RESULTS AND DISCUSSION

4.1. Effect of MET, *Bt* alone and their combination on rat plasma biochemical parameters

4.1.1. Liver functions:

4.1.1.1. Plasma transaminases:

Results of plasma transaminases activities are presented in Table 8 and Figures 12 and 13. These results showed that MET, *Bt* exposure alone and their combination to the rats, caused a significant ($P < 0.05$) increase in plasma AST and ALT activities when compared with control group. Plasma AST increases in such cases and escapes to the plasma from the injured hepatic cells. In addition, plasma ALT level is also useful in indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in plasma when cellular degeneration or destruction occurs in this organ (Hassoun and Stohs, 1995). Therefore the elevation of these enzymes is an indicative of MET, *Bt* exposure alone and their combination toxicity and thus indicating possible hepatocellular damage that could extert through alternations in liver functions. These findings are consistent with a pervious study in which AST and ALT activities were increased in serum rat by methomyl exposure (Djeffal *et al*., 2015). This could be due to hepatotoxicity, which leads to increase in permeability of plasma membrane and leakage of lysosomal enzymes (El-Demerdash *et al.*, 2012), while Abdo *et al.* (2014) illustrated that *Bt* Corn, Genetically Modified Crops (GMC), elevated the activities of AST and ALT in serum rat after 3 months of treatment, suggested these changes might be due to the endotoxins that were produced in the *Bt* corn.

Table, (8): Effect of MET, *Bt* alone and their combination on the AST, ALT and ALP activities in plasma of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
AST (IU/L)	53 ± 1.69^{a}	71 ± 1.92^{ab}	68 ± 1.58^{b}	75 ± 1.81^{c}
ALT (IU/L)	66.22 ± 1.880^{a}	86.23 ± 1.774^{b}	81.74 ± 2.818^{b}	97.87 ± 1.995^{c}
ALP (IU/L)	60.07 ± 1.895^{c}	81.63 ± 1.875^{ab}	78.34 ± 1.455^{b}	86.28 ± 1.700^{a}

Values are expressed as mean ± standard error (SE); n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

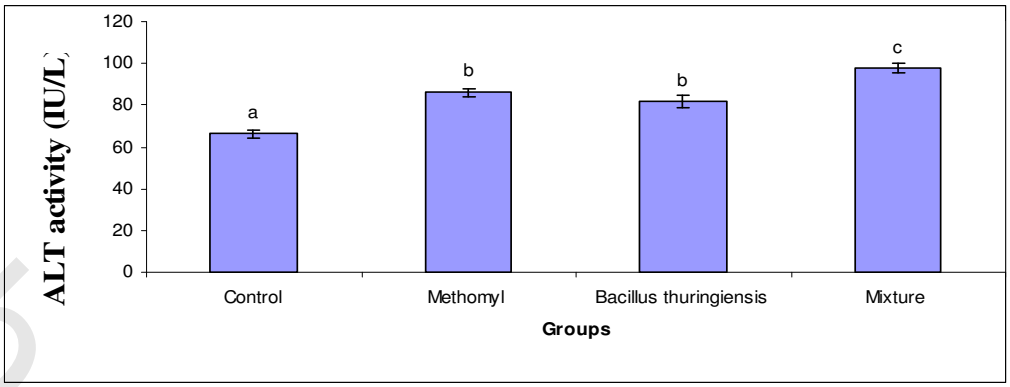

Fig.12: Effects of MET, *Bt* alone and their combination on the ALT activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

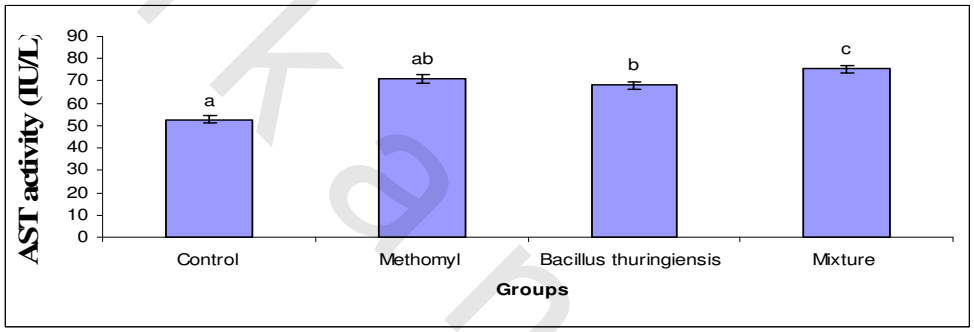

Fig. 13: Effects of MET, Bt alone and their combination on the AST activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.1.2. Plasma alkaline phosphatase:

Plasma alkaline phosphatase activity was found to be significantly ($P < 0.05$) increased in MET, *Bt* alone and their combination-treated animals in comparison to untreated normal control as shown in Table 8 and Figure 14. ALP is an indicator of liver injury when the liver cell membrane is damaged; varities of enzymes normally located on the cytosol (cellular enzymes) are released into blood stream (Awad *et al*., 1998). Also, Szilagyi *et al*. (1994) referred the high levels of plasma ALP to an increased osteoblastic activity, provoked by the disturbance of bone formation. The alterations in ALP activity in rat plasma caused by methomyl are in accordance with the finding reported by Ksheerasagar and Kaliwal (2006) and Patil *et al*. (2008).

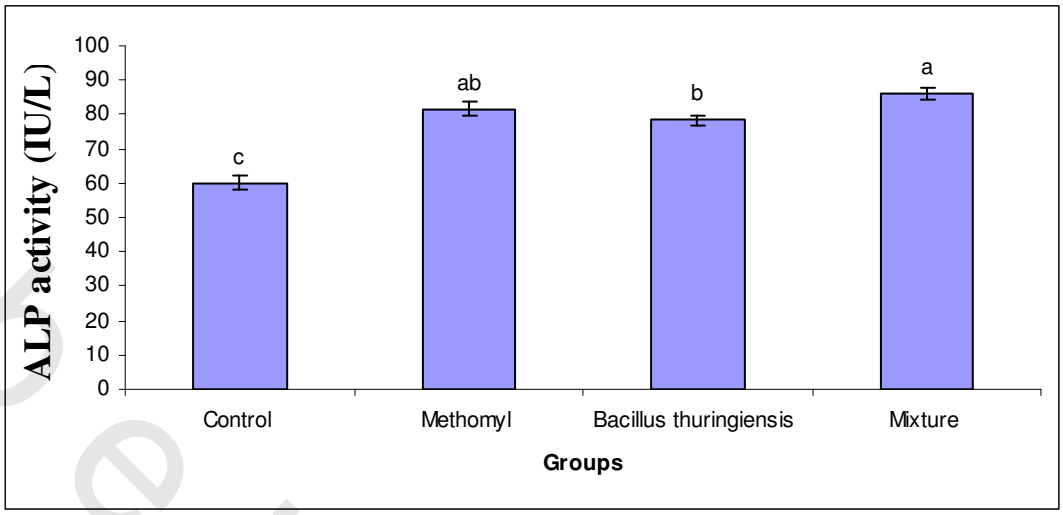

Fig.14 Effects of MET, *Bt* alone and their combination on the ALP activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.1.3. Plasma protein

Data listed in Table 9 and Figure 15 showed a significant ($P < 0.05$) decrease in plasma total protein level in male rats treated with MET, *Bt* alone and their combination when compared to control group. Similar finding of decrease in the levels of total protein was reported in pervious study as a result methomyl administration (Abdel Aziz and Zabut, 2014). Kiliçgün *et al*. (2013) reported that total protein content was decreased in *Bt* maize (GMC) treated group in rats. The decrease in the levels of protein in treated groups rats might be due to changes in protein synthesis and/or metabolism (El-Demerdash and Nasr, 2014).

Table, (9): Effect of MET, *Bt* alone and their combination on the protein and albumin content in plasma of male rats after treatment.

Parameters (g%)	Control	methomyl	*Bacillus thuringiensis*	Mixture
Total protein	7.74 ± 0.247^{a}	5.02 ± 0.156^{c}	5.73 ± 0.168^{b}	4.45 ± 0.165^{d}
Albumin	2.77 ± 0.096^{a}	1.87 ± 0.063^{b}	1.97 ± 0.070^{b}	1.61 ± 0.048^{c}

Values are expressed as mean ± SE; n = 7 for each treatment group.
^{abcd} Means with different superscript letters are significantly different ($P < 0.05$).

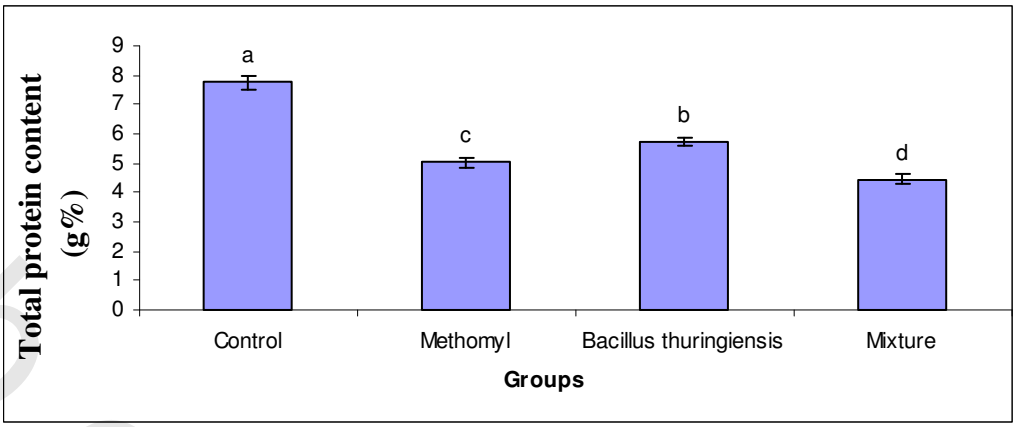

Fig.15: Effects of MET, *Bt* alone and their combination on the total protein content in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, ($P < 0.05$).

4.1.1.4. Plasma albumin:

Plasma albumin content was found to be significantly ($P < 0.05$) decreased in MET, *Bt* alone and their combination – treated animals in comparison to untreated normal control (Table 9 and Figure 16). The albumin fraction is the most important in maintaining this normal colloidal osmotic or oncotic pressure in blood. Thus decrease in plasma albumin concentration to low levels implies that water will diffuse from the blood vessels and enter interstitial fluid and the tissues, leading to the accumulation of water in such tissues (Malomo *et al*., 2007). Albumin is the most abundant blood plasma protein and is produced in liver. The reduction of its level suggests liver disease. This reduction could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (Ncibi *et al*., 2008). Concerning albumin values, their quantification shows a decrease after methomyl administration that agrees with the report of Abdel Aziz and Zabut (2014), whereas Eissa and Zidan (2009) reported that *Bacillus thuringiensis* revealed no significant changes in albumin content of sexually mature wistar rats.

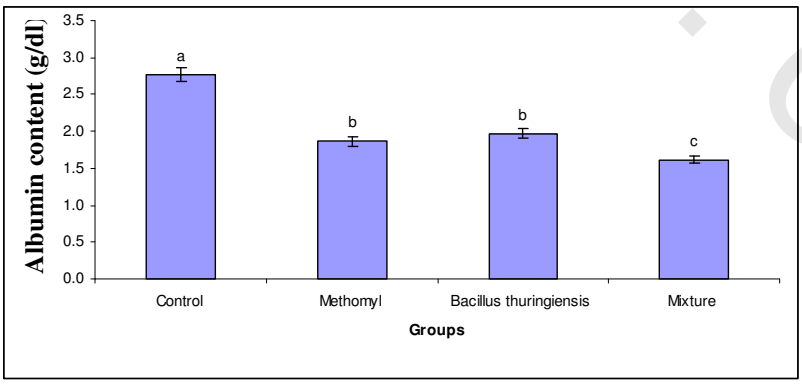

Fig.16: Effects of MET, *Bt* alone and their combination on the albumin content in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.2. Plasma renal functions:

Data listed in Table 10 and Figures 17 and 18 showed a significant ($P < 0.05$) increase in plasma urea and creatinine levels in rats treated with MET, *Bt* alone and their combination compared to control group. The elevation in plasma urea and creatinine levels is considered as a significant marker of renal dysfunction and it may be related to metabolic disturbances in liver function, as urea is the end-product of protein catabolism. Furthermore, xenobiotics intensify the acid-secretory function of kidney and change the transport of sodium (Rudenko *et al*., 1998). These findings are in agreement with the previous studies in which renal dysfunction was occasioned in experimental animal models exposed to methomyl (Abdel Aziz and Zabut, 2014; Djeffal *et al*., 2015; El-Demerdash *et al*., 2013a). Kiliçgün *et al*. (2013) and Schrøder *et al*. (2007) reported that serum urea level was increased in *Bt* maize and rice, GMC, treated rats, respectively. While these studies reported that *Bt* maize and rice treated rats, caused no significant changes in creatinine level.

