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ANTI-XENOBIOTIC ACTIVITY OF GREEN TEA

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ABSTRACT

The present study was conducted to evaluate the effect of green tea on albino rats for a long period (120 days), and the interaction between the green tea supplementation in the presence of ADI dose of pesticide toxicity induced from Profenofos pesticide. To achieve this, 48 male albino rats with similar weights and ages were used in the present study, and were divided into four groups as follows: first group used as control (non treated); second group (treated with aqueous green tea extract GTE); third group treated with pesticide (ADI dose) and fourth group treated with both GTE and ADI dose of pesticide. Data obtained revealed that green tea supplementation have a positive potential effect against pesticide toxicity. Nevertheless, it improves the biochemical plasma parameters either alone or combined with pesticide.

Keywords: Green Tea (GT), Profenofos, Rats, Cholinesterase, Blood parameters, Reduced glutathione.

INTRODUCTION

Tea is one of the most popular beverages consumed world wide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green or black tea. Green or black tea are processed differently during manufacturing. To produce green tea, freshly harvested leaves are steamed to prevent fermentation, yielding a dry, stable product (Crespy and Williamson 2004).

Human are continuously exposed to various kinds of environmental chemicals such as pesticides, Food additives, industrial chemicals and other undesirable contaminants in the air, food, soil, consumer products and drinking water (Hasegawa *et al*., 1995).

Green tea is a very rich source of catechin flavonoids: (-)epicatechin, (-) epicatechin gallate, (-)epigallocatechin and (-)epigallocatechingallate which are condensed during manufacturing of black tea. These compounds have been shown to exert protective effects on many kinds of chemical-induced tumorigenesis in organs/tissues such as the skin, forestomach, lung, liver and large intestine (Katiyar *et al*., 1992,1993,and 1999; Yin Pingzhang *et al*., 1994).

The majority of xenobiotics that enter the body tissues are lipophillic, a property that enables them to penetrate lipid membranes and to be transported by lipoproteins in the body fluids.

Profenofos is a broad-spectrum organophosphours insecticide and acaricide is still used in a wide scale and used widely for agricultural and household purposes. Moreover, samples treated with subleathal concentrations of Profenofos revealed satallite associations and chromatide breaks and gaps, indicating its effect on chromosomes. (Prabhavathy *et al.,*

2006). Profenofos is neurotoxic and alters the apparent Km values widely in a concentration-dependent manner, resulting in a competitive type of inhibition (Venkateswara *et al*., 2003).

The current work was achieved to elucidate and predict whether green tea might exert preventive effects on pesticide toxicity induced by oral administration of Profenofos pesticide parallel with daily intake of green tea extract for a long period (120 days) on male albino rats.

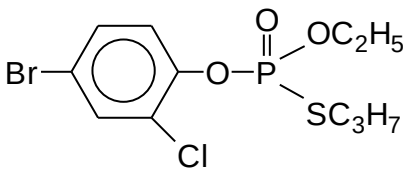
MATERIALS AND METHODS

Experimental Materials:

Green Tea and extraction

Green tea were purchased from Agricultural Spices and Medicinal Plants Co. (Harras), Cairo, Egypt. Powdered sample from green tea extracted with pre-boiled water for 10 minutes at room temperature, then the extract was filtered.

Pesticide: Profenofos:
Chemical name: O-(-4-bromo-2-chlorophenol)-O-ethyl-S-n-propyl phosphorothioate
Commercial name: Profenofos:
Molecular formula: C11H15BrClO3PS



Chemical structure

Experimental Protocol:

Animal maintenance:

The present study included 48 male albino rats (190 ± 10 g). Animals were brought from the stock of animal house National Organization for Drug Control and Research (NODCAR), Giza Egypt. The animals were kept under normal environmental conditions, housed in stainless steel wire bottomed cages, 21± 2^{o}C, humidity 60% and 12-hour light/dark cycle for two weeks before the initiation of the experiment. The animals were allowed free access to water and fed on standard diet.

Treatments:

After acclimation to laboratory conditions, the animals were divided into 4 groups (12 rats each) as follows: the first group, control, fed only on basal diet; the second group, orally intubated with green tea extract (dose calculated according to Paget and Barnes, 1964); the third group, Pesticide group treated with Profenofos with ADI dose; the fourth group treated with both GTE & ADI and considered the interaction group between pesticide and green tea to evaluate the protective effect of green tea and the adverse effect

of Profenofos (ADI dose). This dose represents the Acceptable Daily Intake (ADI) [0.06 g / kg b.w.] as a low dose. The experiment employed daily an oral administration of ADI dose of pesticide after one hour of oral administration of green tea extract (except two days weekly) for 120 days (four months).

Profenofos (Dose)

Acceptable Daily Intake (ADI) per kilogram rats body weight (b.W.) [0.06 g/kg b.w. rats]

Green Tea (Dose)

[0.7 g/kg b.w. rats]

Blood Sampling:

Rats were ether-anesthetized, then blood samples were collected in heparinized centrifuge tubes from retirobulbar venous plexus by the mean of fine capillary glass tubing in accordance with the procedure of Schermer, (1967). Plasma was separated after centrifugation at 5000 rpm and kept in refrigerator until used for biochemical analysis.

Biochemical Markers

Blood and Plasma parameters were subjected to analysis according to the following methods: Blood Haemoglobin (Hb) measured according to the method described by Van-Kampen and Zijlstra (1961) using Randox Company kits. Plasma total protein was measured according to Doumas (1975). Activity of cholinesterase was determined by the method adopted by Elleman *et al*. (1961) using kits QCA Co, Spain. Activity of alkaline phosphatase was determined by the method adopted by Hausman *et al*. (1967) using kits QCA Co, Spain. Albumin concentration was determined according to the method described by Doumas *et. al*. (1971) using kits Human Co, Germany. Plasma urea was determined according to the method described by Tobacco *et al*. (1979) using kits Human Company, Germany. Creatinine was determined according to the method described by Tietz, (1995) using kits Cromatest Company. Creatine kinase activity was determined according to the method described by Schumann, (2003) using kits Human Company, Germany. Total lipid was carried out according to the method of Knight, *et al*., (1972). Total cholesterol was determined according to the method described by Schettler and Nussel (1975) using kits Human Company, Germany. Triglycerides were determined according to the method described by Schettler and Nussel (1975) using kits Human Company, Germany. HDL-cholesterol was determined according to the method described by Gordon, *et al*. (1977) using kits Human Company, Germany. The glucose was determined according to the method described by Trinder, (1969) using kits Spinreact Company, Spain. Glutathione (GSH) level in the whole blood was determined according to the method of Beutler, *et al*. (1963).

