The Possible Protective Effect of Different Concentrations of Aqueous Green Tea Extract (AGTE) Against Hepatic Toxicity Induced by DDT in Rats

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Abstract

Background:

Cell death is the main component of many response patterns of living tissues to xenobiotics including the organochlorine insecticide, dichlorodiphenyl- trichloroethane (DDT). One of the possible ways to ameliorate this response is through interference with the process of oxidative stress which can be fulfilled by many candidate substances like green tea.

Objective:

The present study is designed to evaluate the influence of simultaneous administration of Green Tea, a protective and antioxidant agent, on the status of lipid peroxidation products (MDA) and glutathione (GSH) in liver tissue with measuring the activity of serum liver enzymes (AST, ALT and ALP) in rats treated with a toxic dose of DDT.

Methods:

Twenty four white Albino rats of both sexes, weighing 200- 250gm were used in this study; these animals classified into four groups as follow:

Group I: Six rats received single oral dose of corn oil (3 ml) using gavage tube. The animals were anesthetized by ether and sacrificed by decapitation 24 hr after administering corn oil dose .This group served as negative control.

Group II: Six rats received single oral dose of DDT 100mg/kg (dissolved in corn oil) by gavage tube. The animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose. This group served as positive control of DDT- induced hepatic toxicity.

Group III: Six rats were utilized to study the possible protective effects of AGTE against DDT- induced liver damage which received oral dose of green tea

(1.25% AGTE as their sole source of drinking fluid) given daily by feeding bottle for 7-days (started 7-days prior to and during the period of treatment with DDT , at day 7 DDT dose (100 mg / kg /day)was given, then the animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose) .

Group IV: Six rats were utilized to study the possible protective effects of AGTE against DDT induced liver damage which received oral dose of green tea (10% AGTE as their sole source of drinking fluid) given daily by feeding bottle for 7-days(started 7-days prior to and during the period of treatment with DDT, at day 7 DDT

dose (100 mg / kg /day)was given, then the animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose) .

At the end of treatment all animals were sacrificed to measure the oxidative stress markers, malondialdehyde (MDA) contents and glutathione (GSH) levels in liver tissue homogenate. Serum was obtained for the assay of liver enzymes activity [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)].

Results:

In this study, we investigated the changes in the content of end product of lipid peroxidation (MDA) and the level of glutathione (GSH) which are critical determinants of tissue susceptibility to oxidative damage in liver tissue homogenate as a result of DDT administration.

Rats treated with a single oral dose 100mg/kg/day of DDT showed (48%) elevation of MDA contents and (42%) depletion of GSH levels in liver tissue homogenate compared to control group($p<0.05$) .

Rats orally treated with different concentrations (1.25% and 10%) of AGTE for 7 days prior to and during DDT treatment , showed a significant decrease in MDA contents in liver tissue homogenate (30% and 31.6%) respectively and a significant increase in GSH levels in liver tissue homogenate (59% and 48.3%) respectively compared to DDT-treated group($p<0.05$) , also showed a non significant compared to control group($p>0.05$) .

Rats treated with a single oral dose 100mg/kg/day of DDT produced a significant increase in the serum levels of AST, ALT and ALP(77.5% , 182% and 68%)compared to control groups($p<0.05$) indicating a considerable hepato-cellular injury .

Rats treated with different oral concentrations (1.25% and 10%) of AGTE for 7 days prior to and during DDT treatment produced a significant decrease in the levels of serum AST , ALT and ALP[(46.2% , 72.7% ,53%) and (44.2% , 64.2% and 45.4%)] compared to DDT treated group ($P<0.05$) .

Conclusion:

According to the results obtained in this study we can conclude that Green Tea have the ability, through a mechanism related to it's antioxidant property to provide protective effects against DDT-induced hepatotoxicity .By [the reducation of hepatic oxidative stress (reduction in MDA contents with the elevation of GSH levels) with subsequent improvement in liver functions manifested by reduction in the serum levels of (AST , ALT and ALP)], that improving the damage in liver tissue and makes it a good candidate to be tried clinically in this respect.