Table, (10): Effect of MET, *Bt* alone and their combination on the urea and creatinine levels in plasma of male rats after treatment.

Parameters(mg/dl)	Control	methomyl	*Bacillus thuringiensis*	Mixture
Urea	38.14 ± 1.27^{c}	52.76 ± 1.55^{ab}	49.88 ± 1.62^{b}	55.38 ± 1.69^{a}
Creatinine	0.77 ± 0.026^{c}	1.04 ± 0.022^{a}	1.01 ± 0.023^{a}	1.09 ± 0.021^{b}

Values are expressed as mean ± SE; n = 7 for each treatment group.
^{abcd} Means with different superscript letters are significantly different ($P < 0.05$).

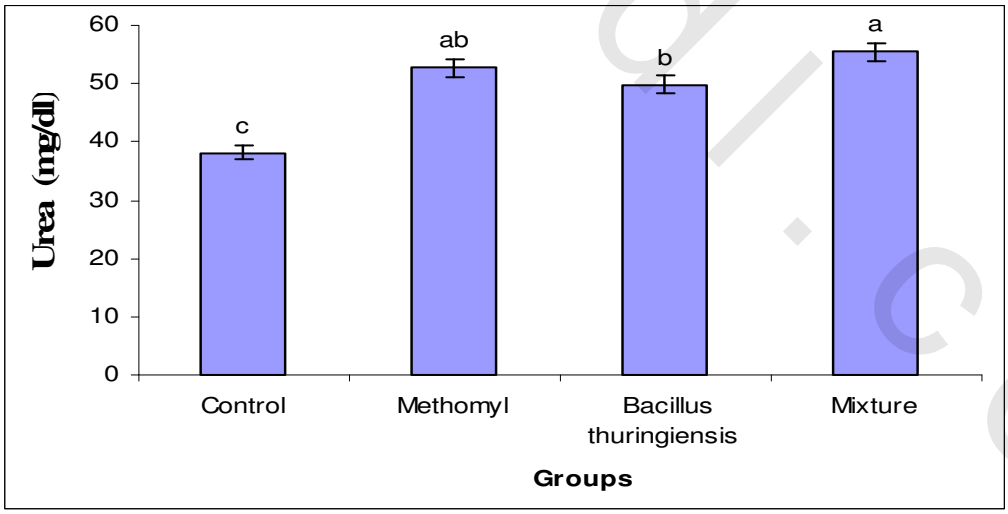

Fig.17: Effects of MET, *Bt* alone and their combination on the urea level in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

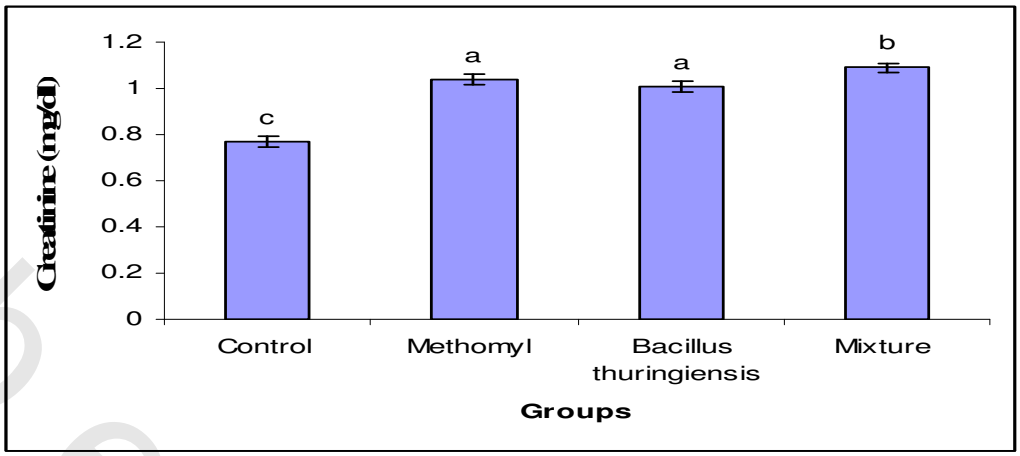

Fig.18: Effects of MET, *Bt* alone and their combination on the creatinine level in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.3. Plasma lipids profile:

Results in Table 11 and Figures 19,20,21,22 and 23 show that exposure of rats to MET, *Bt* either singly or in combination, showed a significant increase the levels of cholesterol, triglycerides, LDLand VLDL while the level of HDL was decreased. The observed increase in the level of plasma cholesterol may be due to an increased cholesterol synthesis in the liver or it may be a sign of liver damage that can be attributed to the effect of pesticides on the permeability of liver cell membrane (Adham *et al*., 1997). Also, the increase in plasma total cholesterol level may be attributed to the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum (Zaahkouk *et al*., 2000). The elevation in plasma triglycerides has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins (Goldberg *et al*., 1982). Similarly, Djeffal *et al*. (2015) reported that the increase in levels of cholesterol and triglycerides after methomyl administration. HDL is mainly synthesized in the liver and intestinal cells. It plays an important role in cholesterol efflux from tissues and carries it back to the liver for removal as bile acids (Shakoori *et al*., 1988). It has been established that the elevated serum or plasma HDL levels are antiatherogenic (McGill *et al*., 1981), whereas the reduced levels are associated with an increased risk for coronary artery disease (Stein, 1987). Supporting the present study, Kiliçgün *et al*. (2013) found that cholesterol, triglyceride, VLDL and LDL levels were increased in *Bt* maize (GMC) treated group in rats.

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Table, (11): Effect of MET, Bt alone and their combination on the levels of cholesterol, triglycerides, LDL, VLDL and HDL in plasma of male rats after treatment.

Parameters (mg/dl)	Control	methomyl	*Bacillus thuringiensis*	Mixture
Total Cholesterol	107.11 ± 3.61[b]	137.51 ± 4.43[a]	133.06 ± 4.21[a]	144.52 ± 4.82[a]
Triglycerides	62.36 ± 1.95[b]	80.85 ± 3.02[a]	78.01 ± 1.54[a]	84.88 ± 2.91[a]
HDL-C	45 ± 1.26[a]	33 ± 0.91[b]	32 ± 1.17[b]	30 ± 0.73[b]
LDL-C	47.63 ± 0.78[b]	62.48 ± 1.95[a]	60.69 ± 2.24[a]	65.68 ± 2.46[a]
VLDL-C	12.47 ± 0.421[b]	16.17 ± 0.605[a]	15.60 ± 0.307[a]	16.98 ± 0.581[a]

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

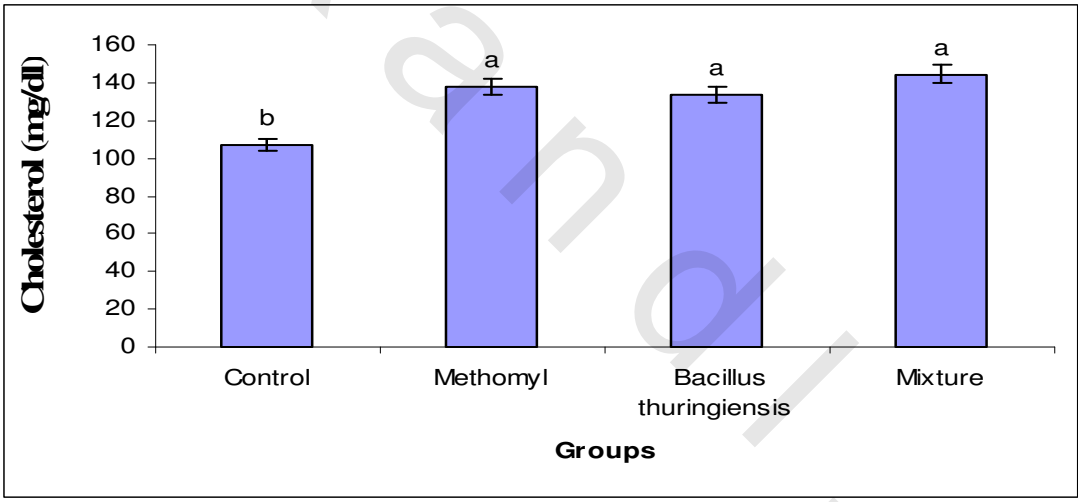

Fig. 19: Effects of MET, *Bt* alone and their combination on the level of cholesterol plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b) were significantly different, ($P < 0.05$).

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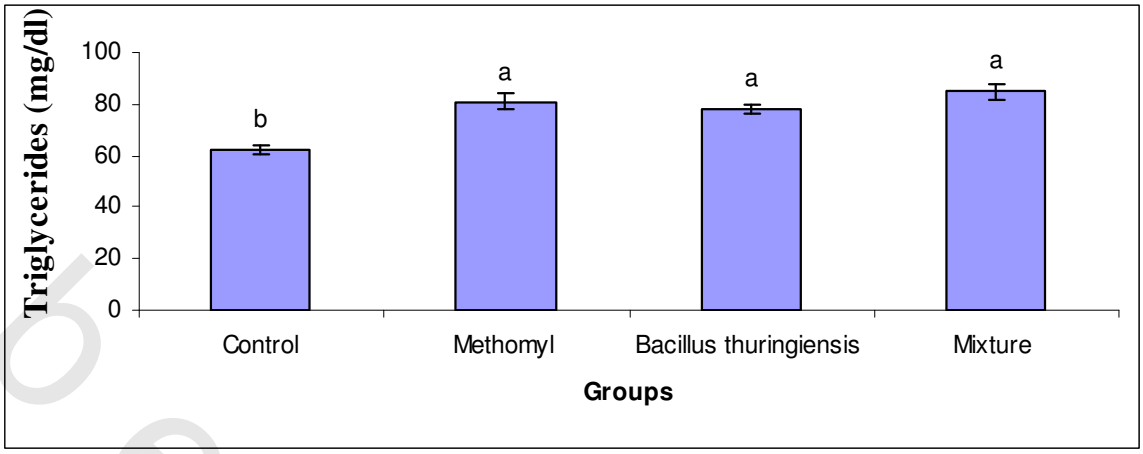

Fig.20: Effects of MET, *Bt* alone and their combination on the level of intriglycerides in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b) were significantly different, ($P < 0.05$)

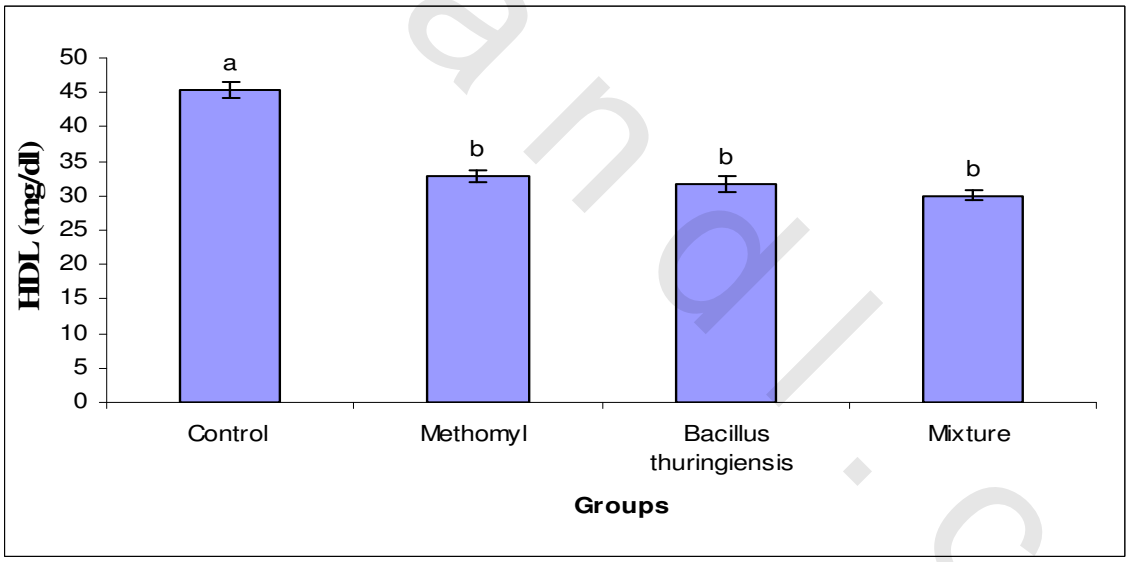

Fig.21: Effects of MET, *Bt* alone and their combination on the level of HDL in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b) were significantly different, ($P < 0.05$)