Statistical analysis:

Data were statistically analyzed according to the method of Gad and Weil (1989).

RESULTS AND DISCUSSION

Effect of Green Tea Extract (GTE), Pesticide administration and the interaction between GTE-Pesticide on:

Body weight

Data represented in Fig.1 showed that body weight gain of control group was revealed higher values among all treated groups through experimental periods and elevated with time. On the other hand, green tea administration resulted in significant decrease in all experimental periods except the first one. The same trend was shown with pesticide treatment but the lowering was more than that found in green tea group. However, in GT-Pesticide (forth group) showed that green tea extract could elevate body weight to reach the green tea body weight line. Generally green tea restore the lowering in body weight gain which found due to pesticide treatment and improve body weight gain in the fourth group (GT-Pesticide interaction group).

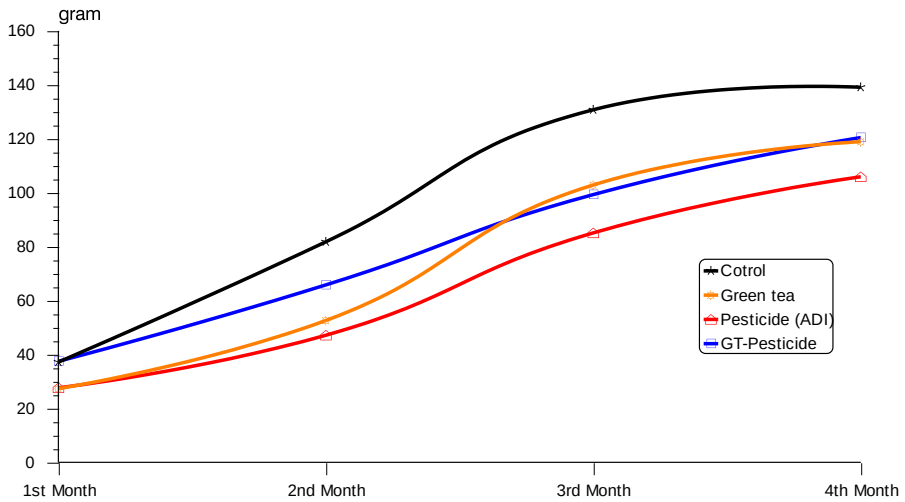


Fig. 1. Body Weight Gain for Control, Green Tea, Pesticide Profenofos and GT-Pesticide Groups through four months.Organs weight (Liver & Kidney)

Liver weight

Fig.(1) illustrated the liver weight of groups under this study. Liver weight was not significantly affected due to green tea treatment. However, little reduction was found in the liver weight of pesticide group, but this reduction is not a significant reduction. Generally, this reduction was decayed and restored due to the oral administration of GTE associated with pesticide ADI dose in a combined group. This means that GT could prevent or maintain liver weight without affected or reduced by pesticide toxicity in ADI dose. These result are coincided with Crespy and Williamson (2004) who mentioned that there are some previous studies reported that GT prevents hepatotoxicity.

Kidney weight

Regarding to Kidney weight (Fig.2), it is shown that kidney was markedly affected with oral administration of pesticide ADI dose which reflected in significant reduction of kidney weight among all groups, in comparison with control group. In contrary, oral administration of GTE resulted in little elevation in kidney weight corresponding to control group. However, GTE-Pesticide interaction showed significant increase in kidney weight. The increase of kidney weight in this case pointed out to the biorole of green tea in inhibiting the reduction effect of pesticide in ADI dose group. Also, these data indicate to the power activity of green tea in stopping the reduction firstly and led to some enlarge in kidney weight secondly. This role could be taken under consideration especially the weight of kidney increased more than both control and green tea groups, which means that GT was more effective in the presence of pesticide ADI dose to increase kidney weight rather than alone; or may be due to the more effort of kidney to remove the pesticide and filter the blood from toxic molecules and the metabolites of pesticide.

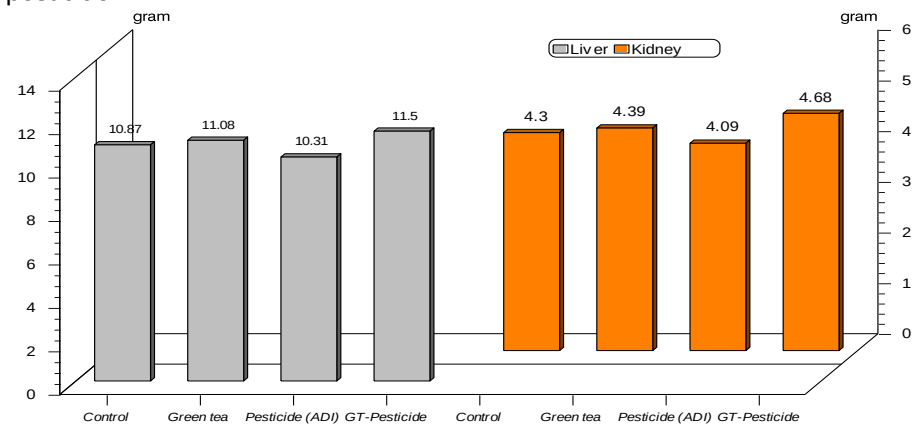


Fig.2. Correlation between Liver and Kidney weight responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months

Effect of Green Tea Extract (GTE), Pesticide administration and the GTE-Pesticide interaction on Biochemical Markers:

Blood Haemoglobin (Hb)

Data represented in Table (1) revealed that blood haemoglobin was not affected with green tea extraact (GTE) during experimental periods except some reduction was observed in first period. On the other hand, significantl decrease in haemoglobin levels was found due to pesticide treatment in both pesticide and GT-Pesticide groups in 2nd & 3rd periods. However, GTE could not restores this lowering in these two periods. Meanwhile the last period showed significant increease of hemoglobin in both groups. This results are in accordance with Chin *et al.* (1982) who reported that oxidative stress has been shown to cause changes in the structure and function of haemoglobin.

