence homology with EGF (Epidermal growth factor). It can bind to EGF receptors on hepatocytes plasma membranes and within the cells stimulating also hepatocyte proliferation (19).

d-The transforming growth factor-beta (TGF- β 1) induces the production of extra cellular matrix protein by liver cells and has been implicated in the pathogenesis of hepatic fibrosis in laboratory animals (20).

Having a rapid glance on the huge amounts of published data that concentrate on liver damage owing to enhanced oxidative brunt, role of endogenous (enzymes, vitamins, and others) and exogenous (e.g. curcumin and vitamins) antioxidants, and the resultant effects that emerge from the existing balance between the two opposing forces. (oxidant- Antioxidant balance) have to be evaluated. Together with evaluation of these two forces, the role of growth factors (HGF, TGF- α and TGF- β) in restoring normal liver architecture and functions has also to be explored.

The aim of the current study focuses on investigation of biochemical and molecular potential of curcumin and other antioxidants like vit E (Tocopherols) on liver growth factors in adult *male rats* in an attempt to understand its mechanism of action. We tried to achieve this, through estimation of Malodandhyde (MDA) as an indicator of the weight of oxidative burden, and plasma antioxidant enzymes i.e. Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD) as a measure of the endogenous antioxidant agents.

Determination of Vit E (tocopherol α) reflects the degree of antioxidant power of endogenous and exogenous compounds

Growth factors i.e. Hepatocyte growth factor (HGF) , transforming growth factor α (TGF α),

and transforming growth factor β (TGF β) were assayed in liver homogenate in order to have closer view for the state of these factors in tissues of organ on which they primarily act ,the liver

Routine Liver function i.e. total and direct Bilirubin, Albumin, GOT and GPT (AST and ALT) were also determined and considered as a mirror reflecting the extent of liver damage in studied animals. This study might provide a clue to search for a compound that is naturally present, and can be domestically given in food with little or no side effects like Curcumin

MATERIAL AND METHODS

This Study was carried out in the department of Biochemistry, faculty of Medicine, Zagazig University, on experimental animals i.e. male albino rats weighing between 200- 250gm. (Average 236 + 12.1 gm). Their ages range from 4 to 5 months (Average 3.1 + 1.3 months). Purchased from the Animal house present in the faculty of medicine, a total number of 70 rats were enrolled in the study.

They were accommodated in clean cages and kept on the standard diets (Casein, 180gm/kgm, corn starch 460 mg/ kgm, sucrose 220 gm/kgm mineral mixture 50 mg/ kgm, vitamin mixture 10 mg / kgm and cellulose 40 gm/ kgm).

All animals had free access to water and kept under the same condition of illumination, temperature ventilation and acoustic noise.

After 10 days of breeding, they were divided in to 7 groups of equal number. Control group animals were kept on the standard diet while other animals in all remaining groups received extra- specific regimen of nutrition that lasted for 8 weeks as follow:

Grouping and treatment

Groups were separated and divided into separate cages each contained 10 animals as follow:

- Group (I): Control group (Received standard diets)
 Group (II): Received thermally treated oil
 Group (III): Received Ethanol
 Group (IV): Received thermally – treated oil + Curcumin
 Group (V): Received Ethanol + curcumin
 Group (VI): Received thermally-treated oil+vit E
 Group (VII): Received Ethanol + vit E

Ethanol was given in a dose of 5 gm/ kg body weight daily prepared as 20% (v/v) solution. This was orally administered using oral catheter.

Curcumin on the other hand, was provided as 100 mg/kg body weight daily, while vit E (tocopherol α) dose was 40 mg/kg and daily

At the end of the experiment period, animals were fasted for 12 hours then sacrificed. Liver and blood samples were prepared.

Blood samples

Whole rat blood was collected from the rat heart -after being anaesthetized, using pentobarbital into dry clean sterile test tubes and divided into two portions:

The first portion to be incubated at 37C for 30 minutes, allowed to clot then centrifuged at 3000 rpm. Sera were separated and kept as aliquots, at liquid nitrogen refrigerators (80°C) till time of analysis.