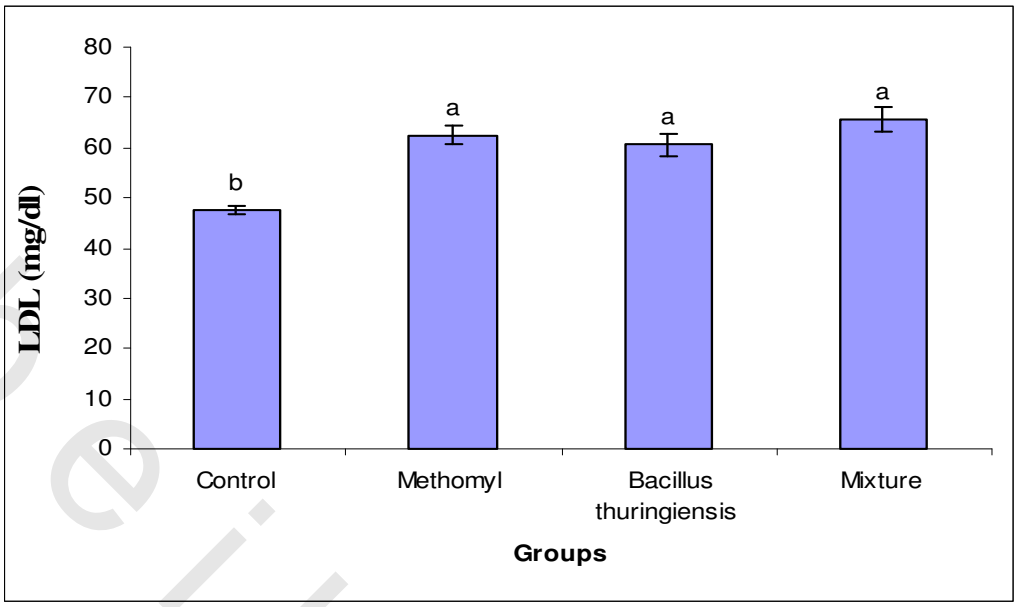

Fig.22: Effects of MET, *Bt* alone and their combination on the level of LDL in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b) were significantly different, (*P* < 0.05)

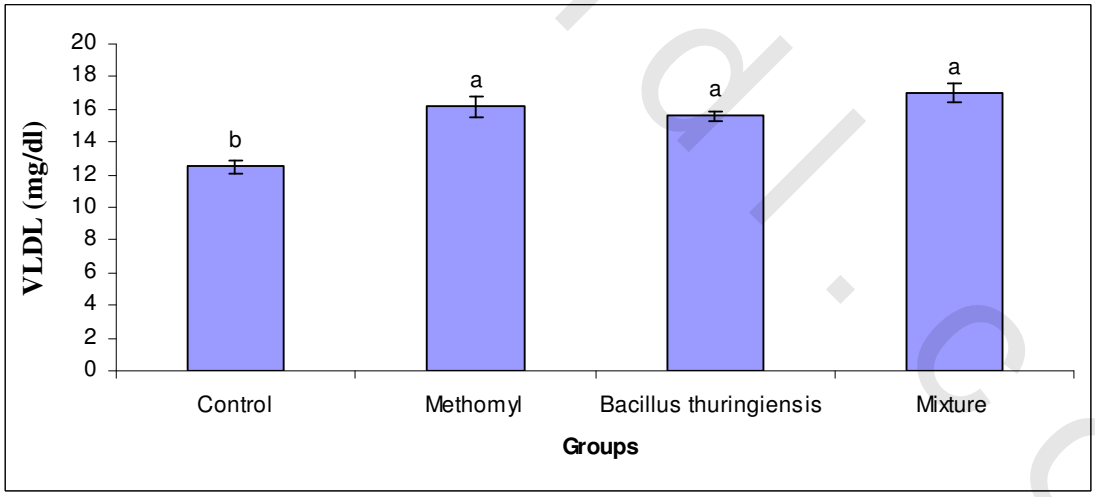

Fig.23: Effects of MET, *Bt* alone and their combination on the level of VLDL in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b) were significantly different, (*P* < 0.05)

4.1.4. Plasma acetylcholinesterase

Acetylcholinesterase (AChE), a critical important nervous system enzyme catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (Yousef *et al*., 2008).

Table (12): Effect of MET, *Bt* alone and their combination on the AChE activity in plasma of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
AChE (µmol/min/mg protein)	2.89 ± 0.05^{a}	1.68 ± 0.06^{c}	2.03 ± 0.03^{b}	1.56 ± 0.09^{c}
% inhibition	-	41.90 %	29.94%	46.50 %

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

Data presented in Table 12 and Figure 24 showed that treatment with MET, *Bt* and their combination caused a significant ($P< 0.05$) decrease in plasma AChE activity as compared to control. Carbamate insecticides reversibly inhibit AChE leading to accumulation of acetylcholine at the nerve endings and subsequently causing parasympathetic overstimulation (Fikes, 1990; WHO, 1996). This effect has been observed previously by other authors in vitro and in vivo (Mansour *et al*., 2009; Mokhtar *et al*., 2013). The decrease in the activity of AChE may result from direct binding of the xenobiotic with the enzyme, modification of erythrocyte's membrane, change in electric charge of membrane layer and increase of ROS (Bukowska and Hutnik, 2006).

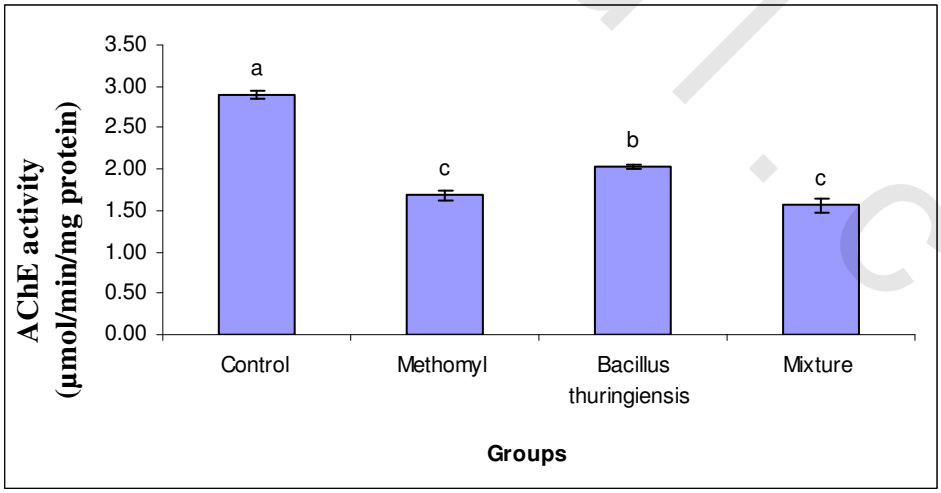

Fig.24: Effects of MET, *Bt* alone and their combination on the AChE activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.5. Plasma Lipid peroxidation

4.1.5.1 Plasma TBARS

Table 13 and Figure 25 showed a significant ($P < 0.05$) increase in plasma TBARS concentrations of male rats exposed to MET, *Bt* alone and their combination as compared to control group. The lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and its occurrence in the biological membranes causes alterations in the membrane structure and function and leads to decrease in membrane fluidity and inactivation of many membrane bound enzymes (Gutteridge and Halliwell, 2000). TBARSs are the degradation products of lipid peroxidation used as a marker to determine quantitatively the extent of lipid peroxidation. An increase in TBARS level is an index of enhanced lipid peroxidation (Kamalakkanan and Prince, 2004). Supporting the present study results, some authors found that methomyl induced LPO and oxidative stress in mice (El-Demerdash *et al*., 2013a; 2012; El-Khawaga, 2005), rat (Garg *et al*., 2009; 2008; Mansour *et al*., 2009) and snail (Salama *et al*., 2005). *Bt* treatment also caused a significant increase in lipid peroxidation. The increased lipid peroxidation evidenced in *Bt* treated rat is in agreement with Shaban *et al*. (2003) in which Dipel (D),a *Bacillus thuringiensis*–based biopesticide was reported to enhanced generation of $O_2^{\cdot-}$ and H_2O_2 that accelerated peroxidation of native membrane lipids. Peroxidation of mitochondrial membrane led to loss of cell integrity, increase in membrane permeability and alteration of Ca^{2+}homeostasis that contribute to cell death due to alteration in the inner membrane potential (Igbavboa *et al*., 1989).

Table (13): Effect of MET, *Bt* alone and their combination on the Lipid peroxidation and antioxidant indices in plasma of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
TBARS (nmol/l)	0.97 ± 0.029^{c}	1.33 ± 0.033^{a}	1.21 ± 0.046^{b}	1.42 ± 0.039^{a}
GSH (mmol/l)	0.175 ± 0.004^{a}	0.116 ± 0.004^{c}	0.128 ± 0.003^{b}	0.107 ± 0.004^{c}
GST (µmol/h)	0.625 ± 0.023^{a}	0.443 ± 0.015^{b}	0.487 ± 0.016^{b}	0.383 ± 0.014^{c}
SOD (U/ml)	1.14 ± 0.033^{a}	0.76 ± 0.022^{c}	0.87 ± 0.013^{b}	0.67 ± 0.017^{d}
CAT (U/ml)	45.83 ± 1.38^{a}	31.70 ± 0.78^{c}	35.51 ± 0.89^{b}	27.40 ± 0.58^{d}

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

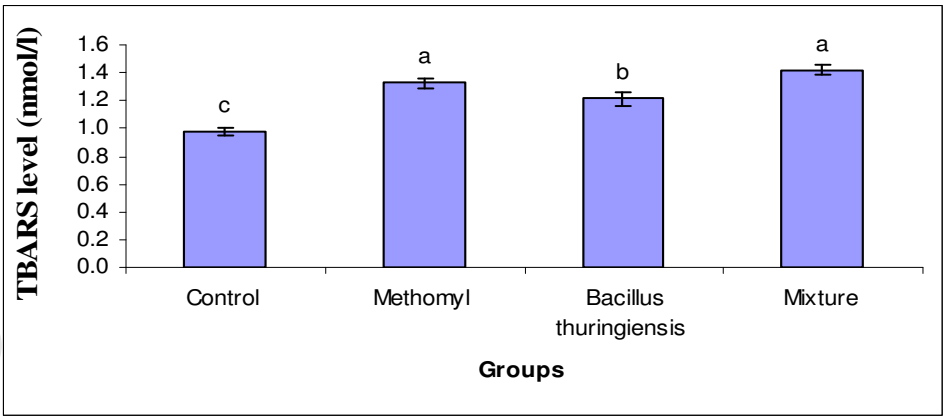

Fig.25: Effects of MET, Bt alone and their combination on the TBARS level in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.6. Plasma non enzymatic and enzymatic antioxidant

4.1.6.1. Plasma reduced glutathione (GSH)

The second line of defense includes the non-enzymatic radical scavenger GSH, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes (Leve De and Kaplowitz, 1991). During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulfide compound, GSSG (oxidized form) (Meister and Anderson, 1983). Table 13 and Figure 26 showed that a significant ($P < 0.05$) decrease in plasma GSH content of male rats exposed to MET, *Bt* alone and their combination as compared to control group. These results are consistent with previous studies in which GSH content was decreased in rats by methomyl exposure (Garg *et al*., 2009; 2008). Exposure of rats to *Bt* caused a significant decrease in levels of GSH. Similarly, Shaban *et al.* (2003) reported that the decrease in GSH level after Dipel (D), a *Bacillus thuringiensis*-based biopesticide administration.