Plasma Glucose

Data in Table (1) showed that plasma glucose increased with time, while GT supplementation induced decrease in plasma glucose and this decrease at fourth period was statistically significant. Meanwhile in pesticide group, significant lowering in plasma glucose was observed in 1st & 4th period, but in 3rd period plasma glucose increased significantly. This disturbance effect may be due to pesticide toxicity in plasma glucose. The same trend was found due to GT-Pesticide interaction.

Table (1). Effect of Green Tea Extract, Profenofos pesticide and GTE-Profenofos interaction on plasma Hemoglobin and Glucose of male albino rats through four months.

Parameter	Month	Control	Green Tea	Pesticide (ADI)	GTE-Pesticide
Haemoglobin (g/dl)	1	14.20 ± 0.29	13.11 ± 0.29*	13.85 ± 0.27	14.76 ± 0.8
	2	13.78 ± 0.48	12.37 ± 0.43	11.56 ± 0.22*	11.13 ± 0.21*
	3	14.50 ± 0.54	14.15 ± 0.48	12.93 ± 0.19*	12.16 ± 0.24*
	4	13.89 ± 0.76	12.93 ± 0.17	18.25 ± 0.51*	16.94 ± 0.35*
Glucose (mg/dl)	1	98.94 ± 2.43	94.99 ± 2.09	83.88 ± 4.05	105.99 ± 4.22
	2	104.32 ± 3.91	92.69 ± 4.24	96.64 ± 6.19	104.05 ± 4.15
	3	97.76 ± 4.06	104.08 ± 4.56	112.32 ± 1.4	116.87 ± 2.03
	4	116.63 ± 3.92	105.64 ± 1.61	94.64 ± 6.84	87.97 ± 2.63

All data were expressed as mean ± SE * significant effect p> 0.05 ** Highly significant p> 0.05

Total protein (TP)

Table (2) showed that plasma total proteins was approximately given the same values in GT group in comparison to control, except the first period which showed a higher increase in TP level. On the other hand, pesticide treatment resulted in some increase in TP while significant decrease was observed in 4th period. Meanwhile, the previous significant lowering was restore to be close to control value due to GTE treatment as it is shown in Table (2).

Albumin

Data obtained in Table (2), showed that plasma albumin was close to control group in 1st, 2nd, and 3rd periods. Respecting to 4th period, it is found that green tea extract resulted in significant increase in plasma albumin level which means the potential effect of green tea in enhancement the biosynthesis of albumin and the healthy role of green tea in this state. However, pesticide showed insignificant effect on albumin level, despite little increase was found in 1st and 4th periods. The interaction between GT-Pesticide showed significant increase in 3rd & 4th periods.

Reduced Glutathione (GSH)

Data obtained in Table (2) showed that GSH was decreased with time duration in control group. While green tea treatment revealed significant increase in GSH level in fourth period. On the other hand, the pesticide led to lowering GSH level significantly in all periods, except the 2nd period. However,

the interaction between GT-Pesticide resulted in restoring this adverse effect whereas GSH level was generally raised and this elevation was significant appeared in the 4th period. This means that pesticide has effect on GSH synthesis or led to GSH utilization due the toxicity effect, Inversely GT has inverse effect to this lowering, that improves and elevates reduced glutathione level in rats.

Table (2). Effect of Green Tea Extract, Profenofos pesticide and GTE-Profenofos interaction on plasma Albumin, Total Protein and Reduced Glutathione of male albino rats through four months.

Parameter	Month	Control	Green Tea	Pesticide (ADI)	GTE-Pesticide
Albumin (g/dl)	1	3.80 ± 0.43	3.61± 0.51	4.60± 0.59	4.04 ± 0.55
	2	4.29± 0.17	4.36± 0.38	4.18± 0.19	3.59± 0.21
	3	4.70 ± 0.08	4.79± 0.08	4.63± 0.05	5.23± 0.19*
	4	3.81± 0.15	4.64± 0.12*	4.11± 0.11	4.56± 0.20*
Total Protein (g/dl)	1	6.05 ± 0.18	7.58 ± 0.71	6.63 ± 0.60	5.25 ± 0.69
	2	5.88 ± 0.47	6.01 ± 0.49	6.29 ± 0.32	5.96 ± 0.59
	3	8.20 ± 0.89	8.30 ± 0.20	8.70 ± 0.22	7.41 ± 0.40
	4	8.60 ± 0.15	8.01 ± 0.19	6.40 ± 0.15*	7.62 ± 0.39
Reduced Glutathione (mg/dl)	1	5.16 ± 0.19	4.92 ± 0.44	2.78 ± 0.20*	3.7 ± 0.6
	2	4.89 ± 0.47	4.87 ± 0.19	5.45 ± 0.38	5.47 ± 0.72
	3	2.0 ± 0.01	1.78 ± 0.20	1.01 ± 0.07*	1.72 ± 0.13
	4	2.9 ± 0.17	3.88 ± 0.20*	1.61 ± 0.08*	6.7 ± 0.06*

All data were expressed as mean ± SE * significant effect p> 0.05 ** Highly significant p> 0.05

Correlation between Plasma Total Protein & GSH

Correlation between plasma total protein and reduced glutathione is illustrated in Fig. (3). It has pointed out that GSH correlated with total protein, whereas an indicator to the increasing in total protein and to high synthesis in GSH which reflect in facing the toxic effect of pesticide. Also, it is interesting to found that green tea is enhanced GSH level more than control, which means that most of protein in this state synthesis proceed toward GSH to face pesticide toxicity.