Introduction:

Cell death is the main component of many response patterns of living tissues to xenobiotics including the organochlorine insecticide [(1)]. DDT (dichlorodiphenyl- trichloroethane) was one of the most commonly utilized organochlorine pesticides which has an important role in many phases of agriculture, control of insects of public health and in the eradication of household pests [(2)].The toxicological effects of DDT in animals and humans have been observed in the CNS, immune system [(3)] reproductive, endocrine system [(4)] in addition to the blood [(5)], liver and kidneys [(6)]. Different mechanisms of DDT poisoning on CNS were suggested involves changes in physical properties of lipid bi-layer of the axonal membrane, decreases membrane fluidity, increases mobility of molecules within the membrane, decreases the dipole potential and causes a decrease in the dielectric constant [(7)]. In addition, DDT and its metabolites (DDE and DDD) may have the ability to affect both oxido-reduction

and energy production in the mitochondria by uncouple oxidative phosphorylation leading to impaired ATP [8] and protein synthesis [9] with elevation of cytosolic Ca^{2+} level by inhibition of Ca^{2+}-ATPase in the endoplasmic reticulum [10]. Furthermore, free radical formation may be involved in DDT toxicity, where the compound produces 1,1- dichloro , 2,2- bis (p-chlorophenyl) ethyl radical by one electron transfer from cyt-P450 , this reactive intermediate may abstract a hydrogen atom from microsomal lipid or undergo a second one electron reduction to yield 1,1- dichloro 2,2- bis (p-chlorophenyl) ethyl carbon ion which undergo protonation gives DDD [11].

One of the possible ways to ameliorate this response is through interference with the process of oxidative stress which can be fulfilled by many candidate substances like green tea.

Tea plant (Camellia sinensis) belongs to the family theacaceae, It represents approximately 20% of world tea consumption. Green tea extracts now days are widely used as dietary supplements [12] and is chemically characterized by the presence of polyphenolic compounds particularly the flavonoids, including catechins (flavan-3-ols), that form nearly 30-40% of the dry weight of green tea [13] Which are : (59%) of (–)-epigallocatechin-3-gallate (EGCG) ; (19%) of (–)-epigallocatechin (EGC); (13.6%) of (–)-epicatechin-3-gallate (ECG) ; (6.4%) of (–)-epicatechin (EC), and Gallic acid (GA) (Figure 1-3) [14] . Other phenolic acids such as caffeic acid, and flavonols such as kaempferol, myricetin and Quercetin [51], volatile oils, vitamins like (B,C, E and folic acid); xanthine bases (caffeine and theophylline); minerals and trace elements (Ca, Mg, Cr, Mn, Fe, Cu, Zn and Se), tannins and amino acids (theanine is the major amino acid present in green tea) [14].

This study was designed to assess whether or not the oral administration of different concentrations of aqueous green tea extract (AGTE) to rats could provide a protective effect against the hepatic oxidative stress and dysfunctions induced by DDT.

Methods:

Twenty four white Albino rats of both sexes, weighing 200- 250gm were used in this study; these animals classified into four groups as follow:

Group I: Six rats received single oral dose of corn oil (3 ml) using gavage tube. The animals were anesthetized by ether and sacrificed by decapitation 24 hr after administering corn oil dose .This group served as negative control.

Group II: Six rats received single oral dose of DDT 100mg/kg (dissolved in corn oil) by gavage tube. The animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose. This group served as positive control of DDT- induced hepatic toxicity.

Group III: Six rats were utilized to study the possible protective effects of AGTE against DDT- induced liver damage which received oral dose of green tea (1.25% AGTE as their sole source of drinking fluid) given daily by feeding bottle for 7-days (started 7-days prior to and during the period of treatment with DDT , at day 7 DDT dose (100 mg / kg /day)was given, then the animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose) .