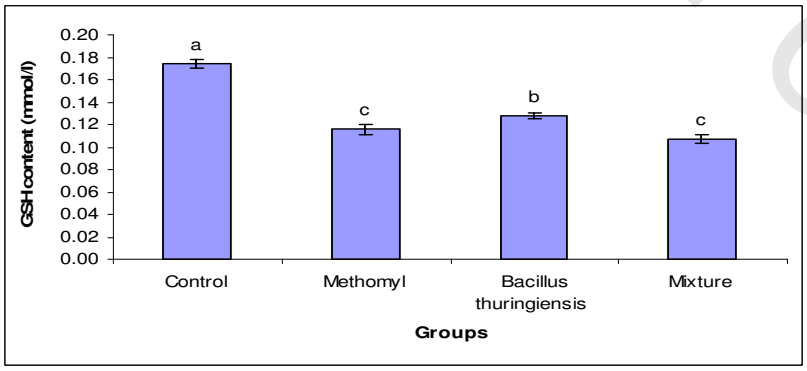

Fig.26: Effects of MET, *Bt* alone and their combination on the GSH content in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$)

4.1.6.2. Plasma glutathione S-transferase (GST)

GST are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (Mansour and Mossa, 2009). Table 13 and Figure 27 showed that MET, *Bt* exposure alone or in combination to the rats, caused a significant decrease in plasma GST activity as compared to control. Consistent with the present results, methomyl was reported to induce glutathione reduction in human lymphocyte in vitro (Lohitnavy and Sinhaseni, 1998). El-Khawaga (2005) reported a significant decrease in the GST activity after a single intraperitoneal dose of methomyl to mice. These results strongly suggested that methomyl has the capability to induce free radicals and oxidative damage as evidenced by perturbations in various antioxidant enzymes (Salama *et al*., 2005). The significant decrease of GST activity in plasma of male rats after administration of MET, *Bt* alone and their combination may indicate insufficient detoxification and is probably related to the decreased GSH levels.

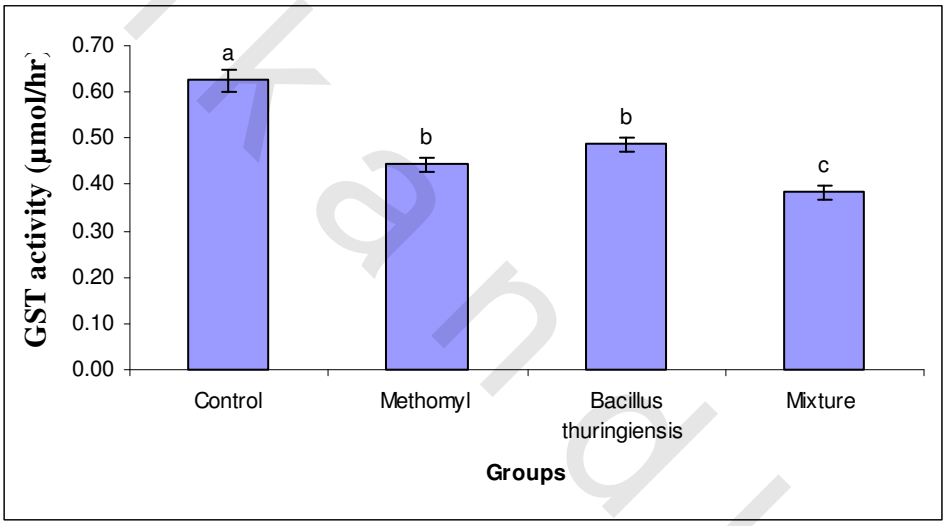

Fig.27: Effects of MET, *Bt* alone and their combination on the GST activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.1.6.3. Plasma superoxide dismutase (SOD)

Results observed in Table 13 and Figure 28 showed that treatment with MET, *Bt* exposure alone and their combination, caused a significant ($P < 0.05$) decrease in plasma SOD activity as compared to control. These findings are in agreement with Mansour *et al*. (2009) who reported that SOD activity was decreased in rat erythrocytes with different concentrations of methomyl. The decrease in SOD activity may be a consequence of increase in the formation of oxy free radicals in order to thwart the free-radical toxicity (Crapo and Tierney, 1997). Moreover, disturbance in SOD activity could also be related to physiological response of the animal in miti-gating the oxidative stress, as SOD plays a vital role in the detoxification of ROS (EL-Demerdash *et al*., 2012).

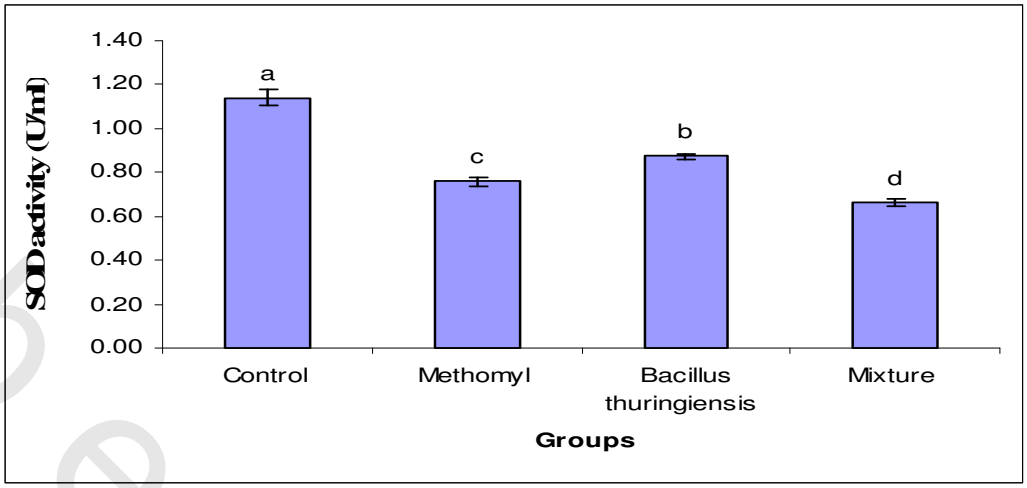

Fig.28: Effects of MET, *Bt* alone and their combination on SOD activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, (*P* < 0.05).

4.1.6.4. Plasma catalase (CAT)

CAT is a haemoprotein, which reduces hydrogen peroxide to molecular oxygen and water (Gutteridge, 1995). Data presented in Table 13 and Figure 29 showed that treatment with MET, *Bt* alone and their combination, caused a significant decrease in plasma CAT activity as compared to control. An increase in CAT might be in response to increased oxidative stress. However, when a condition of oxidative stress strongly establishes, the defense capacities against ROS becomes insufficient, in turn ROS also affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH and decreases the activity of CAT activity (EL-Demerdash *et al*., 2012). Several studies reported that carbamate pesticides inhibited the activities of antioxidant enzymes in vitro (El-Demerdash, 2001; Mansour and Mossa, 2009; Maran *et al*., 2010) and in vivo (El-Khawaga, 2005; Garg *et al*., 2008; Salama *et al*., 2005).

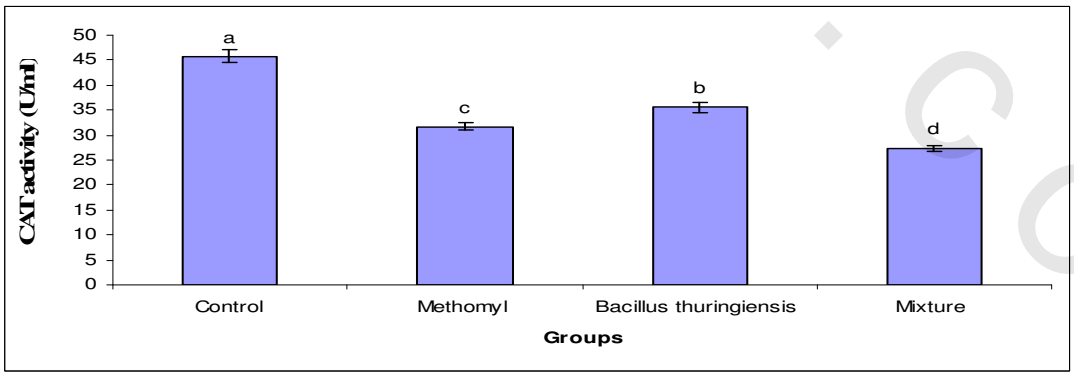

Fig.29: Effects of MET, *Bt* alone and their combination on CAT activity in plasma of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, (*P* < 0.05).

4.2. Effect of MET, *Bt* alone and their combination on rat liver biochemical parameters

The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals and drugs. Hepatotoxicity is therefore an important endpoint in the evaluation of the effect of a particular xenobiotic. Clinical chemistry and histopathological evaluations are commonly used methods for detecting organ-specific effects related to chemical exposure (Travlos *et al*., 1996; Crissman *et al*., 2004).

4.2.1. Liver function:

4.2.1.1. Liver transaminases:

Liver damage was evaluated by the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Results revealed a significant ($P < 0.05$) decrease in liver enzymes after treatment with MET, *Bt* alone and their combination (Table 14 and Figures 30 and 31). These results conincide with El-Demerdash *et al*. (2012) who illustrated that methomyl caused a significant reducation in liver AST and ALT activities. The reduction in aminotransferases may be due to tissue damage, alterations in the permeability of cell membrane and perturbations in protein metabolism (Farag *et al*., 2010).

Table (14): Effect of MET, Bt alone and their combination on the ALT, AST, ALP and LDH activities and on the protein content in liver of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
AST (U/mg protein)	144 ± 2.04^{a}	99 ± 2.81^{b}	105 ± 2.05^{b}	85 ± 1.82^{c}
ALT (U/mg protein)	161 ± 5.08^{a}	99 ± 2.34^{c}	118 ± 3.40^{b}	91 ± 2.04^{c}
AlP (U/mg protein)	379 ± 9.40^{a}	243 ± 8.27^{bc}	267 ± 9.17^{b}	231 ± 8.28^{c}
LDH (U/mg protein)	996 ± 23.28^{c}	1318 ± 33.99^{b}	1307 ± 21.93^{b}	1379 ± 30.34^{a}
Protein(mg/gm Tissue)	189 ± 6.39^{a}	133 ± 3.49^{c}	149 ± 4.41^{b}	122 ± 4.46^{c}

Values are expressed as mean ± SE; n = 7 for each treatment group.
^{abcd} Means with different superscript letters are significantly different ($P < 0.05$).

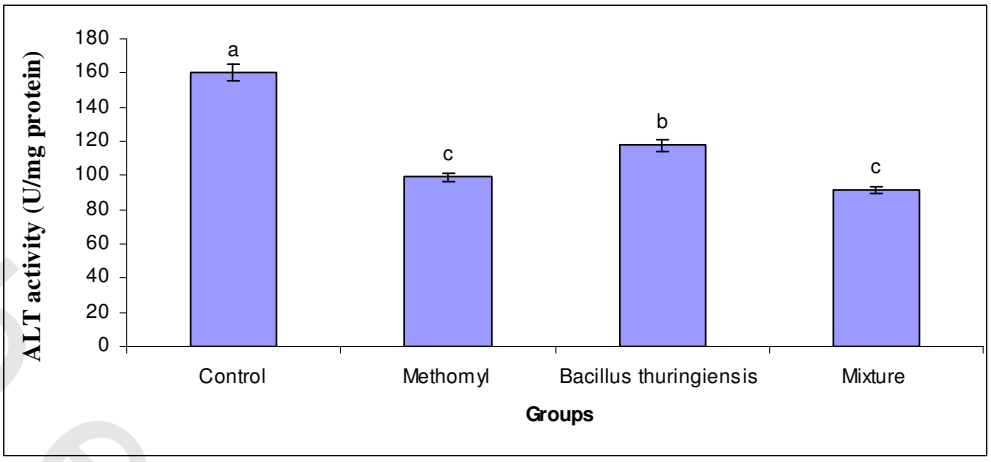

Fig. 30: Effects of MET, *Bt* alone and their combination on the ALT activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

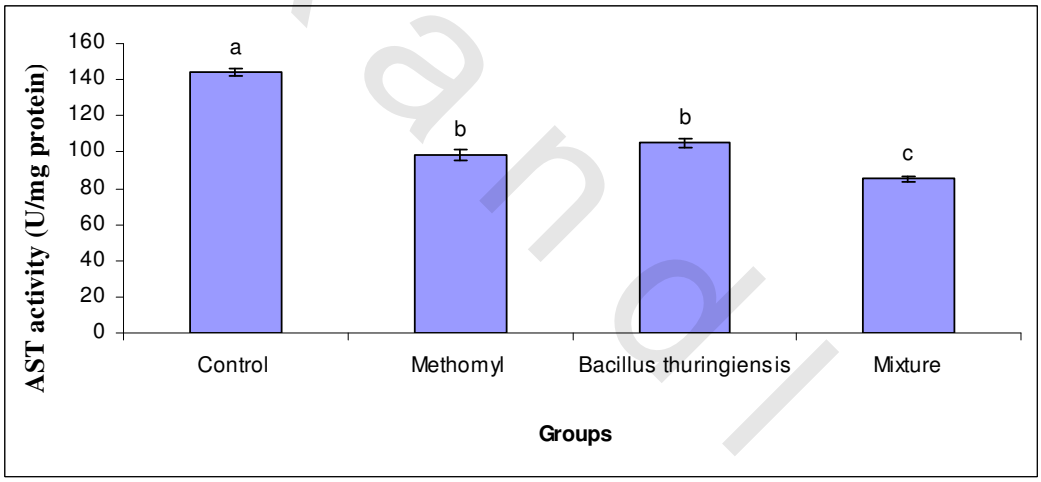

Fig.31: Effects of MET, *Bt* and their combination on the ALT activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.1.2. Liver lactate dehydrogenase

Liver lactate dehydrogenase (LDH) was significantly ($P < 0.05$) increased in rats treated with MET, *Bt* and their combination as compared to control (Table 14 and Figure 32).The enzyme LDH can be used as an indicator for cellular damage, clinical practice, and cytotoxicity of pollutants. LDH activity indicates the switching over of anaerobic glycolysis to aerobic respiration. The changes in the LDH activity may be due to severe cellular damage, leading to increased release of dehydrogenase that impaired carbohydrate and protein metabolism (Sivakumari *et al*., 1997). Movever, carbamates insecticides have been reported to increase LDH activity in liver, testis and epididymis of rats (El-Demerdash *et al*., 2012; Kacker *et al*., 1997). The elevated activity of LDH indicates a

compensatory mechanism by the affected tissue that requires additional energy for its maintenance (El-Demerdash *et al*., 2012).