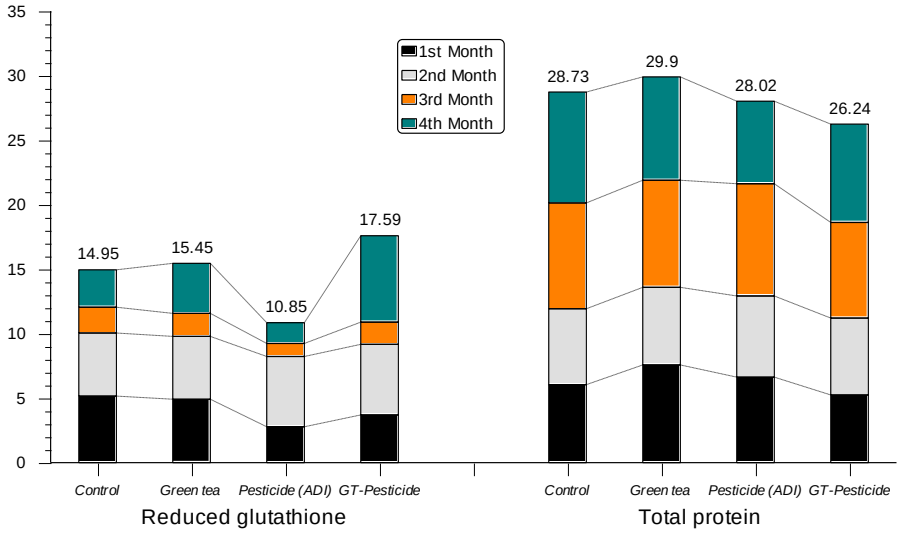


Fig.3. Correlation between plasma GSH-Total Protein responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months.

Enzymes activity

Cholinesterase (ChE)

Data in Table (3) showed that cholinesterase was generally lowered due to GTE or pesticide treatment or the interaction between them, but the lowering of acetyl cholinesterase was different in all cases. The lowering effect was statistically significant in 2nd and 4th period, meanwhile lowering in pesticide group in 4th period was insignificant.

Alkaline Phospatase (ALP)

Data in Table (3) showed that GTE administration led to significant increase of ALP activity in 2nd period and significant decrease in 4th period. This change indicates to that alkaline phosphatase firstly temporary was raised then finally lowered at the end of experiment. On the other hand, pesticide treatment resulted in significant increase in ALP level at 1st, 2nd and 3rd periods, while the ALP was similar to control at the end of experiment. However, GT-Pesticide interaction treatment resulted in significant increase at 1st period, despite some decrease was found in the latest period, but it is statistically insignificant.

Creatine Kinase (CK)

Data in Table (3) showed that CK was increased in 1st period in control rats compared with 2nd, 3rd and 4th periods. Meanwhile, some decrease of CK was noticed through all periods in GT group, but it is insignificant. Regarding to CK values due to pesticide toxicity it is observed that pesticide was resulted in opposite trend corresponding to control in all

experimental periods. Finally, the values were settled and being significantly lowered at 3rd period. Meanwhile, the value was insignificant at 4th period which may be represents some adaptation of rats to pesticide toxicity after long time (120 days). Moreover, data in Table (3) obviously showed that GTE resulted in restoring, and returning CK value to be close to control value at 2nd and 3rd period. However, significant increase was found in 4th period. This increase may be attributed to the high accumulation of pesticide toxicity with time after 120 days.

Table (3). Effect of Green Tea Extract, Profenofos pesticide and GTE-Profenofos interaction on plasma enzymatic activity of male albino rats through four months.

Parameter	Month	Control	Green Tea	Pesticide (ADI)	GT-Pesticide
Cholinesterase (U/L)	1	397.25 ± 17.03	322.57 ± 35.09	375.36 ± 14.84	404.69 ± 51.5
	2	407.44 ± 4.49	197.07 ± 51.18*	215.83 ± 52.67	112.61 ± 32.67*
	3	408.2 ± 27.36	387.87 ± 21.07	380.05 ± 20.78	384.74 ± 39.67
	4	412.02 ± 25.89	154.84 ± 19.86*	333.13 ± 49.98	228.74 ± 15.52*
Alkaline Phosphatase (U/L)	1	100.74 ± 2.78	96.92 ± 8.93	155.1 ± 4.27**	140.68 ± 14.43*
	2	72.24 ± 5.05	106.8 ± 0.7**	132.99 ± 7.94**	97.03 ± 12.19
	3	121.43 ± 11.79	127.9 ± 12.39	164.39 ± 11.84*	132.98 ± 12.39
	4	89.03 ± 11.16	66.69 ± 6.04*	94.2 ± 1.96	61.77 ± 7.12
Creatine kinase (U/L)	1	130.5 ± 13.65	97.3 ± 3.2	112.78 ± 13.1	114.91 ± 9.91
	2	77.74 ± 7.7	72.97 ± 2.09	99.51 ± 9.6	79.61 ± 4.19
	3	89 ± 9.6	74.63 ± 2.5	51.75 ± 3.87*	89.56 ± 11.2
	4	59.7 ± 7.56	48.09 ± 7.6	50.86 ± 1.2	87.9 ± 3.23*

All data were expressed as mean ± SE * significant effect P< 0.05 & ** Highly significant P< 0.01

Correlation between Plasma Acetylcholinesterase & Alkaline Phosphatase

The correlation between Plasma Acetylcholinesterase & Alkaline Phosphatase revealed that both green tea and pesticide lowered AChE activity which means that green tea is similar to pesticide effect on AChE activity, but the effect is not the same. This observation could be interpret through showing the alkaline phosphatase activity which raised in pesticide that mean toxic effect, while at the same time alkaline phosphatase was not elevated in green tea treatment which mean the safety effect. On other words green tea lower AChE which reflect on enhancement body function, while pesticide lowering is a toxic effect because the target is different in these two cases. This results are in accordance with Okello, (2004), who found that both green and black tea inhibited the activity of enzyme acetylcholinesterase (AChE), which breaks down the chemical messenger or neurtransmitter, acetylcholine that dropped in Alzheimer disease. They also added that tea could improve memory which may lead to the development of a new treatment for a form of dementia, which affects an estimated ten million people worldewide.