To the second portion, an anticoagulant was added (K ethylene diamine tetraacetate, Potassium EDTA) to prevent blood clot. Samples were centrifuged and plasma was separated into aliquots and kept at -80°

In serum Samples the following parameters were evaluated

Liver function tests (Bilirubin total and direct, albumin, GPT, and GOT). These parameters were assayed on Hitachi Auto analyzer, Hitachi, Japan)

MDA Malondialdehyde, also by chemical method according to this one adopted by (21). The method depends upon reaction of MDA

with thiobarbiturates in an acidic medium at high temperature producing pink color that can be estimated colorimetrically at two wavelengths 520 and 535 to exclude interfering substances. Compounds that give this reaction are now known as thiobarbiturate reactive substances mostly MDA. Standard curve was plotted from which individual results were read

Vitamin E (Tocopherols)

The ELISA is based on the competitive binding enzyme immunoassay technique adopted by (22).

The micro titer plate provided in this kit has been pre-coated with an antibody specific to vit E, during the reaction, vit E in the sample or standard competes with a fixed amount of biotin-labeled vit E for sites on a pre-coated Monoclonal antibody specific to vit E.

Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm) 2 nm. The concentration of vitamin E in the samples is then determined by comparing the O.D. of the samples to the standard curve.

In plasma samples

Enzymes concerned with anti-oxidation property i.e. superoxide dismutase and glutathione peroxidase were assayed

Glutathione peroxidase (GPX) was assayed according to the method of (23). This is a chemical method that principally depends upon oxidation of reduced glutathione (GSSG) and conversion of NADPH+ to NADP+. This conversion is associated with decrease in the optical density measured at 340 nm which is a measure of the enzyme activity. Standard curve was plotted from which individual sample data were gained.

Superoxide dismutase (SOD) was measured according to the method of (24).

The method depends on the fact that the spontaneous autoxidation of pyrogallol, at alkaline pH, produces superoxide anion (O_2^-) radical, which in turn enhances further oxidation of pyrogallol with a resultant increase in absorbance at 420 nm. From the constructed standard curve, individual data were recorded.

Tissue Samples (liver homogenates)

Preparation of liver samples

Animals were finally sacrificed by cervical dislocation, and then livers were rapidly removed. Part of liver tissues was excised, weighed and homogenized, using glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland), with ice-cooled saline to prepare 25% W/V homogenate. Part of the homogenate was deproteinized with ice-cold 12% trichloroacetic acid (TCA) and the obtained supernatant, after centrifugation at 1000 x g, was used for estimation of growth factors HGF, TGF α and TGF β . Results were expressed as units / gm tissues

Hepatocyte growth factor (HGF)

HGF was assayed in liver homogenate, employing the Enzyme-linked Immunosorbent technique (ELISA), adopted by (25). Kits were commercially purchased from Ray Bio, Human, TGF- β 1, ELISA

The method is an in vitro assay for the quantitative measurement of human HGF in serum, plasma and other biological fluids. This assay employs an antibody specific for human HGF coated on a 96-well plate. Standards and samples are pipetted into the wells and HGF present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated, anti-human HGF antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of HGF bound. The Stop Solution changes the color from blue to

yellow, and the intensity of the color is measured at 450 nm.

From the plotted standard curve, different results were gained

Transforming growth factor α (TGF- α)

TGF- α was assayed in liver homogenate employing enzyme-linked immunosorbent assay technique (ELISA) adopted by (26). Kits were commercially purchased from Ray Bio, Human, TGF- β 1, ELISA

Kit is an in vitro assay for the quantitative measurement of human TGF-alpha in serum, plasma, cell culture supernatants, tissue homogenate and urine. This assay principles are the same as those mentioned for HGF. Standard curve was also plotted and different results were recorded as units/gm tissues.

