5. RESULT AND DISCUSSION

5.1. Effects of cobalt chloride, ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane on hepatic biomarkers

Table 4 and Figure 6, 7, 8, 9, 10, 11 and 12 showed the levels of plasma hepatic marker enzymes in control and experimental rats. Intraperitoneal administration of cobalt chloride caused abnormal liver function in rats. In cobalt chloride treated rats the activities of plasma hepatospecific enzymes such as plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, were significantly ($P < 0.05$) increased, when compared with control rats. But rats treated with cobalt chloride and EDTA significantly ($P < 0.05$) decreased the levels of serum hepatic markers. Also, rats treated with cobalt chloride and Cyclam significantly ($P < 0.05$) decreased the levels of serum hepatic markers.

Table 4

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total protein in plasma of male rats treated with cobalt chloride (Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
AST(U/l)	54.27 ± 1.97^{c}	72.26 ± 1.77^{a}	61.45 ± 1.89^{b}	60.81 ± 1.91^{b}
ALT(U/l)	55.74 ± 1.67^{c}	74.88 ± 1.14^{a}	61.32 ± 0.99^{b}	62.77 ± 1.20^{b}
ALP(U/l)	56.97 ± 1.92^{c}	73.59 ± 2.06^{a}	$63.44\pm2.31^{b,c}$	65.91 ± 2.40^{b}
LDH(U/l)	385 ± 8.03^{c}	514 ± 14.77^{a}	437 ± 15.22^{b}	431 ± 17.95^{b}
Total protein(mg/dl)	7.43 ± 0.21^{a}	5.23 ± 0.13^{c}	6.56 ± 0.22^{b}	6.13 ± 0.18^{b}

Values are expressed as means ±SE; n=7 for each treatment group.

^{abc} Mean values within a row not sharing a common superscript letters were significantly different, $P<0.05$.

Several of soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. The increases in the activities of these enzymes in plasma are indicators of liver damage and thus cause alteration in liver function. (Awad et al., 1998) fond that cell damage exhibited good correlation with the enzyme leakage. Hence, cellular damage caused by toxic substance is frequently accompanied by increasing cell membrane permeability. In our study, the increased activities of plasma AST, ALT, ALP and LDH obviously indicate that liver is susceptible to cobalt toxicity. Cobalt exposure enhances intracellular ROS production and increases lipid peroxidation, which eventually leads to cell damage and leakage of plasma AST, ALT, ALP and LDH. In fact, increased extracellular activity of these enzymes which are intracellular enzymes is the result of disrupted cell membrane integrity, which occurs during the lipid peroxidation, under the influence of free radicals and in the conditions of oxidative stress (Zou et al., 2001). Ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane have the

ability to inhibit radical generation and could further reduce the oxidative stress elicited by cobalt since EDTA and Cyclam chelation therapy is proposed to not only remove metals but also have antioxidant properties (Lamas and Ackermann ,2000)