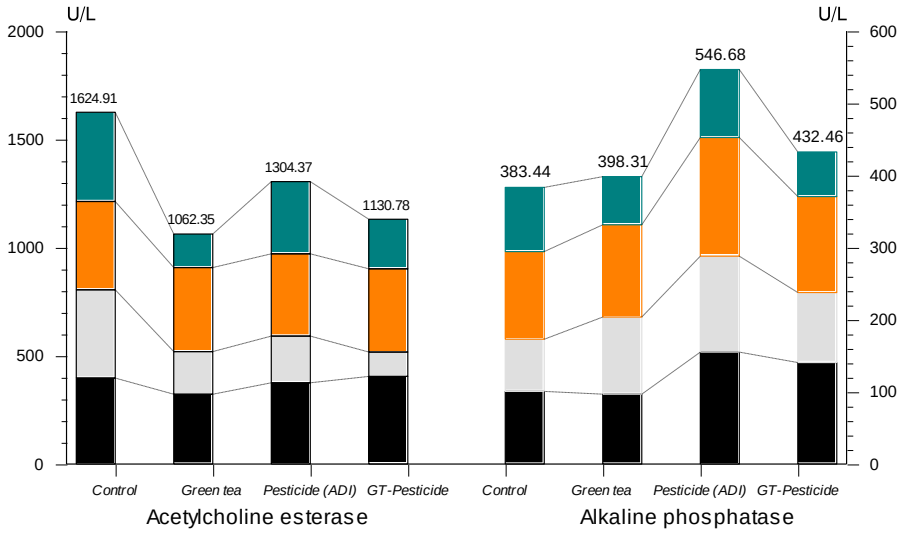


Fig.4.Correlation between plasma AChE and Alkaline phosphatase responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months.

Lipid profile

Total Lipids (TL)

Data In Table (4) revealed that total lipids were increased with time, while green tea reduced this elevation significantly in all periods through experimental time. On the other hand, the pesticide treatment lowered total lipids in all periods except the second period was not significantly affected. However, the interaction between GT-Pesticide resulted in significant lowering in both 3rd and 4th periods.

Triglycerides (TG)

Data in Table (4) showed that TG was increased with time, while GTE administration led to decrease TG significantly in 4th period. That means GT effect was markedly shown with long period not short time. On the other hand, TG was not affected with ADI dose of pesticide through experimental time. Meanwhile, the interaction between GTE and pesticide revealed significant decrease found in all periods except the first one. This result indicates that GT was responsible to this lowering mainly rather than the pesticide effect.

Total Cholesterol (TC)

Regarding to Table (4), it is shown that total cholesterol was not changed significantly with time during experimental period in control rats group. Meanwhile, GTE resulted in TC reduction significantly in all periods except in the second period some lowering was found, but it is not statistically significant. On the other hand, pesticide treatment showed that total cholesterol was not significantly affected. Meanwhile GT-Pesticide

combination led to significant lowering in total cholesterol in all periods except the first one. This means that green tea has lowering capability towards total cholesterol. These results confirmed that green tea has more effect on lowering total cholesterol with long time.

HDL-Cholesterol (HDL-C)

Respecting HDL-C (Table, 3) it has been found that HDL-C was increased due to GT supplementation especially at the 3rd and 4th period. However, pesticide resulted in significant reduction in HDL-C at 4th period. This harmful in this state was restore due to GT supplementation whereas HDL-C was increased due to interaction effect of GT with pesticide, and the HDL-C was revealed higher value reached to 53.08 mg/dl and 56.18 mg/dl in 3rd and 4th periods. That means GT has powerful potential to reflect the toxicity resulted from pesticide administration.

Table (4). Effect of Green Tea Extract, Profenofos pesticide and GTE-Profenofos interaction on plasma Lipids profile of male albino rats through four months.

Parameter	Month	Control	Green Tea	Pesticide	GTE-Pesticide
Total Lipids (mg/dl)	1	340.20 ± 5.96	254.60 ± 8.4**	315.6 ± 2.38*	353.40 ± 13.33
	2	315.89 ± 7.64	278.50 ± 8.3*	317.0 ± 4.63	327.70 ± 36.50
	3	344.58 ± 6.09	210.38 ± 9.1**	272.75 ± 7.69**	206.48 ± 9.43**
	4	366.91 ± 22.64	127.25 ± 24.8**	94.51 ± 13.43**	134.46 ± 29.0**
Triglycerides (mg/dl)	1	53.88 ± 2.78	52.36 ± 2.76	65.21 ± 3.38*	51.45 ± 9.32
	2	43.07 ± 5.68	31.50 ± 4.30*	35.84 ± 11.26	23.13 ± 2.70*
	3	162.62 ± 10.23	169.1 ± 31.55	158.0 ± 14.8	72.30 ± 5.05*
	4	140.1 ± 18.4	73.41 ± 15.70*	137.38 ± 15.4	68.76 ± 2.70*
Total Cholesterol (mg/dl)	1	102.9 ± 6.65	63.65 ± 5.01*	98.03 ± 17.3	99.43 ± 6.58
	2	80.30 ± 8.29	48.57 ± 8.61	83.28 ± 6.02	45.33 ± 6.38*
	3	77.68 ± 4.65	45.35 ± 1.11*	70.67 ± 2.58	55.22 ± 3.24*
	4	72.71 ± 2.58	46.66 ± 1.74*	70.92 ± 6.35	49.40 ± 1.19*
HDL-C (mg/dl)	1	42.01 ± 0.48	43.23 ± 1.44	36.56 ± 0.51*	43.81 ± 2.22
	2	30.86 ± 0.66	29.56 ± 0.79	30.88 ± 5.46	29.56 ± 2.78
	3	34.29 ± 3.28	53.08 ± 1.26*	32.40 ± 3.08	49.19 ± 2.90*
	4	46.92 ± 2.79	56.18 ± 0.30*	36.15 ± 2.09*	46.65 ± 1.15

All data were expressed as mean ±SE * significant effect P< 0.05 & P<0.01 ** Highly significant p> 0.05

Correlation between Plasma Total Lipids and Triglycerides

Correlation between plasma total lipids and triglycerides, depicted in Fig. (5), revealed that green tea behaved the same trend in lowering total lipids and triglycerides either alone or in combined with pesticide. In addition its effect on TG was more than total lipids as shown in combined interaction (group 4).