Group IV: Six rats were utilized to study the possible protective effects of AGTE against DDT induced liver damage which received oral dose of green tea (10% AGTE as their sole source of drinking fluid) given daily by

feeding bottle for 7-days(started 7-days prior to and during the period of treatment with DDT, at day 7 DDT dose (100 mg / kg /day)was given, then the animals were anesthetized by ether and sacrificed by decapitation 24hr after administering DDT dose) .

At the end of treatment all animals were sacrificed to measure the oxidative stress markers, malondialdehyde (MDA) contents [15] and glutathione (GSH) levels [16] in liver tissue homogenate. Serum was obtained for the assay of liver enzymes activity [aspartate aminotransferase (AST) [17], alanine aminotransferase (ALT) [17] and alkaline phosphatase (ALP) [18]].

Results:

In this study, we investigated the changes in the content of end product of lipid peroxidation (MDA) and the level of glutathione (GSH) is a critical determinant of tissue susceptibility to oxidative damage and the changes in liver enzymes activity (AST,ALT and ALP) in liver tissue homogenate as a result of DDT administration .

Table 1: The Effects of treatment with different concentrations of aqueous green tea extract (AGTE) on the contents of MDA and GSH in rats' liver homogenate, serum AST, ALT and ALP activities compared to DDT-treated and control groups.

Groups	MDA (µmol/gm tissue)	GSH (µmol/gtissue)	Serum AST U/L	Serum ALT U/L	Serum ALP U/L
Control (n=6)	2.3442 ± 0.1763	35.614 ± 10.68	201.33 ± 50.839	39.167 ± 8.819	41.12 ± 17.586
DDT (100mg/kg) (n=6)	3.4645 ± 0.2144 (*)	20.724 ± 5.099 (*)	357.33 ± 32.191 (*)	110.33 ± 15.126 (*)	69.085 ± 9.259 (*)
DDT +AGTE 1.25% (n=6)	2.433 ± 0. 2836 (S)	32.904 ± 4.338 (S)	192.33 ± 90.13 (S)	30.133 ± 6.55 (S)	32.6 ±8.82 (S)
DDT +AGTE 10% (n=6)	2.3712 ± 0.1638 (S)	30.738 ± 3.497 (S)	199.28 ± 65.76 (S)	39.5 ±16.68 (S)	37.73 ± 10.53 (S)

Data represented mean ± SEM: n= number of animals:*P<0.05 with respect to control group: S= significant difference with respect to DDT-treated group