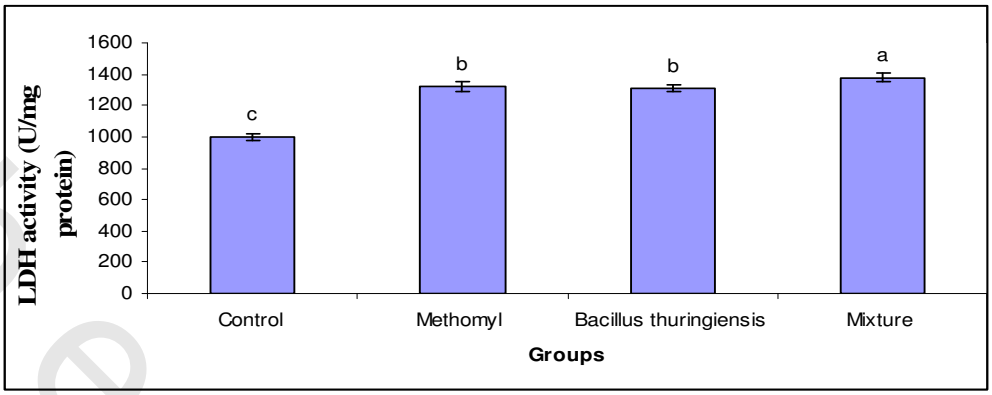

Fig.32: Effects of MET, *Bt* and their combination on the LDH activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.1.3. Liver alkaline phosphatase:

Biochemical parameters are sensitive index to changes due to pollutant and can constitute important diagnostic tools in toxicological studies. Phosphatases are important critical enzymes in the biological system, responsible for detoxification, metabolism, and biosynthesis of energetic macromolecules for different essential functions. Interference with these enzymes leads to biochemical impairment, lesions of tissue, and loss of cellular function (Enan *et al*., 1982). Table 14 and Figure 33 demonstrated that effect of MET, *Bt* and their combination on liver ALP activity in rat. The data revealed a significant ($P < 0.05$) decrease in ALP activity in rat liver. In agreement with the present results, El-Demerdash *et al*. (2012) reported that methomyl caused a significant decrease in liver ALP activity in rat. Rahman *et al*. (2000) suggested that the decrease in the ALP activity in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, showing the stress condition of the treated animals.

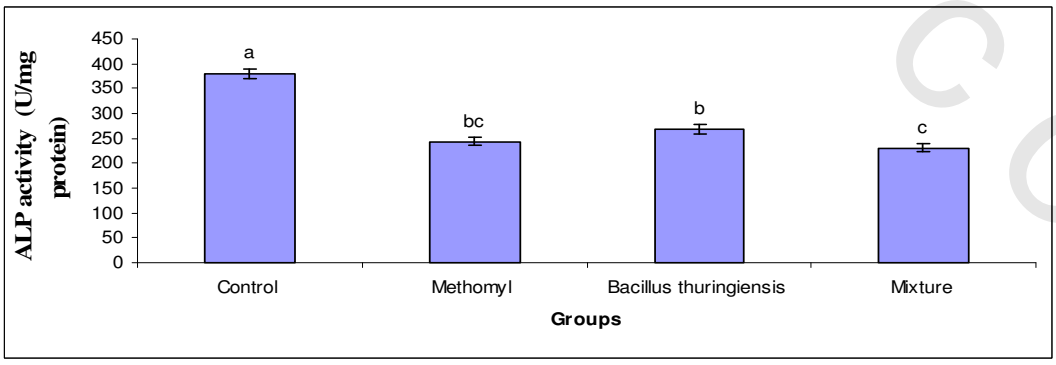

Fig.33: Effects of MET, *Bt* and their combination on the ALP activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.1.3. Liver protein:

Data presented in Table 14 and Figure 34 showed a significant ($P < 0.05$) decrease in protein content in liver male rat treated with MET, *Bt* and their combination as compared to control. The decrease in the levels of protein in rat might be due to changes in protein synthesis and/or metabolism in addition to increased excretion of high molecular weight protein (inhibition of the synthesis of liver protein and decreased liver function) (Christopher, 1991). Also, Bradbury *et al*., (1987) pointed out that the decreased protein content might also be attributed to the destruction of cellular function and consequent impairment in protein synthetic machinery. Protein depletion in tissues may constitute a physiological mechanism and may play a role in compensatory mechanism under xenobiotic stress (El-demerdash *et al*., 2013b).

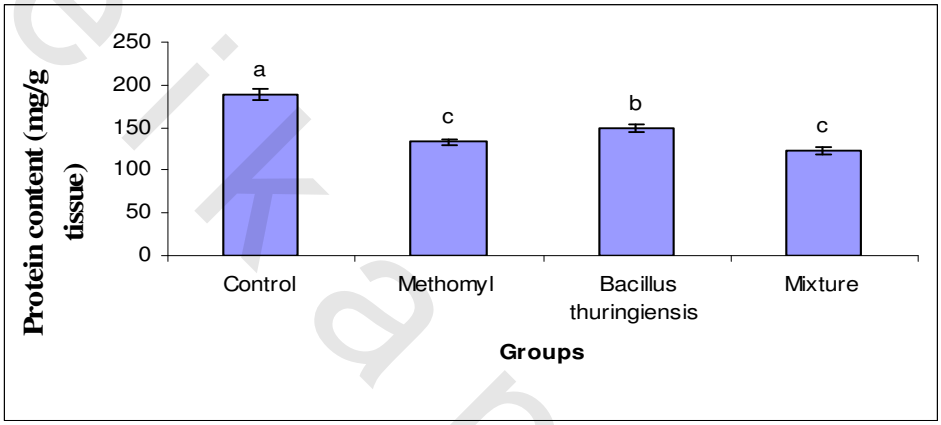

Fig.34: Effects of MET, *Bt* alone and their combination on the protein content in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.2. Liver lipid peroxidation

4.2.2.1. Liver TBARS

The data presented in Table 15 and Figure 35 showed a siginificant increase in TBARS concentrations in rat treated with MET, *Bt* alone and their combination as compared to control group. Carbamates have been observed to accentuate oxidative stress in rat tissues, which lead to the overproduction of free radicals that exert deleterious effects on liver, kidney, brain and heart (Banerjee *et al*., 1999; Eraslana *et al*., 2009; Kamboj *et al*., 2006). The biopesticide *Bacillus thuringiensis* also has the ability to induce oxidative stress in rats, stimulate hepatic lipid peroxidation through the formation of free radicals that attack the cell membranes (Shaban *et al*., 2003).

Table (15):Effect of MET, *Bt* alone and their combination on the lipid peroxidation and antioxidant indices in liver of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
TBARS (nmol/g tissue)	32.84 ± 0.999^{c}	45.14 ± 0.735^{b}	44.96 ± 1.352^{b}	49.02 ± 1.531^{a}
GSH (mmol/mg protein)	2.13 ± 0.053^{a}	1.39 ± 0.041^{c}	1.63 ± 0.028^{b}	1.23 ± 0.037^{d}
GST (µmol/h/mg protein)	1.12 ± 0.033^{a}	0.74 ± 0.019^{bc}	0.78 ± 0.023^{b}	0.67 ± 0.025^{c}
SOD (U/mg protein)	76.80 ± 1.62^{a}	51.14 ± 1.97^{c}	59.37 ± 1.69^{b}	44.69 ± 1.79^{d}
CAT (U/mg protein)	49.29 ± 1.55^{a}	33.34 ± 0.67^{c}	36.83 ± 1.23^{b}	27.52 ± 0.92^{d}

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

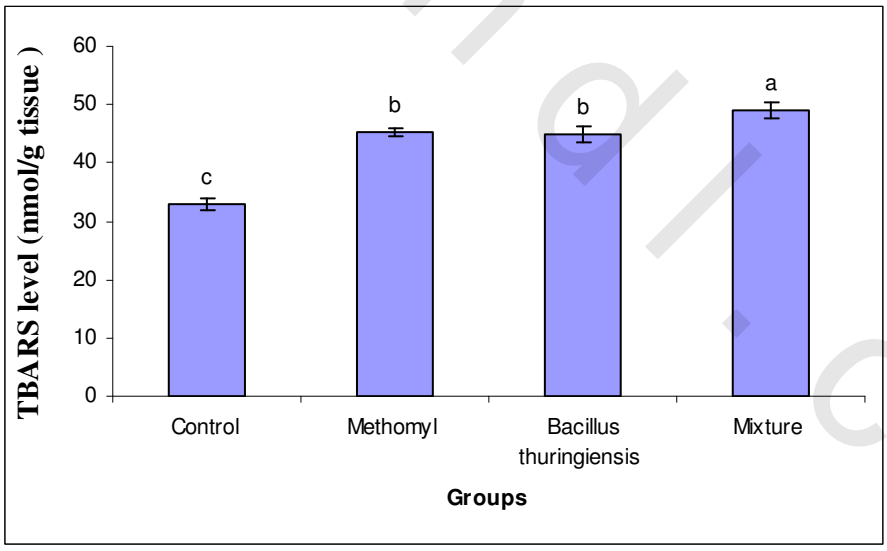

Fig.35: Effects of MET, *Bt* alone and their combination on the TBARS level in liver tissue of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.3. Liver non enzymatic and enzymatic antioxidant

4.2.3.1. Liver reduced glutathione

Liver GSH content was significantly decreased as compared to control in rats treated with MET, *Bt* alone and their combination as compared to control group (Table 15 and Figure 36). GSH is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine (which is attached by normal peptide linkage to a glycine). GSH plays an important role in protecting the cells against radical and oxy-radical induced damage. Relationship between extent of lipid peroxidation and glutathione status of the liver tissue has a significant inverse relation (Younes and Sieger, 1981). During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulfide compound, GSSG (oxidized form) (Meister and Anderson, 1983). The decrease in GSH levels could be due to the presence of free radicals produced by insecticides (El-Demerdash, 2011). In addition, GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme GST. Glutathione and other thiol containing proteins play a crucial key role in cellular defense against pesticides toxicity (Halliwell and Gutteridge, 1999). These effects have been previously observed by other authors in vitro and in vivo in which GSH content was decreased in rats by carbamates exposure (Maran *et al*., 2009; Manawadi and Kaliwal, 2010). The decrease in GSH level after *Bt* administration may possibly be due to increased demand of the tripeptide for lipid hydroperoxide metabolism by GPx and interaction of GSH with *Bt* derived free radicals in the same manner as reported by Barros *et al*. (1988) about insecticide lindane. GPx catalyses the formation of oxidized glutathione (GSSG) during the reduction in hydroperoxides (Huang and Sultatos,1993; Parke, 1991). GSH can be regenerated from GSSG by glutathione reductase (Parke, 1991).