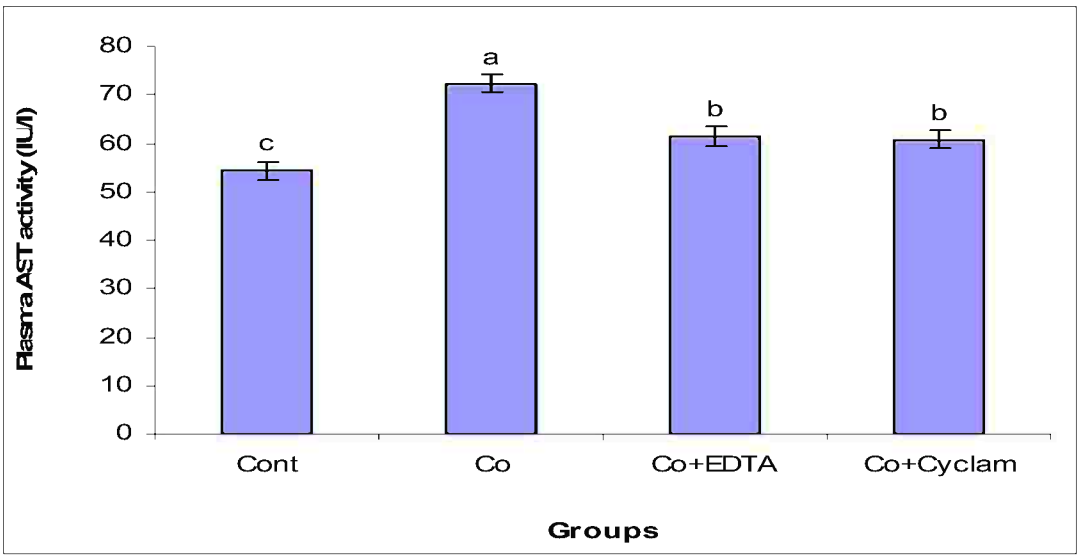

Fig.6. changes in aspartate aminotransferase activity (AST) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ±SE of seven rats in each group.

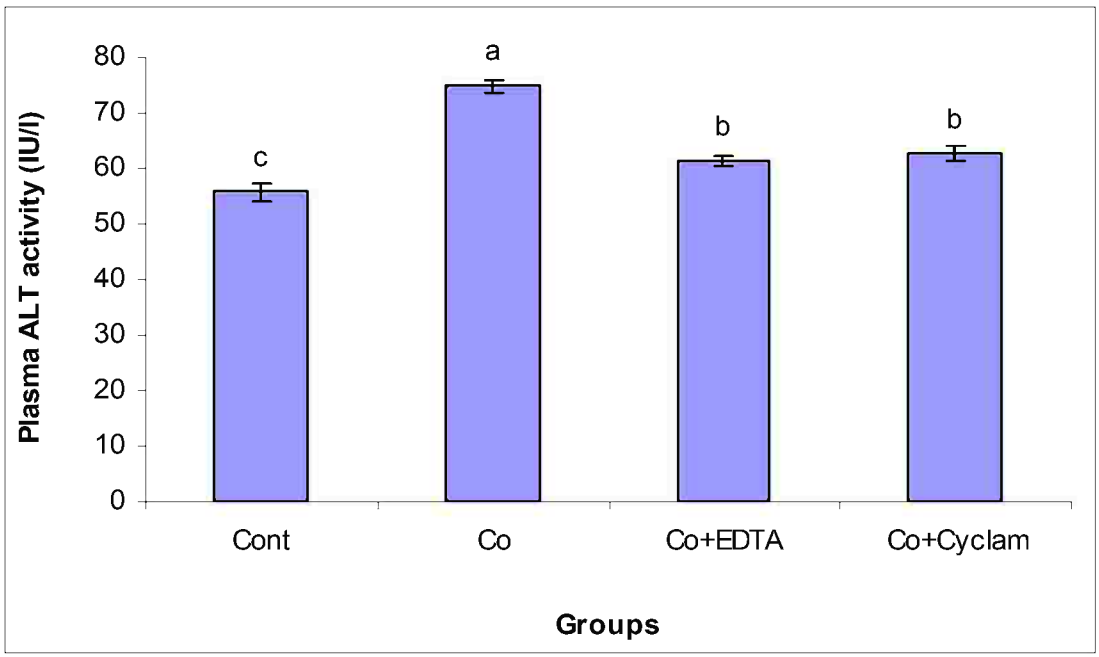

Fig.7. changes in Alanine Aminotransferase activity (ALT) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