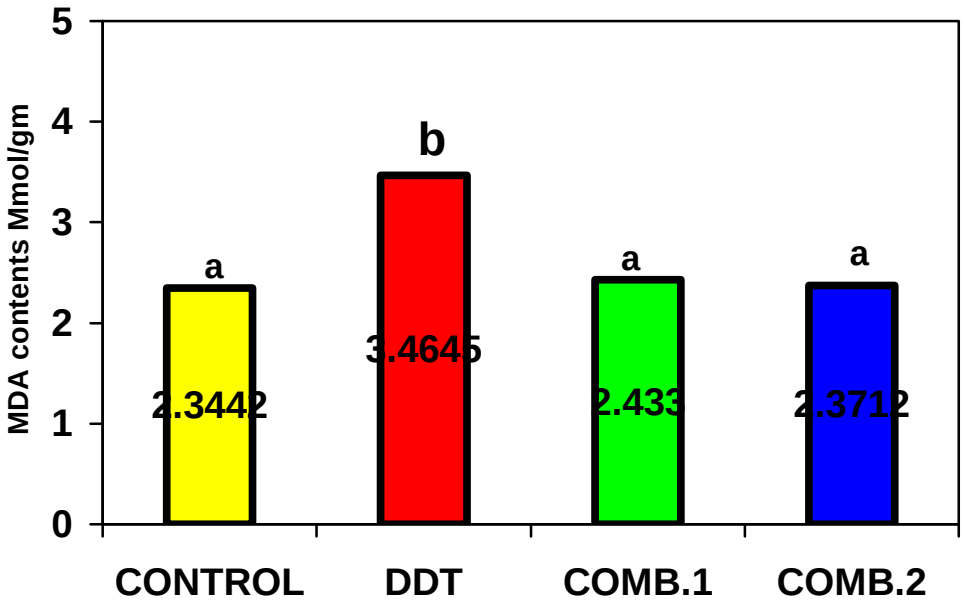

Figure-1: Bar chart comparing the MDA contents in liver tissue homogenate in different groups of rats [values with non-identical superscripts (a and b) are considered significantly different (p< 0.05)].
COMB-1: DDT + AGTE 1.25%; COMB-2: DDT + AGTE 10%

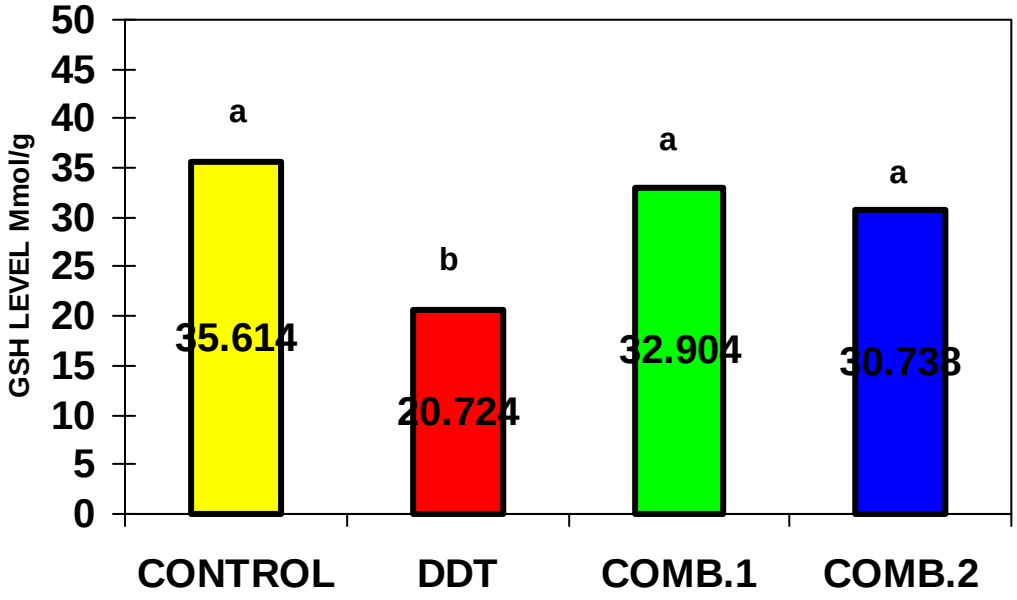

Figure-2: Bar chart comparing the GSH level in liver tissue homogenate in different groups of rats [-values with non-identical superscripts (a and b) are considered significantly different (p< 0.05)].
COMB-1: DDT + AGTE 1.25%; COMB-2: DDT + AGTE 10%