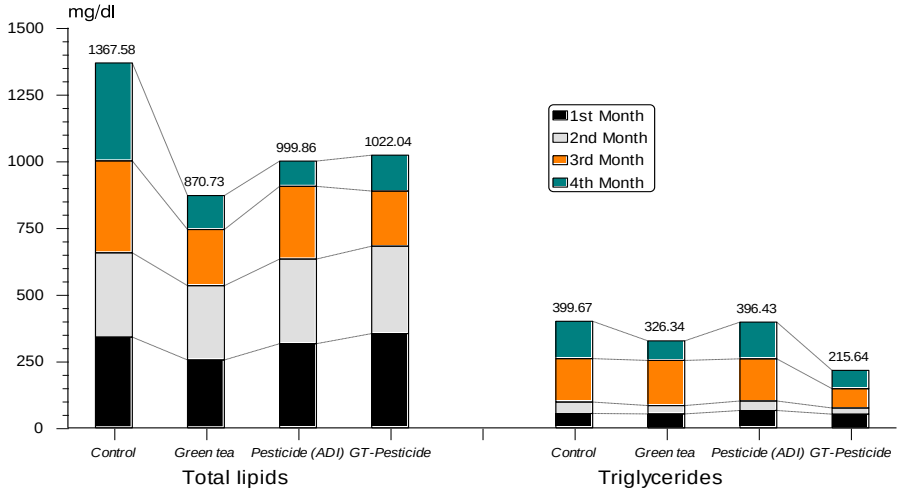


Fig.5.Correlation between plasma total lipids and triglycerides responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months.

Correlation between Plasma Total Cholesterol & HDL-C

Figure (6) revealed the negative correlation between Cholesterol and HDL-C which appeared clearly due to green tea group compared with control rats. Whereas cholesterol lowering is attributed to HDL-C elevation which resulted from green tea effect.

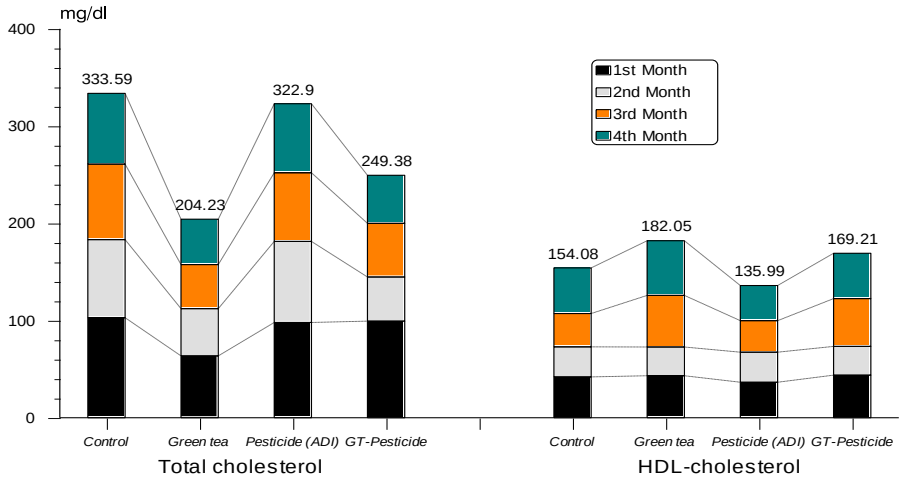


Fig.6.Correlation between plasma Cholesterol and HDL-cholesterol responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months.

Kidney function

Urea

According to Table (5), it was found that urea was raised in control rats with time, while GT supplementation resulted in urea lowering through experimental duration and this lowering was significantly at the fourth period. Also, pesticide administration resulted in urea lowering in 3rd and 4th periods and the lowering was markedly found due to green tea interaction with pesticide and was clearly at the 3rd and 4th periods.

Creatinine

Plasma creatinine was not significantly changed during experimental period due to green tea supplementation, but the significant decrease was found in plasma creatinine at the latest period as shown in Table (5). However, plasma creatinine revealed significant increase due to pesticide at first period which decayed with time at second period, then significant decrease was observed in 3rd period and still to the end of experiment. Regarding to GT-Pesticide interaction, it has been found significant increase in the second period due to the disappearance of pesticide effect. Moreover, plasma creatinine was decreased at 3rd and 4th periods, which means kidney function was returned to work better after affecting by pesticide toxicity.

Table (5). Effect of Green Tea Extract, Profenofos pesticide and GTE-Profenofos interaction on Kidney function of male albino rats through four months.

Parameter	Month	Control	Green Tea	Pesticide (ADI)	GT-Pesticide
Urea (mg/dl)	1	25.55 ± 3.78	21.46 ± 0.50	30.76 ± 0.48	27.90 ± 0.58
	2	23.57 ± 2.24	27.41 ± 0.68	26.14 ± 0.26	30.51 ± 3.87
	3	28.43 ± 1.16	25.75 ± 0.88	23.20 ± 1.55*	15.01 ± 0.47**
	4	23.90 ± 0.11	20.57 ± 1.22*	18.90 ± 0.90*	17.24 ± 1.12**
Creatinine (mg/dl)	1	0.60 ± 0.03	0.60 ± 0.02	0.73 ± 0.02*	0.50 ± 0.06
	2	0.66 ± 0.02	0.70 ± 0.10	0.58 ± 0.02	0.70 ± 0.03
	3	0.60 ± 0.04	0.60 ± 0.03	0.40 ± 0.01**	0.48 ± 0.02*
	4	0.60 ± 0.02	0.38 ± 0.06*	0.40 ± 0.01**	0.36 ± 0.02**

All data were expressed as mean ± SE * significant effect P<0.05 & ** Highly significant P< 0.05

Correlation between Plasma Urea & Creatinine

As it is shown in Fig.7 both creatine kinase and urea were affected due to pesticide which reflected in decrease in creatine kinase activity associated with urea lowering level. Lowering in creatine kinase in plasma due to green tea effect, indicates that green tea maintains this enzyme from leaving the cells and from its increase in plasma which may be found due to pesticide toxicity in second period.