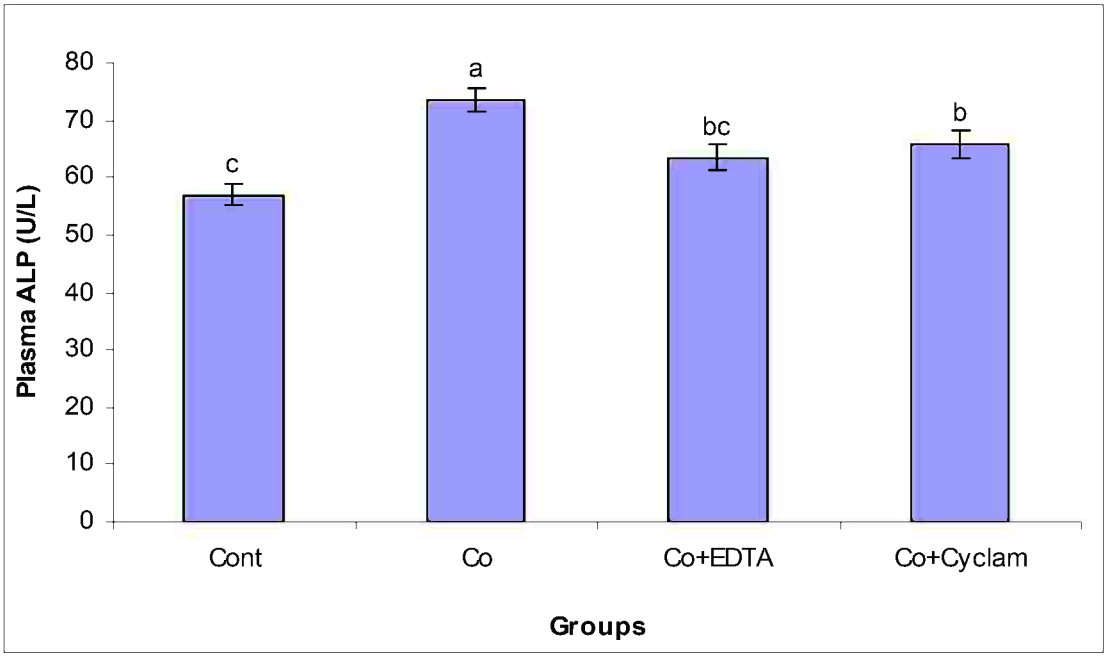

Fig.8. changes in alkaline phosphatase activity (ALP) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