Transforming growth factor. Beta (TGF- β 1)

TGF- β 1, was determined in plasma in liver homogenate employing Enzyme-linked immunosorbent assay (ELIS) adopted by (27). Kits were commercially purchased from Ray Bio, Human, TGF- β 1, ELISA. TGF- β is a stable, multifunctional polypeptide growth factor. It exists in at least five isoforms, known as TGF β 1, TGF β 2, TGF- β 3, TGF- β 4, TGF- β 5. Their amino acid sequences display homologies on the order of 70-80%. The various TGF- β iso-types share many biological activities and their actions on cells are qualitatively similar in most cases although there are a few examples of distinct activities. TGF- β 1 is the prevalent form and is found almost ubiquitously while the other isoforms are expressed in a more limited spectrum of cells and tissues. It is normally secreted as an inactive, or latent, complex.

The Ray Bio® Human TGF- β 1 ELISA (Enzyme-Linked immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay that employs an antibody specific for human TGF- β 1 coated on a 96-well plate. Standards and samples procedure is the same applied for HGF. Standard curve was plotted and results were tabulated antibody.

Statistical Analysis

The data were expressed as means \pm SEM. The student "t" test was used for comparison of paired sets of data, while one way ANOVA (ANOVA) followed by Tukey and Kramer, multiple comparison test was used to judge the difference between the different group. AP value ≤ 0.05 was considered significant.

RESULTS

Table (1) Shows that there was a Significant increase ($P \leq 0.05$) in the mean value of serum (MDA) in all studied groups as compared to control. On the other hand, results showed also a significant decrease in the mean value of (MDA) in groups IV, V, VI, VII when compared to groups II and III. This decrease however was not restored to control levels.

Table (1) also shows a significant decrease ($P \leq 0.05$) in the mean value of Vit E, in groups II and III -as compared to control.

The same finding was also documented in groups IV, V or in other words, those animals which received treatments by curcumin.

Finally mean value of Vit E in groups VI and VII showed a significant increase ($P \leq 0.05$) as compared to groups II and III.

It worth mentioning that the vit E status in groups VI and VII is a direct result of receiving vit E as a line of antioxidants treatment.

Table (1) Showed a Significant decrease ($P \leq 0.05$) in the mean value of plasma (GPx) in all studied groups as compared to control.

On the other hand, results showed also a significant decrease in the mean value of (GPx) in groups IV, V, VI, VII when compared to groups II and III. This decline was not restored to control.

Nearly the same findings were also observed when Superoxide dismutase enzyme (SOD) was considered.

Same table showed also that, there was a significant increase ($P \leq 0.05$) in the mean values of liver homogenates HGF in all studied groups as compared to control. Groups II and III presented maximum values because the rats included in these groups did not receive any treatment.

In groups IV, V, VI, and VII, mean HGF values showed significant decline ($P \leq 0.05$) as compared to groups II and III, which is easily attributed to the received treatment with the powerful antioxidant Curcumin or vit. E. The Same statistical findings were also observed when the other two growth factors TGF α and TGF β 1 were investigated.

The trial by antioxidants supplied to normalize growth factors levels to match with the control, does not come to an end i.e. control level was not -however -restored.

Table (2) shows the results of liver functions assay in all groups.

Liver function is considered as a mirror reflecting state of all parameters affected the liver. There was significant increase in GOT and GPT mean values in all groups compared to control ($P \leq 0.05$). This rise affected mostly groups II and III. Treatment with vit E or Curcumin improved the state of enzymes level mostly in groups treated with Curcumin. On the other hand same finding was also seen when albumin level was considered. Lowest significant decline was observed in groups II and III, Treatment improved the levels in groups IV, V, VI and VII. Curcumin is more efficient than vit E in this respect. Regarding Bilirubin assay, total and direct, significant rise was documented in all groups compared to the control ($P \leq 0.05$), especially in animals treated with Curcumin. It has to be mentioned that the rise of serum bilirubin though significant but it did exceeds unity i.e. 1 mg/dl in all circumstances.