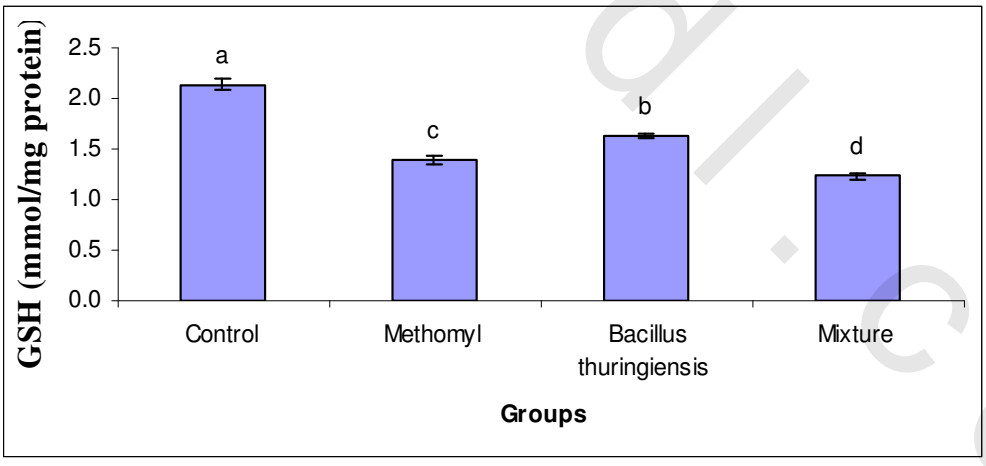

Fig.36: Effects of MET, *Bt* alone and their combination on the GSH content in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, (*P* < 0.05)

4.2.3.2. Liver glutathione S-transferase

Results revealed a significant decrease in liver GST activity after treatment with MET, *Bt* alone and their combination as compared to control group (Table 15 and Figure 37). The inhibition of this enzyme activity and an increase in lipid peroxidation probably lead to the intracellular accumulation of ROS with subsequent development of liver injury. These results are in agreement with those of Manawadi and Kaliwal (2010). The toxicity of many xenobiotics is associated with the production of free radicals that are not only toxic themselves but are also implicating in the pathophysiology of many diseases (Abdollahi *et al*., 2004).

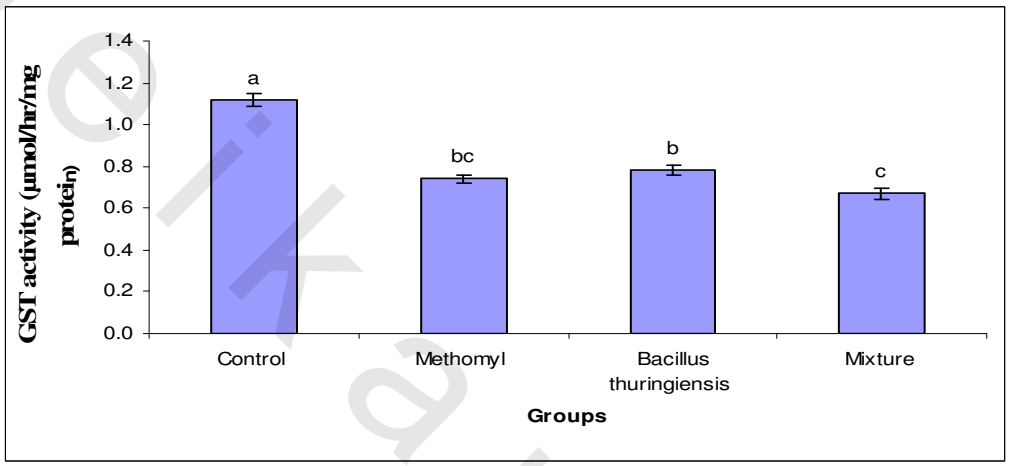

Fig.37: Effects of MET, *Bt* alone and their combination on the GST activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.2.3.3. Liver superoxide dismutase (SOD)

Data listed in Table 15 and Figure 38 revealed a significant decrease in liver SOD activity after treatment with MET, *Bt* alone and their combination as compared to control group. SOD has an antitoxic effect against the superoxide anion. SOD accelerates the dismutation of superoxide to H_2O_2 which is removed by catalase (Usoh *et al*., 2005). Several studies reported that methomyl insecticide decreased the liver SOD activity in rat (Djeffal *et al*., 2015) and mice (El-Demerdash *et al*., 2012). Also, Shaban *et al.* (2003) reported that inhibition of liver SOD activity in rats was due to the direct interaction of Dipel (D), a *Bacillus thuringiensis*–based biopesticide, with the enzyme molecules and modification of the post-transcriptional or post-translational steps in the enzyme synthesis or the action of $O_2^{\cdot-}$, in free state or after their transformation to H_2O_2, an oxidation of the cysteine in the enzyme.

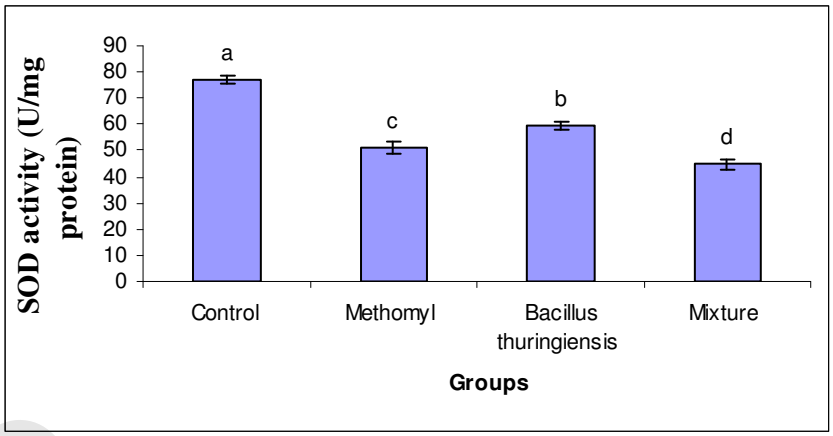

Fig.38: Effects of MET, *Bt* alone and their combination on the SOD activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, ($P < 0.05$).

4.2.3.4. Liver catalase (CAT)

Data concering liver antioxidant CAT activity in presented in Table 15 and Figure 39. It is well established that exposure of rat liver to MET, *Bt* alone and their combination produces a significant ($P < 0.05$) reducation in the antixodiant enzyme activities. Catalase is responsible for breakdown of hydrogen peroxide, an important ROS, produced during metabolism. CAT catalyzes the removal of H_2O_2 formed during the reaction catalyzed by SOD (Ramanathan *et al*., 2002). These results are consistent with a pervious study in which liver CAT activity was reduced in mice by methomyl exposure (El-Demerdash *et al*., 2012). The inhibition of the antioxidant enzymes activities and an increase in lipid peroxidation probably lead to the intracellular accumulation of ROS with subsequent development of tissue injury. The toxicity of many xenobiotics is associated with the production of free radicals that are not only toxic themselves but are also implicating in the pathophysiology of many diseases (Abdollahi *et al*., 2004).

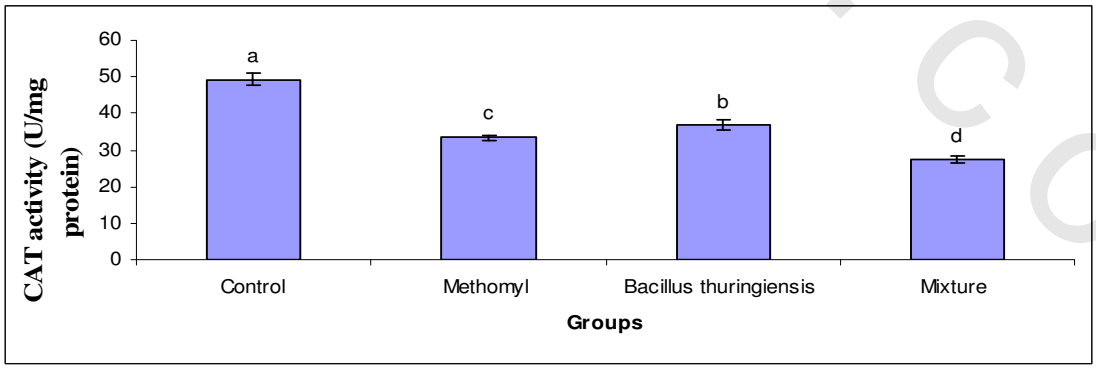

Fig.39: Effects of MET, Bt alone and their combination on the CAT activity in liver of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, (P < 0.05).

4.2.4. Histopathological changes in the liver

The histological examination of the liver sections is presented in Figure 40. Control rats showed normal histological structure of the hepatocytes and portal tracts (Fig. 40A). Histopathological studies revealed several abnormalities in the liver tissue of rats treated with MET, *Bt* and their combination. A hydropic change in hepatocytes, portal fibrosis and inflammation was observed in liver of methomyl-treated rats (Fig. 40B). These lesions were similarly reported by El-Demerdash *et al*. (2012). They reported that liver of mice treated with methomyl for 10, 20 and 30 days showed dilated central vein and sinusoids between hepatocytes with pycnotic nuclei. Also, Ksheerasagar and Kaliwal (2006) suggested that the carbosulfan has adverse effects on liver functions leading to histological and physiological impairment. This could be due to morphological and chemical induced injury that can manifest itself in different ways. Among frequent liver lesions and alterations as the result of the methomyl treatment was increased apoptotic feature (Radad *et al*., 2009).

Examination of liver sections of *Bacillus thuringiensis* -treated rats showed piecemeal necrosis, portal fibrosis and inflammation (Fig. 40C). Previous studies have also revealed that *Bt* toxins in mammal liver cell cultures increase the production of lactate dehydrogenase (LDH), which damages the endothelial cells of the sinusoids, leading to dilation of the sinusoidal lumen at doses of approximately 2.0 ng/ml after incubation for 24 and 48 h (Sun *et al*., 2001; Shimada *et al*., 2003). Also, Adetunji and Anyanwu (2011) suggested that exposure of male rat to *B. thuringiensis* at 10 Colony-forming unit (CFU) caused a periportal hepatic necrosis of the liver. In addition, Shaban *et al*. (2003) and Ito (2006) have demonstrated that the insecticide Dipel (*B.thuringiensis* var. *kurstaki*) has the ability to alter the defense behavior of liver cells, induce oxidative stress, stimulate lipid peroxidation through the formation of free radicals and damage the membranes of liver cells in rats, leading to an inflammatory reaction and activity of the Kupffer cells.

Liver of rats treated with both MET and *Bt* showed more portal fibrosis and inflammation (Fig. 40D). This is confirming that altered hepatic cell membrane permeability can lead to changes in the enzyme activity in liver as observed in the present study.