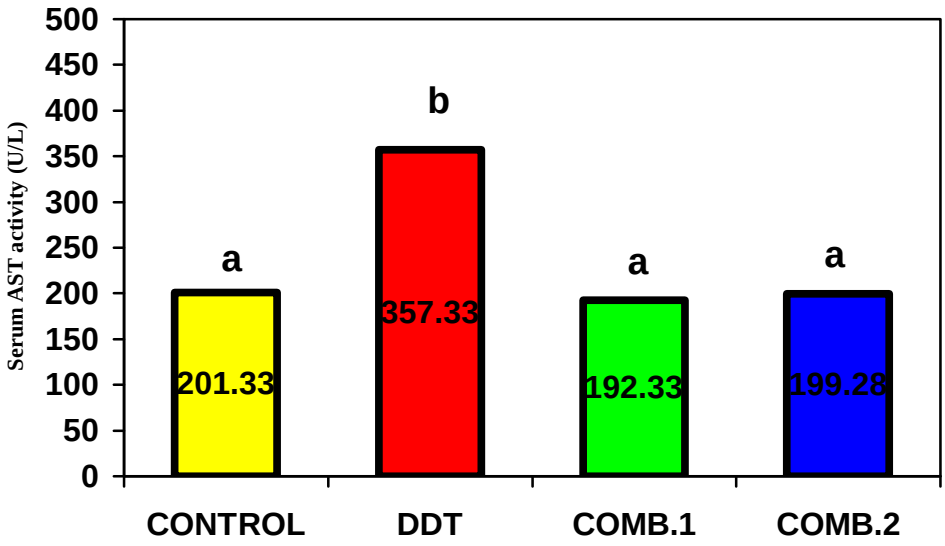

Figure-3: Bar chart comparing the serum aspartate aminotransferase (AST) activity in different groups of rats [values with non-identical superscripts (a and b) are considered significantly different ($p < 0.05$)].
COMB-1: DDT + AGTE 1.25%; COMB-2: DDT + AGTE 10%

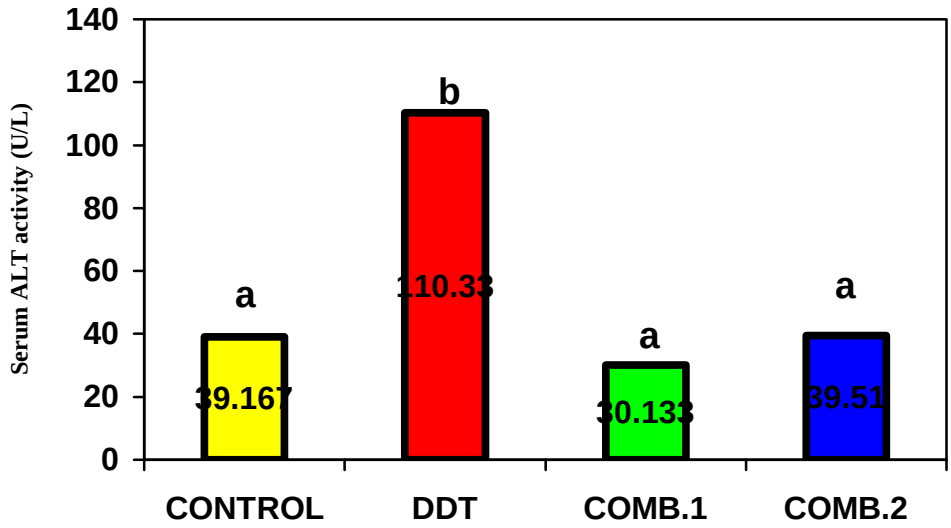

Figure-4 : Bar chart comparing the serum alanine aminotransferase (ALT) activity in different groups of rats[values with non-identical superscripts (a and b) are considered significantly different ($p<0.05$)].
COMB-1: DDT + AGTE 1.25%; COMB-2: DDT + AGTE 10%