Table 1. Mean values \pm S.E of all assayed parameters in all studied groups

Group	I Control Group	II (thermal treated oil)	III Ethanol	IV Thermally treated oil +curcumin	V Ethanol+ Curcumin	VI Thermally treated oil +Vit E	VII Ethanol + Vit E
HGF ng/g tissue	0.149 \pm 0.03 ^g	6.103 \pm 0.027 ^a	5.917 \pm 0.023 ^b	2.988 \pm 0.0204 ^f	4.806 \pm 0.029 ^c	3.342 \pm 0.013 ^e	4.116 \pm 0.036 ^d
TGF α Pg/g tissue	5.530 \pm 0.157 ^f	31.85 \pm 0.1767 ^b	51.174 \pm 0.33 ^a	21.71 \pm 0.167 ^e	23.403 \pm 0.17 ^d	28.26 \pm 0.191 ^c	21.58 \pm 0.342 ^e
TGF β ng/g tissues	130.97 \pm 1.58 ^e	233.72 \pm 0.96 ^a	221.6 \pm 1.84 ^b	194.18 \pm 2.92 ^c	196.35 \pm 1.37 ^c	201.15 \pm 1.24 ^c	181.08 \pm 6.43 ^d
MDA nmol/ml	51.45 \pm 0.86 ^d	154.46 \pm 2.09 ^a	151.89 \pm 2.77 ^a	70.47 \pm 1.83 ^c	69.72 \pm 2.43 ^c	75.54 \pm 3.70 ^c	84.12 \pm 1.10 ^b
Vit E umol/l	7.33 \pm 0.351 ^a	2.491 \pm 0.165 ^d	3.624 \pm 0.233 ^c	5.031 \pm 0.106 ^b	4.527 \pm 0.232 ^b	6.090 \pm 0.135 ^{cd}	7.043 \pm 0.125 ^{cd}
GPx U/l	25.628 \pm 0.27 ^d	7.798 \pm 0.82 ^a	8.29 \pm 0.70 ^a	16.08 \pm 0.56 ^c	17.75 \pm 0.58 ^{bc}	18.00 \pm 1.91 ^{bc}	19.773 \pm 0.49 ^b
SOD U/l	47.057 \pm 0.60 ^f	20.252 \pm 0.32 ^b	22.663 \pm 0.60 ^a	38.757 \pm 0.56 ^c	32.233 \pm 0.67 ^e	41.66 \pm 0.51 ^c	40.408 \pm 1.83 ^{cd}

*Data are presented as mean \pm S.E

*Means within the same rows carrying different superscripts are significant a ($P \leq 0.05$).

*MDA and vit E were assayed in serum, antioxidant enzymes in plasma and growth factors in liver homogenates

Table 2. Liver Function (Bilirubin, total and Direct, Albumin ,GOT(ALT),and GPT(AST) In all Studied Group

Item	I Control Group	II (thermal treated oil)	III Ethanol	IV Thermally treated oil +curcumin	V Ethanol+ Curcumin	VI Thermally treated oil +Vit E	VII Ethanol +Vit E
Bilirubin Total	0.522 \pm 0.026d	0.954 \pm 0.025a	0.875 \pm 0.046b	0.6160 \pm 0.0326c	0.604 \pm 0.01013d	0.587 \pm 0.0094c	0.5950 \pm 0.0159d
Bilirubin Direct	0.172 \pm 0.021d	0.389 \pm 0.002a	0.3090 \pm 0.011b	0.296 \pm 0.004c	0.328 \pm 0.03d	0.291 \pm 0.005c	0.23 \pm 0.008d
Albumin	4.220 \pm 0.198a	3.196 \pm 0.02029e	3.806 \pm 0.0245b	3.89 \pm 0.01389b	3.501 \pm 0.00971d	3.718 \pm 0.0236c	3.397 \pm 0.0181e
GPT	28.400 \pm 0.669 g	77.300 \pm 0.7a	73.50 \pm 0.6540b	42.40 \pm 0.896f	50.7 \pm 0.955d	55.40 \pm 0.763c	48.20 \pm 0.611e
GOT	60.60 \pm 1.90e	133.30 \pm 2.160a	111.40 \pm 2.140b	72.40 \pm 1.620d	82.10 \pm 2.33c	78.100 \pm 1.353c	70.80 \pm 1.84d

* Data are presented as mean \pm S.E

* Means within the same rows carrying different superscripts are significant at ($P \leq 0.05$).