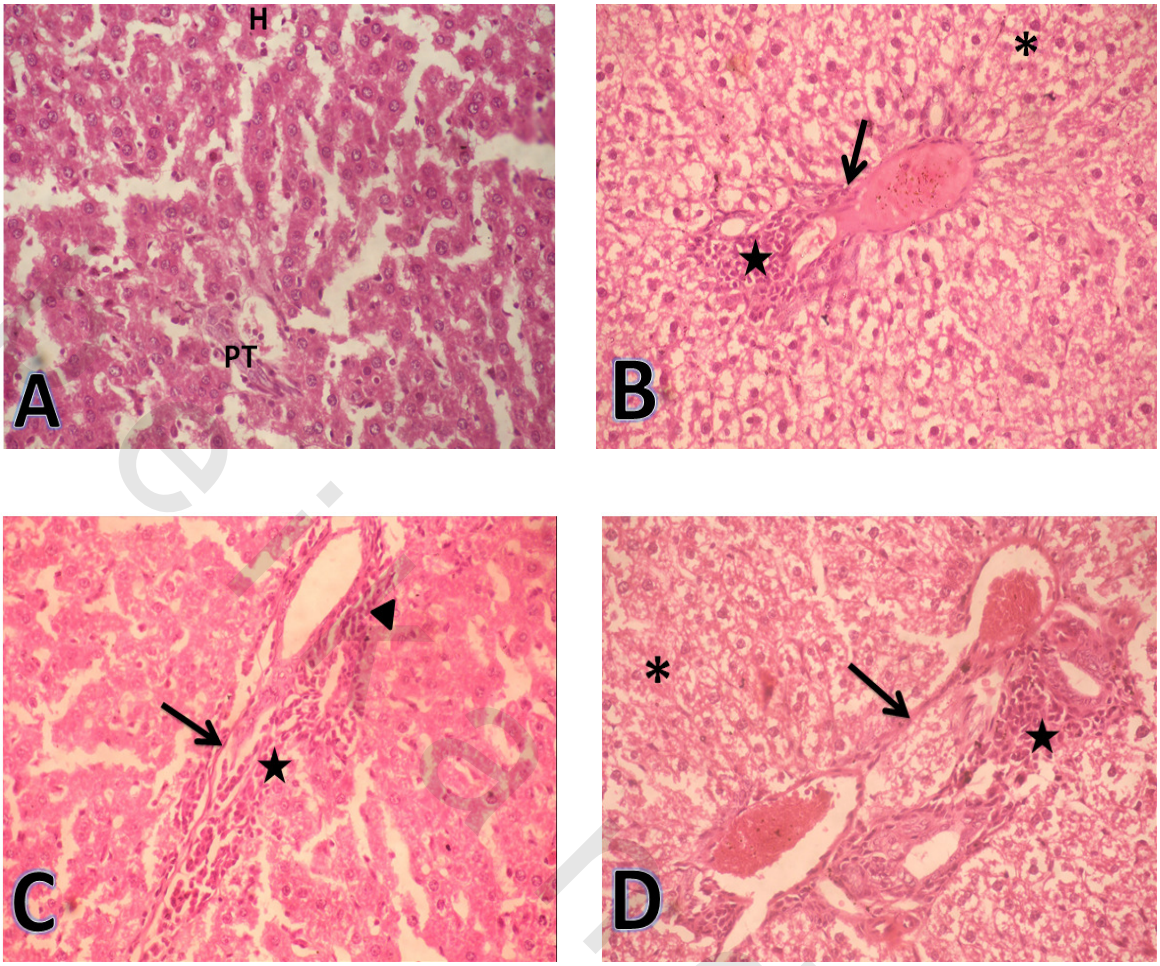

Figure 40. Photomicrographs of section of rat's liver (H&E x400): A. Control rats showing the histological structure of the normal hepatocytes (H) and portal tracts (PT). **B.** MET treated rats showing hydropic change in hepatocytes (*), portal fibrosis (arrow) and inflammation (★). **C.** *Bt* treated rats showing piecemeal necrosis (◀) portal fibrosis (arrow) and inflammation (★). **D.** MET in combination with *Bt* treated rats showing hydropic change (*), portal fibrosis (arrow) and inflammation (★).

4.3. Effect of MET, *Bt* alone and their combination on rat kidney biochemical parameters

The kidney is the critical target organ for xenobiotic compounds which produce a variety of renal toxic effects involving tubular cells and glomerulus (Mohamed *et al*., 2003). These compounds inhibit the incorporation of amino acid into protein causing an increase in urea levels which is the major nitrogen-containing end product of protein metabolism (Pollak and Harsas, 1982). In the present study, increased plasma creatinine and urea levels reflect the diagnosis of renal failure. Moreover, elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production (Fetoui *et al*., 2010).

4.3.1. Effect on kidney ALP activity and protein content

4.3.1.1. Kidney Alkaline phosphates:

Alkaline phosphatase (ALP) is a biochemical parameter, sensitive index to changes due to pesticide toxicity. ALP is an important and critical enzyme in the biological process; it is responsible for detoxification, metabolism and the biosynthesis of energetic macromolecules for various essential functions. Data listed in Table 16 and Figure 41 showed that treatment with MET, *Bt* and their combination, caused a significant decrease in kidney ALP activity as compared to control. Rahman *et al*. (2000) suggested that the decrease in the ALP activity in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, showing the stress condition due to insecticide treatment.

Table, (16): Effect of MET, *Bt* alone and their combination on the ALP activity and on the protein content in kidney of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
ALP (U/mg protein)	203 ± 4.71^{a}	119 ± 3.55^{bc}	128 ± 3.73^{b}	109 ± 3.57^{c}
Protein (mg/g tissue)	74.29 ± 2.72^{a}	49.04 ± 1.70^{c}	56.28 ± 2.06^{b}	43.27 ± 1.08^{c}

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

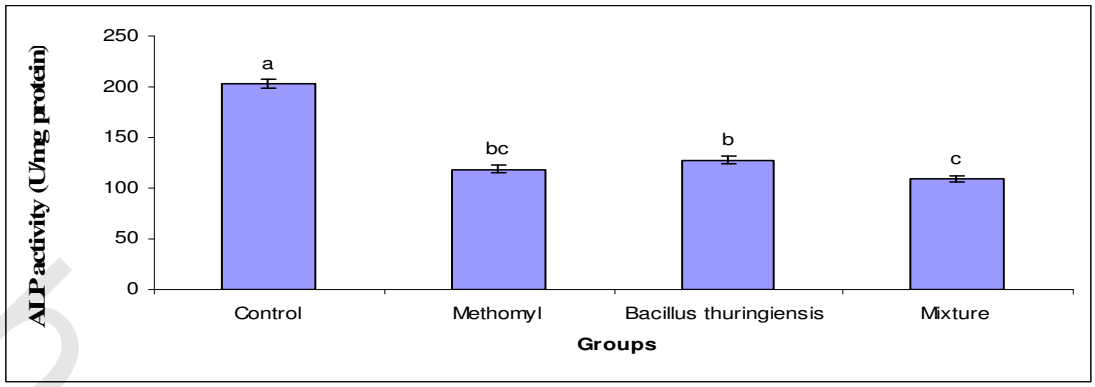

Fig.41: Effects of MET, *Bt* alone and their combination on the ALP activity in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.3.1.2. Kidney protein content:

Table 16 and Figure 42 showed that treatment with MET, *Bt* and their combination, caused a significant decrease in protein content in rat kidney as compared to control. Protein is one of the main cellular components susceptible to damage by free radicals (El-Demerdash, 2012). The decrease in protein content in rat kidney homogenate treated rats is in agreement with the findings of Shakoori *et al*. (1990) and El-Demerdash (2001). Protein depression was reported to be mainly due to excessive loss through nephrosis (Rahman *et al*., 1990). Additionally, the decrease in protein could be due to the reduction of protein synthesis or increased proteolytic activity or degradation (Shakoori *et al*., 1990).

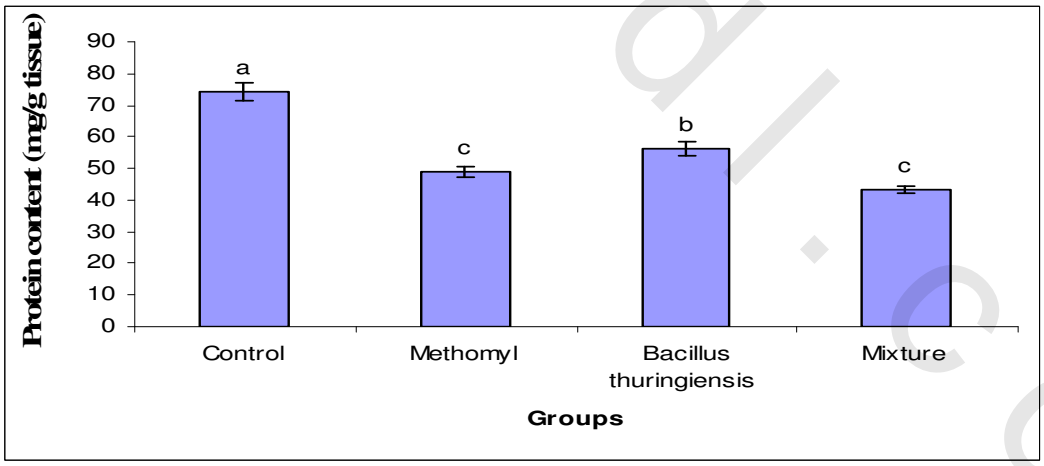

Fig.42: Effects of MET, *Bt* alone and their combination on the protein content in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.3.2. Kidney lipid peroxidation

4.3.2.1. Kidney TBARS

Data of TBARS concentration measured in rat kidney are presented in Table 17 and Figure 43. A significant ($P < 0.05$) increase in TBARS concentrations was evident in rat kidney exposed to MET, *Bt* alone and their combination as compared to control group. These results corroborated with the previous finding which demonstrated that MET exposure, caused a significant increase in MDA content, suggested that MET activated the formation of free radicals in hepatic and kidney tissues (Djeffal *et al*., 2015).

Table, (17): Effect of MET, Bt alone and their combination on the lipid peroxidation and antioxidant indices in liver of male rats after treatment.

Parameters	Control	methomyl	*Bacillus thuringiensis*	Mixture
TBARS (nmol/g tissue)	23.79 ± 0.70^{c}	31.95 ± 1.07^{ab}	29.59 ± 0.91^{b}	33.46 ± 1.12^{a}
GSH (mmol/mg protein)	2.47 ± 0.086^{a}	1.70 ± 0.063^{b}	1.86 ± 0.068^{b}	1.48 ± 0.054^{c}
GST (µmol/h/mg protein)	0.567 ± 0.017^{a}	0.373 ± 0.013^{c}	0.420 ± 0.010^{b}	0.327 ± 0.011^{d}
SOD (U/mg protein)	0.054 ± 2.04^{a}	45.60 ± 0.99^{c}	50.14 ± 0.83^{b}	39.02 ± 0.87^{d}
CAT (U/mg protein)	51.95 ± 1.37^{a}	35.05 ± 1.10^{c}	39.25 ± 0.83^{b}	30.74 ± 0.98^{d}

Values are expressed as mean ± SE; n = 7 for each treatment group.
[abcd] Means with different superscript letters are significantly different ($P < 0.05$).

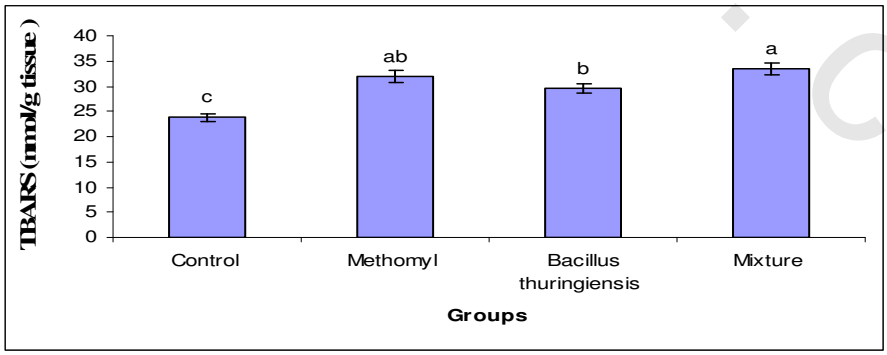

Fig.43: Effects of MET, *Bt* alone and their combination on the TBARS level in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.3.3. Kidney non enzymatic and enzymatic antioxidant

4.3.3.1. Kidney reduced glutathione

Data of GSH measured in rat kidney are presented in Table 17 and Figure 44. A significant a significant ($P < 0.05$) decrease in GSH content was evident in kidney rat exposed to MET, *Bt* alone and their combination. GSH is well known for its antioxidant properties and its role as redox modulator in related enzymes. The resultant lipid peroxides may also react with GSH and lead to a decrease in GSH content and its related enzyme activities. Oxidative damage, therefore, maybe attributed to the consequence of insufficient cellular antioxidant potential due to pesticides exposure acting on the membranes, oxidizing its lipid components and enhancing TBARS production during their exposure. It appears that elevation in lipid peroxidation is a consequence of depleted GSH stores, which are otherwise capable of moderating the levels of LPO (Garg *et al*., 2009). Therefore, reduced level of GSH enhances the toxic effect, because GSH plays an important role in detoxification of ROS. These results are consistent with pervious studies in which GSH content was decreased in kidney rat and mice by methomyl exposure, which reflects its consumption through the oxidative stress (Djeffal *et al*., 2015; El-Demerdash *et al*., 2013a)

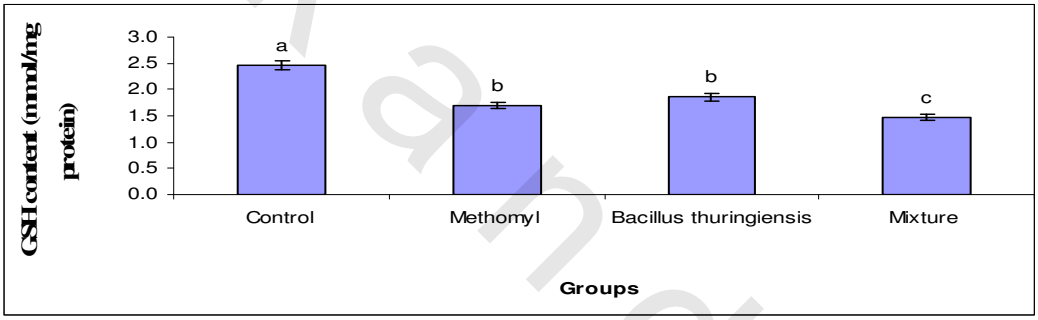

Fig.44: Effects of MET, *Bt* alone and their combination on the GSH content in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c) were significantly different, ($P < 0.05$).