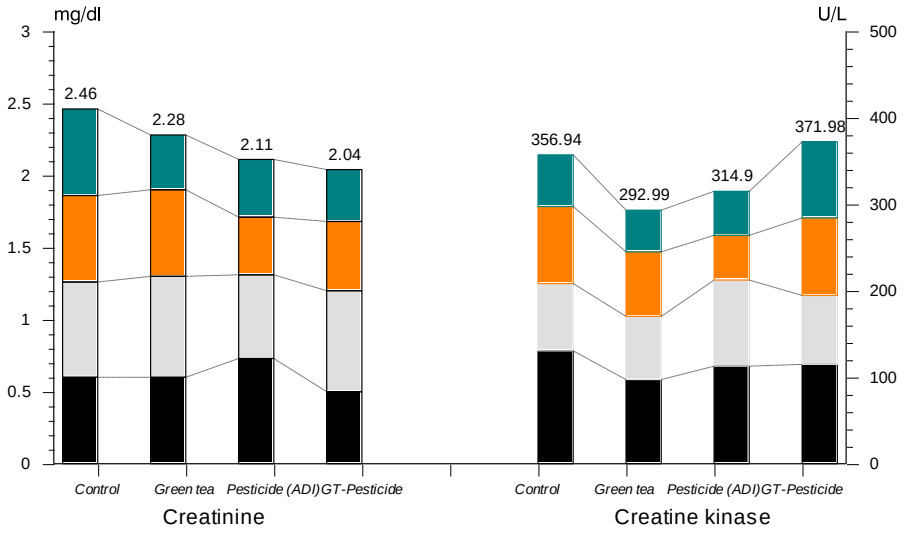


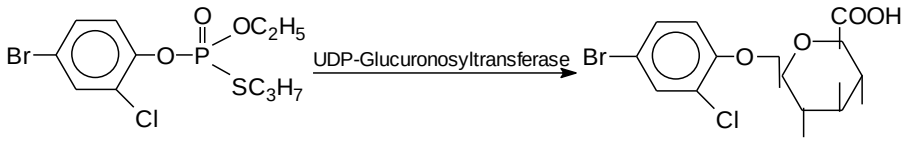
Fig.7.Correlation between plasma Creatinine and creatine kinase responsive to green tea extract and pesticide toxicity and the interaction between green tea and pesticide through four months.

Showing to body weight it was revealed that reduction was found due to green tea extract which coincided with Kuo, *et al*. (2005) who reported that rats fed on green tea leaves for 30 weeks, body weight suppression was found. They also added that green tea lowered the level of triglycerides, moreover GT was more efficient than black tea in lowering the level of total cholesterol.

According to lipids profile it has been found that lipids were significantly affected with green tea extract which could maintain the level to some extend lower than control. This foundation are in agreement with Yeh *et al*. (2003) who stated that a significant suppression of fatty acids synthease by tea and polyphenols has been demonstrated. The present study demonstrated that GTE could maintained the blood cholesterol level within the normal range which reflected in or can reduce the risk of having the pathological changes especially atherosclerosis and coronary heart disease. This fact was found in change the risk value TC/HDL to be close to the control value as a result of green tea treatment and confirmed also, in the treated group which turned also to show that it is affected by GT administration. These results were in accordance with Yang & Koo (1997) who reported that tea could lower plasma cholesterol. Also, Lin *et al*. (1998) mentioned that the long-term feeding of green powder to rats could reduce the blood level of triglycerides and other lipids. Also, the lowering of lipid profile especially triglycerides could be explain on the fact which mentioned by Loest *et al*. (2002) and Raederstorff *et al*. (2003) who found that green tea intake decrease the absorption of triglycerides and cholesterol. In addition Yeh *et al*.

(2003) stated that a significant suppression of fatty acids synthease by tea and polyphenols has been demonstrated. Nevertheless, GT ingestion decrease LDL cholesterol (Yokozawa *et al*., 2002). Concurrently, HDL cholesterol increases, showing that green tea polyphenols exert an antiatherosclerotic effect (Miura *et al*., 2001).

Regarding to plasma creatinine it is obviously observed that plasma creatinine level at 3rd period was 0.6 mg/dl in both control and green tea groups, meanwhile this value was decreased to 0.4 mg/dl as a result of pesticide toxicity, after that GTE supplementation raised this value to 0.5 mg/dl. These data means that GT restore kidney function to normal state as the effect of GTE. The role of GT in protecting kidney from toxicity may be attributed to that GT, as well known that, tea increase urine output due to the diuretic action of the caffeine present in tea. The diuretic effect of caffeine was originally based on acute studies in caffeine naïve individuals, which led to elevation in urine excretion volume due to GT drinking and so high amounts of water will pass through kidney carry out antioxidant substances of GT contents associated with pesticide molecules and metabolites which represents the harmful compounds found in blood. In this case, toxicity of toxic substances and metabolites found in blood will inhibit due to high antioxidant capacity found from polyphenolic compounds of green tea extract absorbed and interact with pesticide molecules or metabolites and so, the effect of GT in this state will be double: firstly increasing excretion of pesticide by elevating urine volume; secondly, passing of antioxidants to kidney which protect it from damage of pesticide molecules or metabolites; and thirdly, UDP-Glucuronosyltransferase was increased as a result of catechin found in GT, which could be summerized the role of this enzyme from the following equition:



Profenofos Complex of Profenofos metabolite and Glucuroic acid

Fig.8. Role of green tea in protect the body from toxic substances through enhancement of UDP-Gluronosyltransferase whereas 2-chloro-4-bromo-phenol (profenfose metabolite) binds with glucuronic acid and excreted in urine (Proposal Mechanism).

On the other hand, the significant increase in alkaline phosphatase activity at 1st & 2nd & 3rd periods following pesticide treatment could be interpret on the fact that, after oral administration of organophosphorous pesticide, the pesticide absorbed and enter blood stream, after that, elevation of the pesticide level inforce the body to hydrolyze it. Hence, the alkaline phosphatase represents the enzyme which release the inorganic phosphate group from many of estrified organomonophosphate compounds. However, under hydrolyzed conditions, this enzyme hydrolyzes the esterified phosphate

linkage in Profenofos pesticide and release the 2-chloro-4-bromophenol. At this point, presence or high amount of catechin of green tea in blood stream resulted in enhancement to synthesis UDP-glucuronosyltransferase which catalyze binding of the released metabolite of Profenofos (2-chloro-4-bromophenol) with glucuronic acids in blood. Thus, it could be explain that why alkaline phosphatase was elevated firstly and decreased at latest period after long time with oral supplementation of Green Tea treatment dose.