DISCUSSION

Oxidative stress occurs when there is an imbalance between generation of reactive oxygen species (ROS) and inadequate antioxidant defense systems leading to cell damage either directly or through altering signaling pathways. The consequence of oxidative stress may be the oxidative damage of lipids, proteins and DNA with subsequent disease development and aging (28).

Free radicals scavengers or the anti-oxidants represent an important component of body defense mechanism against oxygen – centered free radical mediated injuries so that the boosting of these defenses by pharmacological means may emerge important therapeutic tool. The role of Vit E, Vit A, ascorbic acid, salicylates, barbiturates, superoxide dismutase, glutathione peroxides, poly unsaturated fatty acids and anti-inflammatory agents in addition to plants like curcumin- in ameliorating free radical damage are just but few examples of the importance of anti oxidants in protecting tissues against oxidative stress (29).

It can generally be stated that all forms of life must deal with the free radical damage from internal metabolism and from external sources. It is not surprising that plant substances usually have some amount of antioxidant activity. Some plant substances such as centella asiatica are potent antioxidants (30).

We have reported before that-in agreement with majority of researchers – all types of liver damage are associated with oxidative stress and hence release of free radicals which are known with their cellular damage. Antioxidants including enzymes, vitamins, chemicals and others. and their role in combating ROS was explained before (31).

Let us now consider our rat models in this study. These experimental animals were the subject of liver injuries induced by feeding thermally-treated oil in one group and ethanol in the other. Liver injuries was then expected

Did administration of exogenous antioxidants (Curcumin & tocopherols) in

addition to the role played by endogenous factors – had a role to play in reducing or even complete abolishment of oxidative liver injuries?

This was the point of our concern

Numerous therapeutic activities have been assigned to turmeric for wide variety of diseases and conditions. The hallmark of all conditions is curcumin antioxidant activities. It has also anti-inflammatory, Anti-viral, anti-fungal and anti-Cancer activities. These effects are due to the regulation of transcription factors, growth factors (cytokines), protein kinases and other enzymes (32).

Results of our study showed that, there was significant increase in the mean level of thiobarbiturate-reactive substance, or Malonaldehyde (MDA) -which is the final product of oxidative processes- in serum of both group II and III rats, as compared to control ($P \leq 0.05$). These findings are in agreement with those reported by many authors (4).

In a trial to investigate the effect of supplementation of exogenous antioxidants (namely curcumin and vitamin E) on the rate of MDA production, both agents were fed to rats (groups IV, V, VI and VII) for a period that lasted 8 weeks.

There was marked improvement in the oxidant / antioxidant status as show by the significant decline ($P \leq 0.05$) of the mean values of MDA level in blood of group IV, V, VI, & VII, which received either curcumin (IV & V) or vit E (VI and VII) – when compared to groups II and II that did not receive any support, or the control.

There was a non- significant change in serum MDA mean level in rats treated by curcumin as compared to those treated by vit E. Does curcumin has antioxidant power more than vit E needs more research.

Numerous reports indicated that curcumin could mediate both pro oxidant and antioxidant roles. First curcumin could induce the expression of ROS, which plays an important

role in the anti-proliferative effect of this molecule (33).

Second curcumin binds to thioredoxin reductase (TR) and converts this enzyme to NADPH oxidase thus leading to production of ROS (34). Because TR is over expressed in tumor cells, curcumin kills tumor cells through this mechanism (35).

Third curcumin suppresses lipid peroxidation (36,37).

Fourth increases the expression of intracellular glutathione (38).

Fifth it could also play an antioxidant role through its ability to bind iron (39)

Reviewing all these report it could be suggested that curcumin has the ability to modulate the redox potential of the cell.