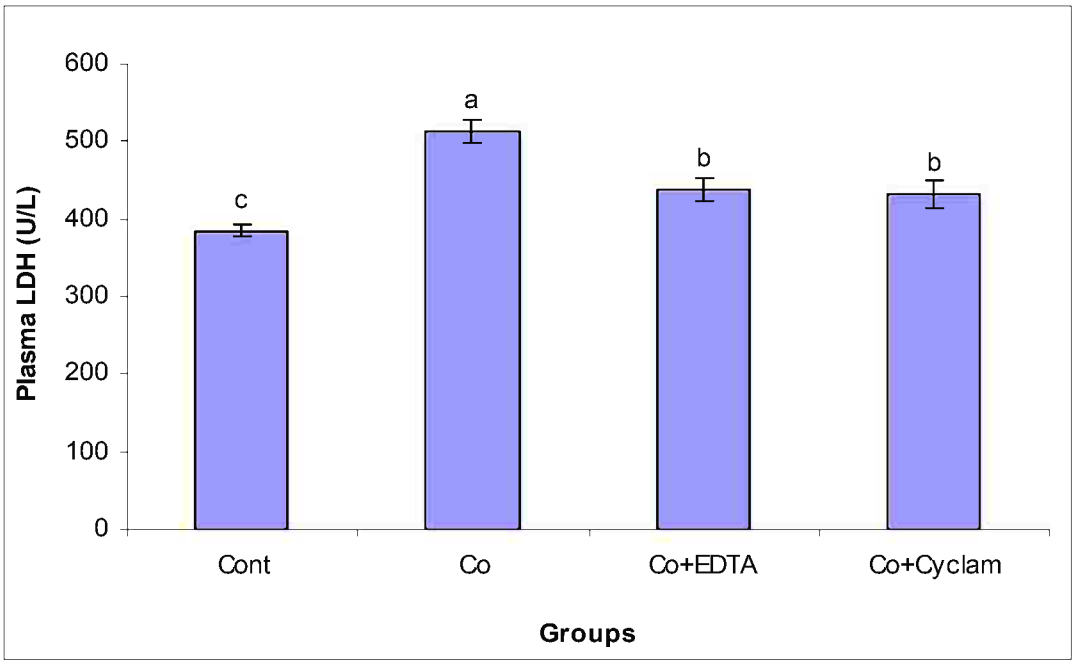

Fig.9. Changes in Lactate dehydrogenase activity (LDH) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

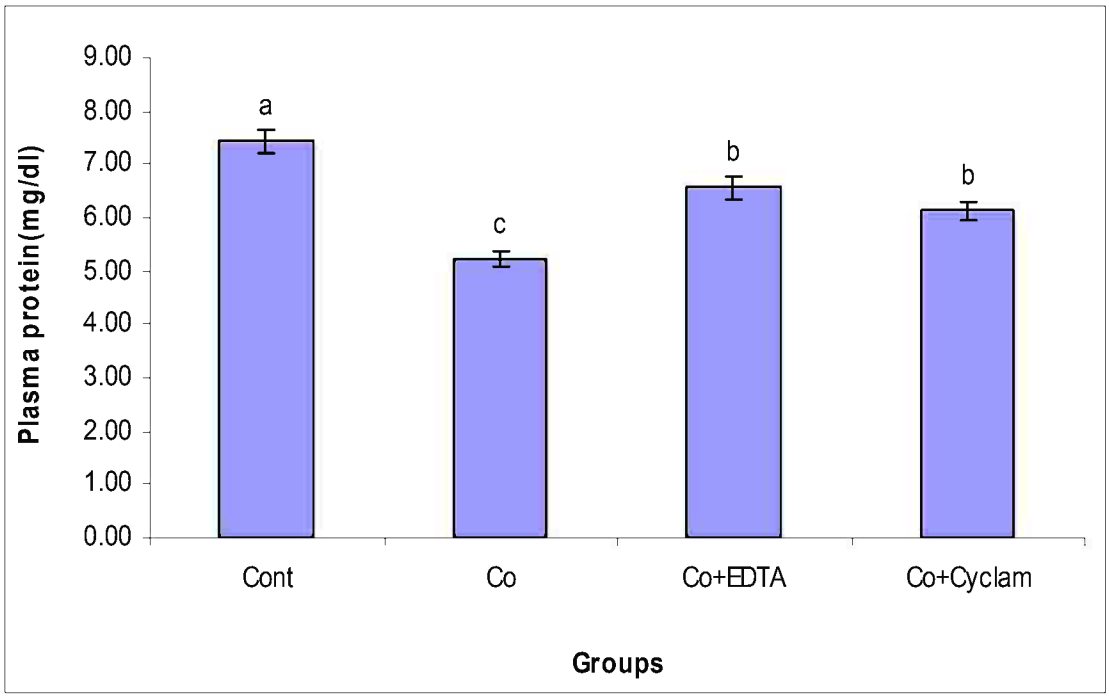

Fig.10. Changes in total protein in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.1.1. Plasma thiobarbituric acid reactive substances

Lipid peroxidation is considered to be a valuable indicator of oxidative damage of cellular component. Most components of cellular structure and function are likely to be potential targets of oxidative damage, and the most susceptible substrates for autoxidation are polyunsaturated fatty acids of the cell membrane, which undergo rapid peroxidation (Yonar and Sakin, 2011). Thiobarbituric acid assay (TBA) was used to measure the extent of lipid peroxidation induced by Co in plasma. Results indicated in Table 4 and Figure 11 showed that thiobarbituric acid reactive substances level was significantly ($P < 0.05$) increased in plasma of rats treated with Cobalt Chloride.