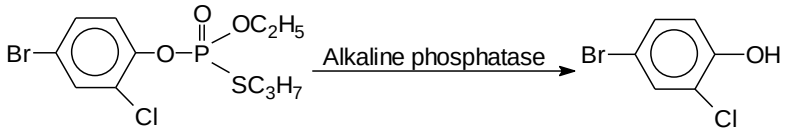


Fig.9. Hydrolysis of Profenofos by Alkaline phosphatase (Proposal Mechamism).

Finally, it could be interpret the biorole of green tea on kidney function and how green tea maintain it to some extend, could be able to excrete and filter the blood from toxic substances, despite the toxicity which found from environmental pollutants. Crespy and Williamson (2004) reported that the blood nitrogen level was markedly decreased due to green tea effect.

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النشاط الحيوى للشاى الأخضر ضد الملوثات

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تهدف هذه الدراسة لمعرفة الدور الذى يلعبه الشاى الأ خضر ضد بعض الملوثات البيئية مثل المبيدات وغيرها من المواد الضارة والمسممة ، وذلك لما يحتويه الشاى الأخضر من مواد فلافونية ومركبات متعددة الفينول . لذلك تم اختيار 48 فأر ألبينو ذكور وقسمت إلى أربعة مجموعات شملت 12 فأراً لكل مجموعة : الأولى كنترول (مقارنة) ، الثانية عوملت بمستخلص مائى للشاى الأخضر ، والثالثة عوملت بمبيد البروفينوفوس بالجرعة الفمية المسموح بها ، أما المجموعة الخامسة فقد جرعت بكل من مستخلص الشاى الأخضر والمبيد واعتبرت مجموعة التفاعل الثنائى .

أظهرت الدراسة قدرة الشاى الأخضر فى خفض وزن الجسم للفئران بينما لم يتأثر وزن الكبد والكلى فى حين أدى المبيد لحدوث نقص فى وزن الكلى الذى استعيد مرة أخرى نتيجة المعاملة بمستخلص الشاى الأخضر مع المبيد وظهر ذلك بوضوح فى مجموعة التفاعل الثنائى (الرابعة) . كما أظهرت الدراسة الدور الواضح لمستخلص الشاى الأخضر فى خفض تأثير المبيد حيث أدى إلى زيادة كل من الليبوبروتينات العالية الكثافة HDL-C والجلوتاثيون المختزل ، كما حدث انخفاض فى نشاط إنزيم الفوسفاتيز القاعدى والذى ارتفع مستواه من جراء المعاملة الفمية للفئران بالمبيد ، مما يعنى حدوث خلل فى الكبد أو ميتابوليزم العظام واستطاع مستخلص الشاى الأخضر أن يحافظ على مستوى قريب من الكنترول فى نشاط الإنزيم ، كما أدى لتحسن واضح فى مستوى الجلوتاثيون المختزل حيث أدى لزيادته بعد النقص المعنوى الذى حدث من جراء تجريع المبيد للفئران بالفم ، علاوة على ذلك فقد ظهر بوضوح العلاقة العكسية المتبادلة بين نسبة الكوليستيرول الكلى ومستوى الكوليستيرول بالليبوبروتين المرتفع الكثافة حيث أدى الشاى الأخضر إلى زيادة مستوى HDL-C مما انعكس على نقص مستوى الكوليستيرول الكلى ، كما انخفضت نسبة اليوريا فى البلازما وحدث تحسن واضح فى بناء الألبيومين من جراء المعاملة الفمية بمستخلص الشاى الأخضر ، وبصفة خاصة وضح تأثير مستخلص الشاى الأخضر على الليبيدات الكلية والجلسريدات الثلاثية التى انخفض مستوى كل منها نتيجة المعاملة بمستخلص الشاى الأخضر. وقد تمكن مستخلص الشاى الأخضر من المحافظة على مستوى القياسات الحيوية بالبلازما بقيم تقارب القيم الطبيعية لمجموعة الكنترول مما يشير للدور الحيوى الذى يمكن أن يقوم به الشاى الأخضر فى المحافظة على بناء حيوى صحى وسليم ومقاوم ضد الملوثات البيئية . وأمكن تعليل هذا الدور الحيوى لمستخلص الشاى الأخضر بقدرته على زيادة تكوين مركب UDP-Glucuronosyltransferase وهذه الزيادة تعمل على التخلص من نسبة كبيرة من المبيد الممثلة بالجسم بزيادة ارتباط نواتج تمثيل المبيد بحمض الجاوكورونيك ، كما أن الشاى له قدرة على زيادة حجم البول نتيجة احتوائه على الكافيين الذى له فعل وتأثير مدر للبول (Diuretic Action) (DA) وبالتالى فإنه يعمل على زيادة الماء المفرز بدلا من إعادة امتصاصه مما يزيد من حجم البول ويزيد من إخراج نواتج التمثيل للمبيد وعدم بقاءه فى الجسم فترة طويلة مما يحافظ على سلامة الجسم ويحسن من قدرة الجسم على التخلص من المواد الضارة ، ولما كان تأثير الكافيين فى زيادة حجم البول يستلزم تواجده بتركيز محدد فى الجسم ، لذلك أظهرت الدراسة أن تأثير مستخلص الشاى الأخضر كان أكثر وضوحاً وفاعلية فى الفترة الطويلة من التجربة نتيجة التجريع اليومى المستمر بمستخلص الشاى الأخضر للفئران ، ومما يدلل على ذلك أن وزن الكلية الذى انخفض فى مجموعة المبيد قد استعيد مرة أخرى نتيجة المعاملة بمستخلص الشاى الأخضر .

وأبرزت الدراسة هذا الدور المحورى الفعال والحيوى الذى يمكن أن يلعبه الشاى الأخضر والذى يمكن أن ينسحب وينطبق على كثير من الملوثات البيئية ، علاوة على أن الشاى الأخضر يحتوى على مجموعة كبيرة من المركبات المتعددة الفينولية Polyphenolic compounds والتى تساهم فى زيادة كفاءة الجسم ضد تلك الملوثات البيئية فى الهواء والماء والغذاء .