That curcumin can modulate the cellular actions of growth factor and other cytokines has also been demonstrated. Curcumin has been shown to down regulate the effect of epidermal growth factor (EGF) through down regulation of the expression and activities of EGF receptors (EGFR). These receptors are closely linked to breast, lung kidney and prostate cancer (40- 42)

Curcumin also suppresses the action of interleukin -6 (IL6) through down regulation of STAT3 activation.

Finally curcumin could suppress angiogenesis through down regulating the expression of vascular endothelial Growth factor (VEGF).

In Carbon tetrachloride CCl_4 - induced liver damage, according to (43) vitamin E, with or without curcumin significantly improved the concentration of total protein and albumin in plasma of rats.

Vitamin E administration also improved renal function as shown by decline in urea and creatinine levels. This later effect may be due to the antioxidant properties of vitamin E- like curcumin- since it has been found that ROS may be involved in the impairment of glomerular filtration rate (GFR) (44,45).

Of the antioxidant enzymes super oxide dismutase, glutathione peroxides and catalase are extremely effective antioxidant enzymes responsible for the catalytic dismutation of highly reactive toxic super oxide radicals to H_2O_2 and for the catalytic decomposition of H_2O_2 to water and oxygen (46).

These data go in hand with our finding of reduced concentration of glutathione peroxides (GPX) and superoxide dismutase (SOD) in plasma of animals exposed to oxidative stress, either by administration of thermally treated oil or ethanol.

We found significant decrease ($P \leq 0.05$) in both SOD and GPX levels in group II and III as compared to control. Restoration of normal level of these enzymes was - however - tried through the action of administered curcumin and vitamin E. In this work may be complete restoration to control levels needed longer periods of administration.

It worth mentioning that sometimes, mixture of vit E and curcumin is need as strong antioxidant double-headed tool.

For example it was reported before by (47) that such mixture effectively ameliorates the L-thyroxine (T4)-induced oxidative stress in the renal cortex of fondles rats whereas curcumin or vitamin E alone was not able to do so. They also reported that their histopathological examinations are in agreement with the biochemical findings.

An interesting finding was reported by (48), that when trying hepatic and nephro-toxicity induced by many factors like ethanol, or carbon tetrachloride, the decline or changes in vit E, and other antioxidant enzymes are due to cadmium toxicity, while vitamin C changes were only due to ethanol toxicity.

Another face of discussion will be now directed towards the state of growth factors HGF, TGF- α and TGF. β .

The capacity of the liver to regenerate after severe viral, drug - induced, hepatitis or administration of toxins as heated oil or ethanol, or even after massive partial hepatectomy is remarkable. Experimentally the

most dramatic and widely studied example of this phenomenon is the regeneration after the performance of 70% partial hepatectomy in rats in which normal liver mass is restored within 10 days. Similar-though slower regeneration occurs in humans

The possibility that hepatic regeneration could be enhanced therapeutically has been an enticing prospect for some years and recent advances of our understanding the process controlling hepatocyte proliferation brings the prospect excitingly close to realization (49).

After partial hepatectomy in rats, the liver regenerates by division of the existing adult cells rather than by stem cell proliferation. There is a surge of DNA synthesis in hepatocytes which peaks after 24 hours of hepatic resection. After a further 24 hours there is a surge of DNA replication in non-parenchymal cells. Interaction included non-parenchymal cells, kupffer cells, and sinusoidal epithelial cells (49).

In accordance with the findings of this work, (50) depicted that at least more than one growth factor-including HGF, TGF α , and others- that can either initiate or enhance hepatocyte proliferation which are generated in the liver or found in higher quantities in the circulation.

Significant elevation of HGF ($P \leq 0.05$) was found in all groups as compared to control. Although there was a decline in HGF level upon giving curcumin or vit E, yet this was insignificant ($P \leq 0.5$).

HGF-as mentioned by (51)- was reported to be present in serum of patients 24 hours often partial hepatectomy, and also form blood of rats exposed to ethanol or oil toxicity.

It is a potent stimulator of DNA synthesis in hepatocytes being active at low concentration as low as 1ng/ ml.