Table 5.

Thiobarbituric acid reactive substances concentration (TBARS), glutathione content (GSH), superoxide dismutase (SOD), Catalase (CAT) and Glutathione S-Transferase (GST) in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
TBARS(nmol/l tissue)	0.096 ± 0.02^{c}	1.29 ± 0.04^{a}	$1.04\pm0.02^{b,c}$	1.06 ± 0.05^{b}
GSH(mmol/l)	0.162 ± 0.005^{a}	0.105 ± 0.005^{c}	0.140 ± 0.006^{b}	0.136 ± 0.005^{b}
SOD(U/ml)	1.16 ± 0.030^{a}	0.76 ± 0.027^{c}	0.96 ± 0.043^{b}	0.94 ± 0.019^{b}
CAT(U/ml)	45.18 ± 1.13^{a}	29.60 ± 0.55^{c}	39.77 ± 0.93^{b}	40.13 ± 1.38^{b}
GST(Umol/h/mg)	0.644 ± 0.021^{a}	0.387 ± 0.009^{c}	0.536 ± 0.018^{b}	0.547 ± 0.020^{b}

Values are expressed as means ±SE; n=5 for each treatment group.

^{abc} Mean values within a row not sharing a common superscript letters were significantly different, $P<0.05$.

Reactive oxygen species (ROS) are products of electron transport chains, enzymes, and redox cycling and their production may be enhanced by exposure to xenobiotics. Reactive oxygen species (ROS) considered as oxidative markers react with all biological macromolecules, i.e. lipids, proteins, nucleic acids, and carbohydrates (Romao et al., 2006). Cobalt exposure enhances intracellular ROS production and increases lipid peroxidation, which eventually leads to cell damage (Zou et al., 2001). Compared to the control value, TBARS level was significantly ($P<0.05$) increased in rats plasma treated with Cobalt Chloride (Co) while its level significantly ($P<0.05$) decrease in plasma of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co).