4.3.3.2. Kidney glutathione S-transferase

Data presented in Table 17 and Figure 45 showed that treatment with MET,*Bt* and their combination caused a significant ($P < 0.05$) decrease in GST activity in rat kidney as compared with control. There is increasing evidence of an inverse correlation between susceptibility to chemical carcinogens and GST (Smith *et al*., 1977) and their ability to protect against lipid peroxidation caused by many environmental xenobiotics (Mahboob and Siddiqui, 2002). Also, the decrease in the GST activity could probably have been due to deficiency in the GSH levels. GST inhibition has been documented to occur under other oxidative stress conditions (Mansour *et al*., 2009; Garg *et al*., 2009, 2008; Djeffal *et al*., 2015).

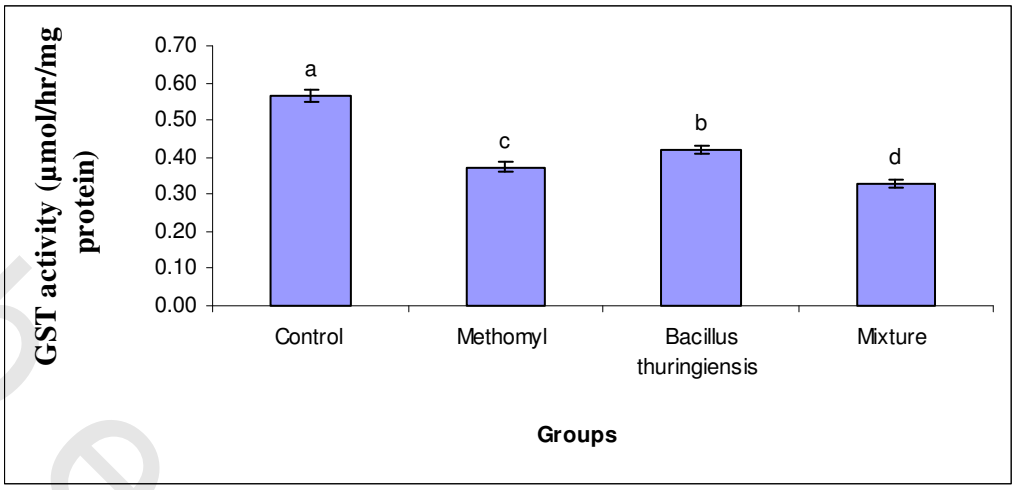

Fig.45: Effects of MET, *Bt* alone and their combination on the GST activity in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, ($P < 0.05$).

4.3.3.3. Kidney superoxide dismutase and catalase

SOD and CAT are the most important defense mechanisms against toxic effects of oxygen metabolism. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, while CAT converts hydrogen peroxide into water. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS (Mansour and Mossa, 2009). SOD and CAT activities were significantly ($P < 0.05$) reduced by MET, *Bt* alone and their combination in treated rat kidney (Table 17 and Figures 46 and 47). Consistent with these results, El-Demerdash *et al*. (2013a) found that methomyl significantly reduced the antioxidant enzymes in mice kidney. The inhibition of enzymes involved in free radical removal led to the accumulation of H_2O_2, which promoted lipid peroxidation and modulation of DNA, altered gene expression and cell death (Calviello *et al*., 2006).

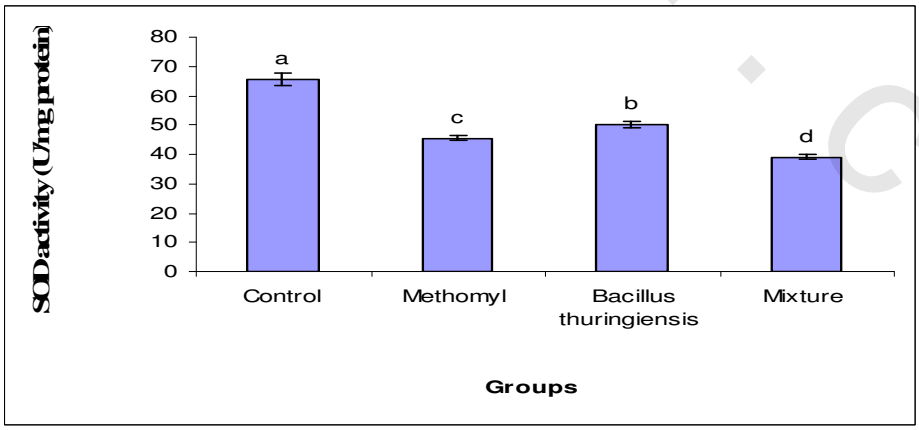

Fig.46: Effects of MET, *Bt* alone and their combination on the SOD activity in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, ($P < 0.05$).

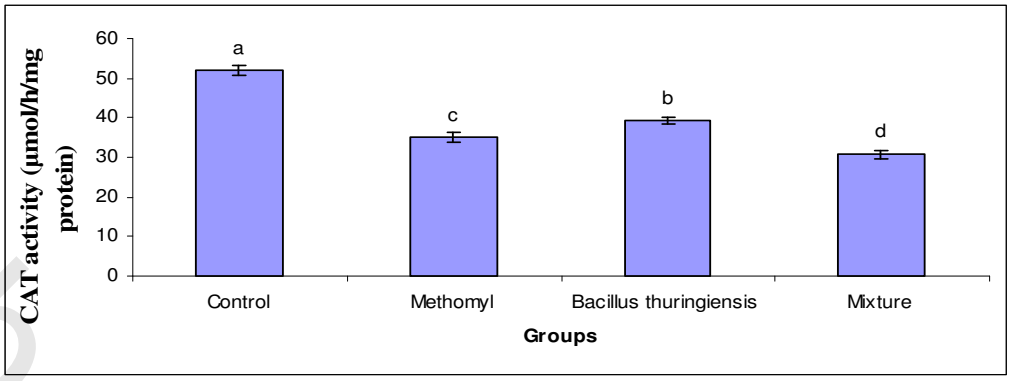

Fig.47: Effects of MET, *Bt* alone and their combination on the CAT activity in kidney of male rats. Data are presented as mean ±S.E for groups of n=7 rats. Mean values not sharing common letters (a, b, c, d) were significantly different, ($P < 0.05$)

4.3.4. Histopathological changes in the kidney

The histological examination of the kidney sections is presented in Figure 48. Control rats showed normal histological structure of the normal renal glomeruli surrounded by renal tubules (Fig. 48A). A congestion with swelling of the endothelial cells and degeneration of the epithelium cells lining the renal tubules was observed in kidney of methomyl-treated rats (Fig. 48B). Similar histological observations were reported in kidney of mice treated with MET for 20-days where severe swelling of the endothelial cells lining glomerular capillaries, as well as, swelling of the degenerated epithelium of the renal tubules were reported (El-Demerdash *et al*., 2013a).

Kidney of rats treated with *Bt* toxins showed histopathological alterations including hydropic change in renal tubules, thickening in glomerular basement membrane with swelling in epithelial and endothelial cells (Fig. 48C). The alterations in the kidneys caused by the biological insecticide reflect the effect of toxins on the immune system through the proliferation of mesangial cells and their infiltration in the tissue. This led to either a reduction or absence of Bowman's spaces, characterizing membranous proliferative glomerulonephritis and thereby reducing the functional capacity of the nephrons (Lemos *et al*., 2013). The effect of the *Bt* toxin on the immune system is reported by Hayakawa *et al*., (2007) who found that strains of the *Bt* bacterium stimulated the activation of lymphocytes in human kidney cell cultures.

Kidney of rats treated with both MET and *Bt* showed hydropic change in renal tubules and thickening in glomerular basement membrane with swelling in epithelial and endothelial cells and in blood vessels (Fig. 48D-E). This is confirming that altered renal cell membrane permeability could lead to changes in the enzyme activity in kidney as observed in the present study.

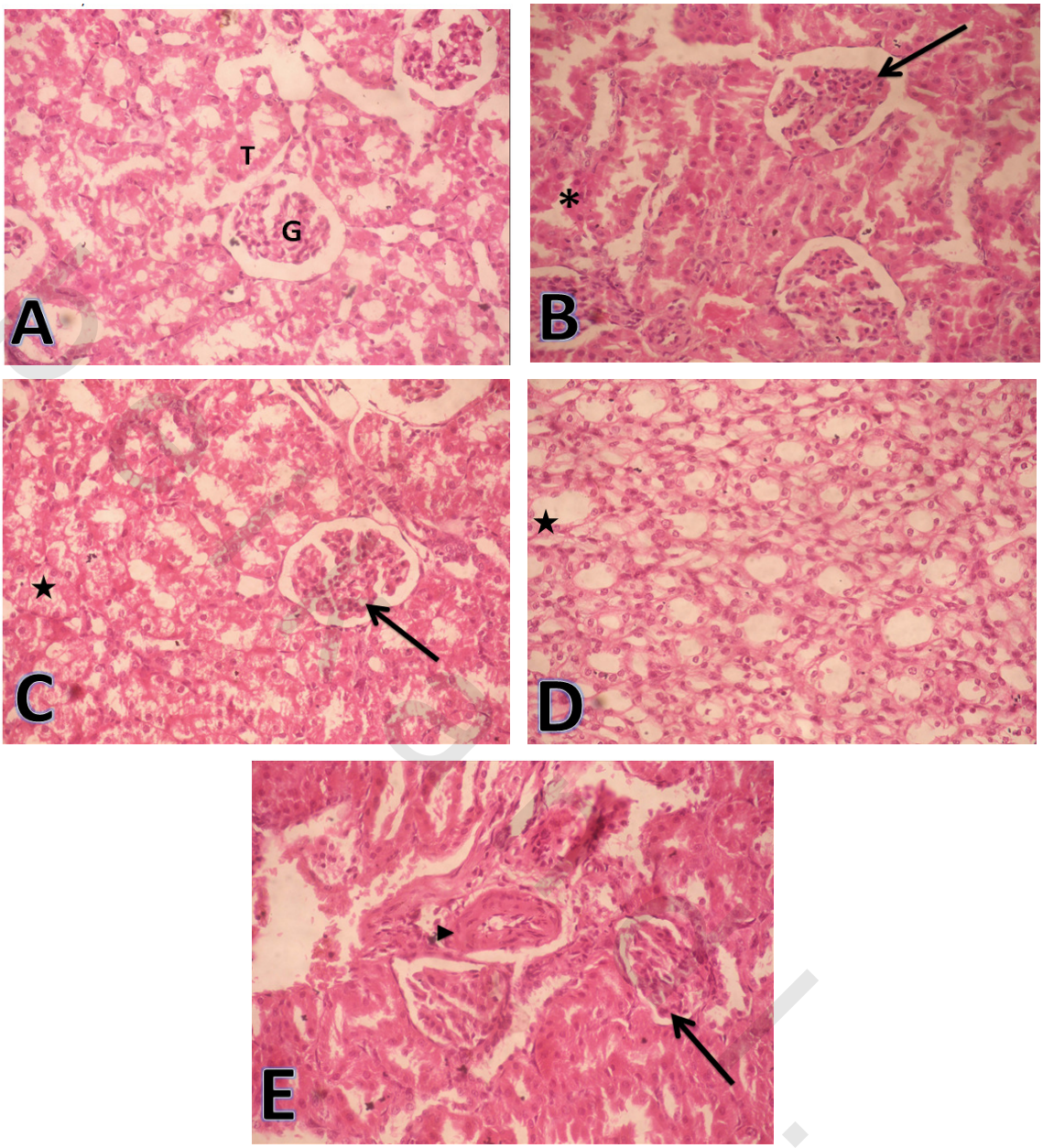

Fig. 48. Photomicrographs of rat kidney sections after methomyl, *Bacillus thuringiensis* and their combination treatments (H&E x400). (A) Control group showing the histological structure of the normal renal glomeruli (G) surrounded by renal tubules (T); (B) kidney of rat after methomyl treatment showing a congestion with swelling of the endothelial cells (arrow) and degeneration of the epithelium cells lining the renal tubules (*); (C) kidney of a rat after Bacillus thuringiensis treatment showing hydropic change in renal tubules (★), thickening in glomerular basement membrane with swelling in epithelial and endothelial cells (arrow) ; (D-E) kidney of rat after methomyl and Bacillus thuringiensis treatment showing (D) hydropic change in renal tubules (★), and (E) thickening in glomerular basement membrane with swelling in epithelial and endothelial cells (arrow) and thickening in blood vessels (arrowhead).