HGF is generated in the liver probably in non-parenchymal cells, (52) and the peak of mRNA expression appears at 10 hours often partial hepatectomy, falling by 24 hours. It has also been shown that mRNA for HGF is strongly expressed in the fetal liver tissues

compared with adult tissues suggesting for the first time that similar mechanisms of liver growth occur both during liver development and in liver repair after damage (49) Once purified materials are available in adequate amounts, rapid advances are likely to occur in all forms of liver damage, especially when (17,53) reported that close similarities between rabbit, rat and human HGF do existing, in other words it is not species specific.

The beneficial effects of HGF on liver injury have been also documented by . HGF reduced to toxicity of α -Naphthylisohiocyanate in rats. And the increased survival of cirrhotic rats subjected to major hepatic resection.

(54) administered a deletion variant of HGF to rats intoxicated with dimethyl nitrosamine-induced liver fibrosis. The variant has the same biologic activity as HGF. Also other group of rats were subjected to liver toxicity be thermally heated oil received this variant.

In both conditions liver histology was restored to normal and collagen content was decreased.

Finally it is interesting to know that 10 years that have passed since the time of purification HGF from plasma of humans with fulminating hepatitis the gene has been cloned (17,53) and this useful immunoassay developed.

These and other studies on experimental animals support the initiating studies of the clinical efficacy of HGF administration various forms of acute and sub acute liver injuries.

TGF- α is a potent hepatocytes mitogen produced in vivo and in vitro by hepatocytes stimulated to proliferate (50,55).

Fausto and Mead (55) showed increases in TGF- α mRNA in hepatocytes of rat liver after partial hepatectomy reaching nine fold levels greater than normal and postulated an autocrine loop by which enhanced TGF α production in regenerating hepatocytes stimulated hepatocyte proliferation (50). The amount of TGF α mRNA returned to normal within four days.

But despite the powerful effect of TGF α in stimulating DNA synthesis and evidence of its enhanced production after partial hepatectomy, recognition of the role of this growth factor by no means completes the story of hepatic regeneration

The time course of TGF α mRNA production peaking at the time of peaked DNA syntheses is too long for the initiation of that process. TGF α might also therefore act on regeneration at the time that hepatocyte have made the transition from G₀ to G₁ and entered the cell cycle (56).

Liver regeneration – like all good things – must come to an end. A major role is emerging for the non- parenchymal cells as controller of the proliferation of hepatocyte population while some of the positive growth factors mentioned while some of the positive growth factors mentioned (HGF, TGF- α) may be generated in non- parenchymal cells the major inhibitor of herpatocyte proliferation

TGF- β has been shown to only produced in non- parenchymal cells starting 24 hours after hepatic injury (Like partial hepatectomy) till the process of liver regeneration ceases. TGF- β that was originally purified from platelets has stimulatory or inhibitory effect on proliferation depending upon the tissue investigated.

It is interesting to know that low levels of TGF- β expression is normally present and it may be that this low production prevents the response of the resting liver to the various positive growth factors normally present in liver and circulation.

TGF- β include 3 isoform is (beta 1, 2 and 3). The three dimensional solution structure of TGF β_1 has been determined using multinuclear magnetic resonance spectroscopy.

TGF- β stimulates the expression of extra cellular matrix proteins and down regulate their degradation by matrix metalloproteinase (MMP) through up regulation of tissue inhibitor of metalloproteinase (TIMP)(57,58). Further more liver regeneration and fibrogenesis are accompanied by an up regulated expression of TGF- β isoforms, reflecting autocrine effect on

experimental fibrosis which can be inhibited by anti TGF- β treatments like neutralizing antibodies. As mentioned TGF- β is an inducer of fibrogenesis and is an important negative regulator of proliferation of hepatocytes .

Based upon the facts documented before about the actions of TGF- β , (59) using an experimental model of schistosomiasis in mice, found an increase in the contents of TGT- β_1 mRNA in the liver of infected animals in parallel with an increase in type I procollagen mRNA.