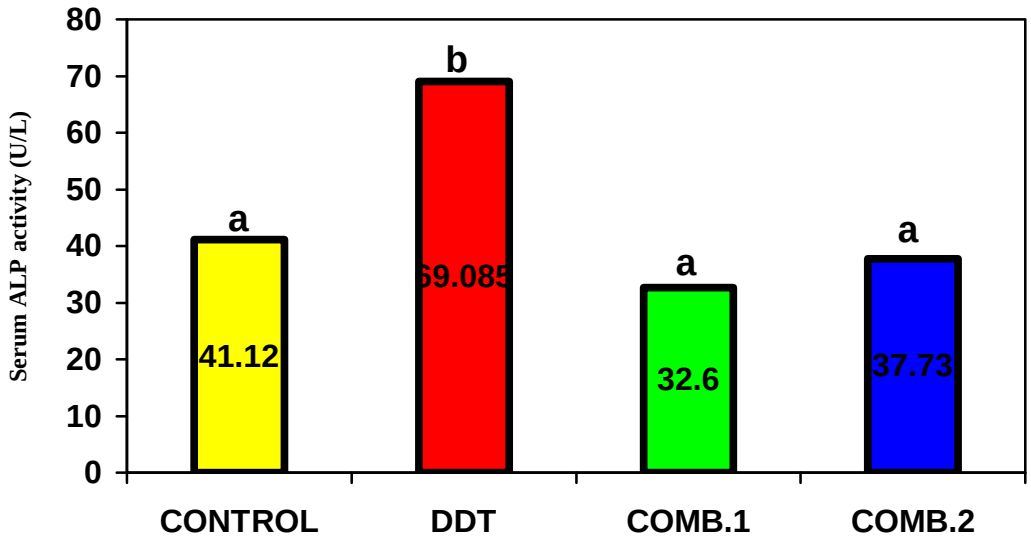

Figure-5 : Bar chart comparing the serum alkaline phosphatase (ALP)activity in different groups of rats [values with non-identical superscripts (a and b) are considered significantly different ($p < 0.05$)].
COMB-1: DDT + AGTE 1.25%; COMB-2: DDT + AGTE 10%

Discussion:

1. Toxic effects of DDT on hepatic tissues:

DDT induce dose - time-dependent oxidative stress and tissue damage in the liver and kidney [19], This damage is due to DDT free-radicals formation by the action of hepatic cytochrome P450 enzyme [20]. These activated radicals injure the hepatocytes by binding covalently to the macromolecules [21] causing degradation of membrane poly-unsaturated fatty acids of endoplasmic reticulum [22], resulting in the formation of lipid peroxides, which in turn causing damage to the membrane, cellular protein, formation of DNA-strand break in hepatic tissues that altering cellular function [23] with the reduction of activities of antioxidant defense mechanisms [24].

Organochlorine insecticides thought to increase phospholipases activity with subsequent increase in fatty acid metabolism through increase B-oxidation of fatty acids. It's increase the activity of superoxide dismutase (SOD) without any change observed in the activity of catalase enzyme These may increase hydrogen peroxide H_2O_2 overload with subsequent increase in tissue lipid peroxidation [25].

In this study, we investigated the changes in the concentrations of end products of lipid peroxidation in liver tissue homogenate as a result of DDT administration. The results showed (48%) elevation of MDA contents in DDT- treated rats compared to controls (Table 1 and Figure 1).

Regarding non-enzymatic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage. Depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals [26] .

It was observed that (42%) a depletion of GSH levels in liver tissue homogenate of rats treated with DDT compared to controls (Table 1 and Figure 2).

The mechanism by which GSH is affected after DDT treatment may be explained by conjugation of GSH with free radicals formed during DDT metabolism through one electron transport of cytochrome-P450 to form DDD or by conjugation with intermediate metabolites [27] by means of glutathione S-transferase where DDT is reported to be a potent inducer for the activity of such enzyme; This lead to increase oxidized glutathione

(GSSG) [28]. The direct interaction of GSH with free radical intermediate that abstract a hydrogen form thieyl radical ($GS^{\cdot}$), glutathione disulfide formation can subsequently occur by dimerization of two ($GS^{\cdot}$) radicals [29].

As a result of increased lipid peroxidation and subsequent degradation of biomembranes, the permeability of the plasma membranes was severely affected and may lead to leakage of AST and ALT, with significant increase in their serum activities (77.5% and 182%) respectively compared to control group (Table 1 and Figures 3 and 4).

The high values of serum activities of both cytosolic enzymes (AST and ALT) may be attributed to the hepatocellular membrane damage probably as a result of binding of toxic metabolite of DDT to the lipid and protein components of the membrane. [20]

The serum activity of alkaline phosphatase (ALP) was also significantly increased in DDT-treated rats (68%) compared to control rats (Table 1 and figure 5).