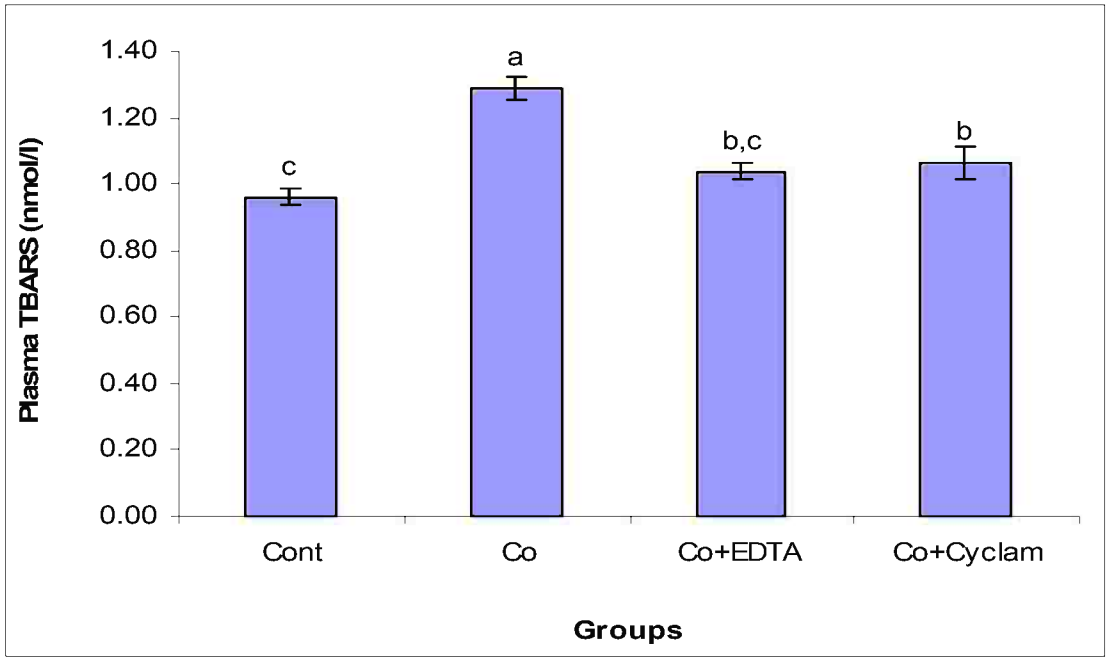

Fig.11. changes in thiobarbituric acid-reactive substances (TBARS) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.1.2. Plasma reduced glutathione (GSH)

Reduced glutathione is the main nonprotein thiol and one of the primary reductants found in cells (Yonar and Sakin, 2011). As reported in Table4 and Figure 12, GSH content was significantly decreased. Glutathione antioxidant system plays a major role against Reactive oxygen species ROS and other oxidant species. The free radicals interact with membrane lipids leading to the production of lipid hydroperoxides. GSH, an electron donor, may be oxidized due to the interaction with the free radicals resulting with the formation of corresponding disulfide compound, GSSG(oxidized form) (El-Demerdash et al., 2009). Hence GSH depletion suggested protection role of GSH to the cells against the adverse effects of Cobalt Chloride (Co). Compared to the control value, GSH level was significantly ($P<0.05$) decreased in rats plasma treated with Cobalt Chloride (Co) while its level significantly ($P<0.05$) increased in plasma of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co). Hence EDTA and Cyclam alleviated the toxic effects and oxidative damage of Cobalt Chloride (Co).

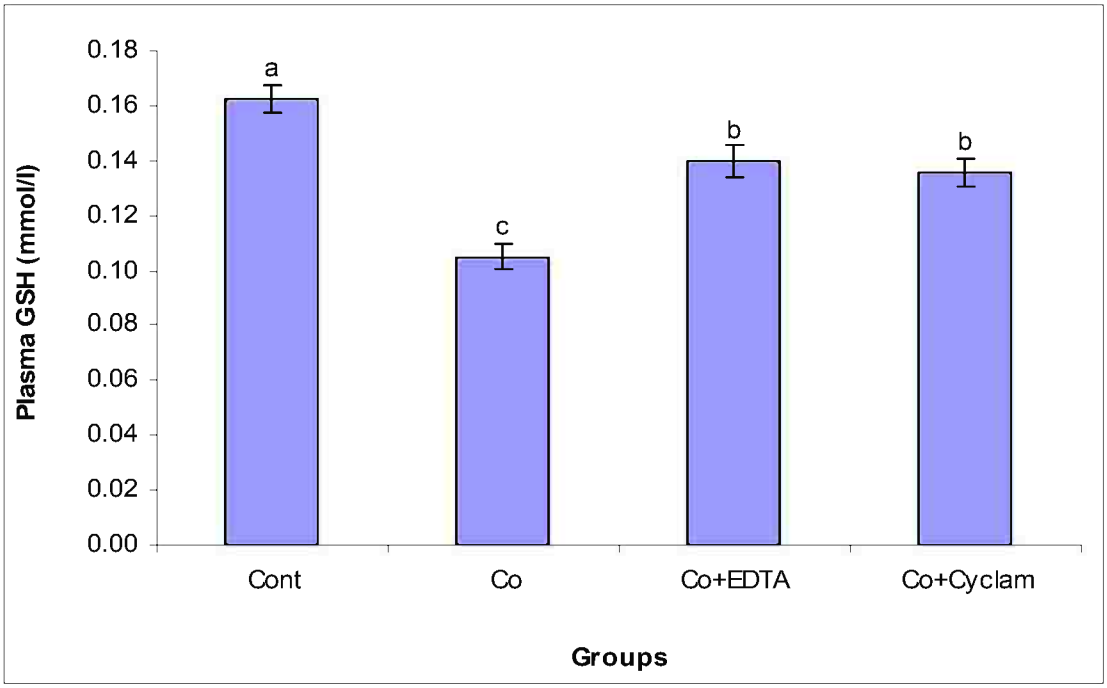

Fig.12. changes in reduced glutathione content (GSH) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.1.3. Plasma superoxide dismutase (SOD)

Superoxide dismutase has an antitoxic effect against the superoxide anion.