TGT- β_1 is capable of activating lipocytes cells that are probably a major site for synthesis of matrix proteins in chronic liver diseases (60- 62).

The loss of sensitivity to growth inhibitory factors is thought to contribute to the unregulated growth which is a character of tumor cells (62, 63).

Transfection of fibroblastic cell lines with the H-ras oncogene leads to both an increase in TGF- β_1 m RNA expression and an altered responsiveness to TGF- β (64,65).

Another fact is that, in addition to its effect on cell proliferation TGT- β_1 can induce the synthesis of proteins important in the regulation of extracellular matrix formation. This effect is however complex and involves both the increase in the level of mRNA as well as alteration in the proteolytic degradation of extracellular matrix protein (66). Moreover it has been shown that TGT- β_1 induces type I procollagen mRNA synthesis in the primary culture of hepatocytes.

it has to be mentioned that TGF- β is unlikely to be the only cell mechanism operating to limit the regenerative process or to prevent growth in normal liver. At least there is one other inhibitory growth factor- distinct from TGF- β -can be isolated from liver cytosol, and cytokines potentially released from the reticulo endothelial cells, macrophages can also inhibit hepatocyte proliferation.

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المخلص العربي

تأثير بعض المواد المضادة للأكسدة علي بعض معامل نمو خلايا الكبد

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حسب ما أتفق عليه معظم الباحثين فان التغييرات المرضيه الكبديه تكون دائما مصحوبه بارتفاع في نسبة المواد المؤكسده الضاره والتي يقع تأثيرها علي البروتينات والاحماض النوويه فضلا عن التأثير علي الغشاء الدهني المزدوج لجميع الخلايا. في هذه الدراسه تمت تغديه فئران التجارب او ذكر الفأر الابيض بالكحول الايثيلي (الايثانول) وكذلك بالزيت المعالج بالحراره وكلاهما يحدث في انسجه الكبد بعض التغييرات المرضيه. وفي نفس السياق تمت تغديه الحيوانات بفيتامين هـ وبماده الكركم الاصفر لدراسه مدي قدرتهما علي التعامل واحباط تأثير تلك الهجمات من المواد المؤكسده. قيست ماده المألون ثنائي الادهيد كمييار لمدي تأثير تلك المواد. ومن حيث ان اعاده الكبد الي نشاطه الطبيعي يتطلب المشاركه مع بعض العوامل الاخرى واهمها عوامل النمو فانه تم قياس ثلاثه عوامل هم : عامل النمو الكبدي وعامل النمو المتحول الفا والنمو المتحول بيتا في المحلول الكبدي المتجانس او مايسمي (جناسه الكبد). اظهرت النتائج انخفاضاً ذو دلالة احصائيه في مستوي انزيمي الجلوتاثيون بيوكسيدير والسوبر اكسيد ديسميوتيز في مصل دم الفئران التي غذيت بالكحول او الزيت المعالج حرارياً . كذلك وجد ان اعطاء المواد المضاده للاكسده فيتامين هـ او الكركم قد حسن من معدل هذه الانزيمات . كان ذلك التأثير اكثر وضوحاً عند التغديه بالكركم . اظهرت النتائج ايضا ان الارتفاع الملاحظ في مستوي عوامل النمو الثلاثه عند اصابه الكبد أثر التغديه بالكحول او الزيت قد انخفض انخفاضاً ذو دلالة احصائيه عند قياس تلك العوامل بمحلول الكبد المتجانس . ان هذا التأثير الحميد كان أكثر ملاحظه عند استعمال الكركم عنه عند استعمال فيتامين هـ كمضاد خارجي للاكسده . أما وظائف الكبد في الفئران فعند قياسها كيميائياً وجد انها لم تكن سوي انعكاس صادق لما عليه حاله الكبد من تغييرات مرضيه أخير فأن الموضوع بحاجة الي المزيد من العمل لاكتشاف واضافه مواد طبيعيه مثل الكركم لها خواص مضاده للاكسده -ومعرفه طريقه عملها- وكيفية اعطائها للمرضي المحتاجين اليها.