There is a significant increase in the activity of Alkaline phosphatase (ALP) in the plasma following an acute, sever insult to the hepatic tissues which is present in the lining membrane of the hepatocytes has a cell membrane location associated with the canalicular membrane damage [30].

The increase in alkaline Phosphatase activity is probably a reflection of remained enzyme molecules on the cell membrane fragments released to the plasma as a consequence of hepatic damage [31].

2. The Effects of Aqueous Green Tea Extract (AGTE) on DDT-Induced Liver Damage in Rats:

In this study green tea used as protective agent against DDT- induced hepatotoxicity. Rats orally treated with different concentrations (1.25% and 10%) of AGTE 7 days prior to and during DDT treatment, showed a significant decrease in MDA contents in liver tissue homogenate (30% and 31.6%) respectively and a significant increase in GSH levels in liver tissue homogenate (59% and 48.3%) respectively compared to DDT-treated group ($p<0.05$), also showed a non significant difference compared to control group ($p>0.05$) (Table 1 and figures 1 and 2) .

The protective effect of green tea is due to its antioxidant properties that scavenging free radicals formed by DDT. It has been shown that green tea contains volatile oils,anti oxidant vitamins like (B,C, E and folic acid); tannins and amino acid (theanine) which is a major amino acid present in green tea [32]. Additionally, polyphenols may also function indirectly as antioxidants through: (a) inhibition of the redox-sensitive transcription factors [33], the antioxidative activity of green tea catechins is related to its ability for chelating redox-active transition metal ions like iron, copper and prevents their participation in Fenton and Haber-Weiss reactions [39].(b)inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase [33]. (c) Induction of phase I and phase II metabolic enzymes, which increase the formation and excretion of detoxified metabolites resulting from xenobiotic metabolism [33]. (d) Induction of antioxidant enzymes such as catalase, superoxide dismutases and glutathione S-transferases [34].

The results of this work showed that both concentrations (1.25% and 10%) of orally administered AGTE to the rats resulted in the reduction of MDA contents and elevation of the level of GSH in liver tissue homogenate which are consistent with the previous studies [35, 36].

2.2. The Effects of Aqueous Green Tea Extract (AGTE) on The Serum Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in DDT treated Rats:

Rats treated with the two oral concentrations (1.25% and 10%) of AGTE 7 days prior to and during DDT treatment produced a significant decrease in the levels of serum AST , ALT and ALP[(46.2% , 72.7% ,53%) and (44.2% , 64.2% and 45.4%)] respectively compared to DDT treated group ($P<0.05$). (Table 1 and figures 3, 4 and 5).

The reduction of the serum activities of AST and ALT by green tea indicates the ability of green tea for stabilizing plasma membrane as well as repair of hepatic tissue damages caused by oxidative stress. This effect is agreeable with the commonly accepted view in which serum levels of transaminase return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes [37].

Alkaline phosphatase (ALP) is an enzyme localized in the bile ducts and is used as indicator for assessment of hepatic excretory action [38, 39], the serum activity of this enzyme is highly elevated in certain liver injury like cholestasis which is a form of liver injury that results from either decrease in the volume of bile formed or an impaired secretion of specific solutes into bile. The ability of green tea for reducing the serum activity of ALP suggests the stability of the biliary dysfunction in rat liver during injury induced by DDT, which are consistent with the previous studies [35, 36].

Conclusion:

According to the results obtained in this study we can conclude that Green Tea have the ability through a mechanism related to direct and indirect antioxidant property to provide protective effects against DDT-induced hepatotoxicity by the reduction of hepatic oxidative stress (reduction in MDA contents) with the elevation of GSH levels with subsequent improvement in liver functions manifested by reduction in the serum levels of (AST , ALT and ALP) that improving the damage in liver tissue and makes it a good candidate to be tried clinically in this respect.

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