Super oxide radicals are produced in mitochondria and endoplasmic reticulum as a consequence of auto-oxidation of electron transport chain components. Superoxide dismutase accelerates the dismutation of superoxide to H_2O_2 which is removed by catalase, thus superoxide dismutase can act as a primary defense and prevents further generation of free radicals. Results observed in Table 5 and Figure 13, show that Compared to the control value, SOD level was significantly ($P<0.05$) decreased in rats plasma treated with Cobalt Chloride (Co) while its level significantly ($P<0.05$) increased in plasma of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co). Hence EDTA and Cyclam alleviated the toxic effects and oxidative damage of Cobalt Chloride (Co).

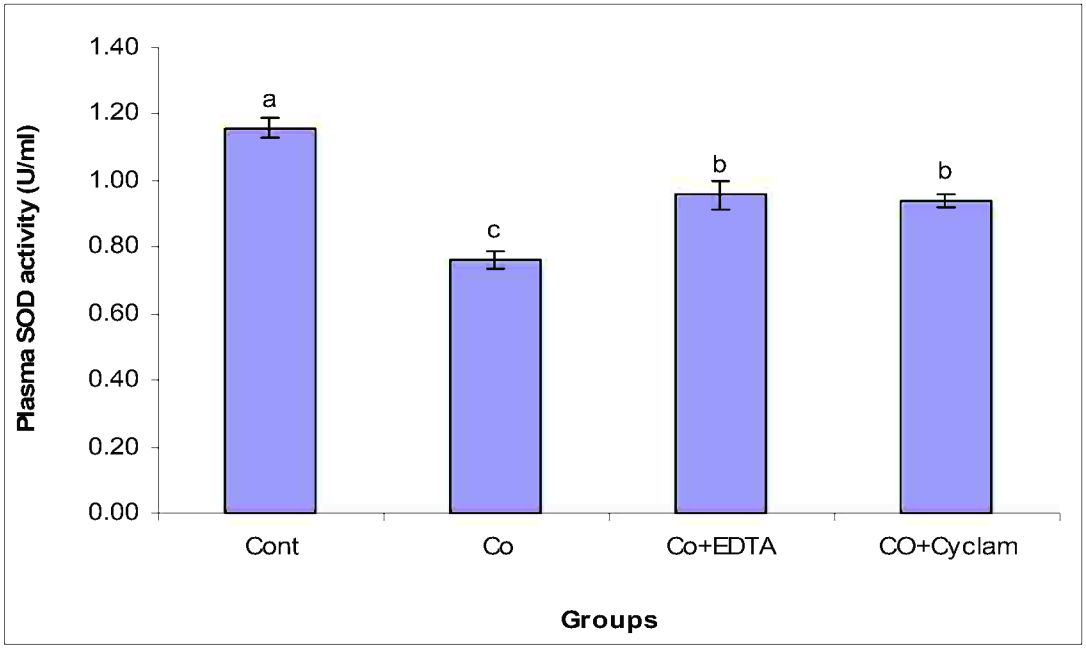

Fig.13. Changes in Superoxide dismutase activity (SOD) in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group

5.1.4. Plasma catalase (CAT)

Catalase is a heme-containing enzyme responsible for breakdown of hydrogen peroxide, an important Reactive oxygen species ROS, generated by superoxide dismutase during metabolism (El- Demerdash et al., 2009). Catalase plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Results observed in Table 4 and Figure 14, show that compared to the control value, CAT level was significantly ($P<0.05$) decreased in rats plasma treated with Cobalt Chloride (Co) while its level significantly ($P<0.05$) increased in plasma of male rats treated with Cobalt Chloride plus ethylene diamine tetraacetic acid (Co+EDTA) and Cobalt Chloride plus 1, 4, 8, 11-tetraazacyclotetradecane (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co). Hence EDTA and Cyclam alleviated the toxic effects and oxidative damage of Cobalt Chloride (Co). The observed reduction in the activity of superoxide dismutase and Catalase indicated impairment in the antioxidant defense system due to free radical generation owing to their excessive utilization. Two mechanisms might play a role in this decrease. The first involved consumption during the breakdown of free radicals and the high level of H_2O_2 or the inhibition of the enzyme by these radicals, while the second involved the direct inhibition of superoxide dismutase and catalase Cobalt Chloride(Co).

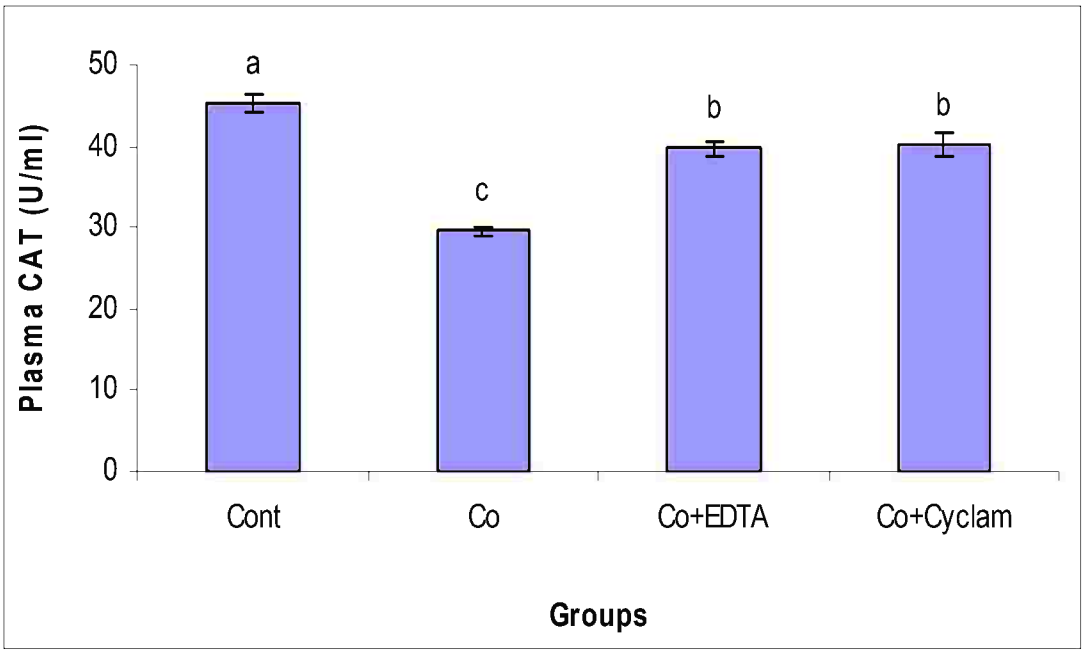

Fig.14.Changes in Catalase activity (CAT) in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.1.5 Plasma glutathione-S-transferase

GST is considered a biomarker due to its change by exposure to xenobiotics; furthermore, it is a phase II enzyme that conjugates and detoxifies metabolites formed by phase I enzymes of the cytochrome P450 mono-oxygenase system. Reduction in glutathione-S-transferase activities in rat plasma is in consistence with the findings of (El-Demerdash. 2007) who reported a reduction in glutathione-S-transferase activity in the rats following its exposure to a mixture of organophosphate and pyrethroid insecticides (El-Demerdash.2011).

Table 5 and Figure 15 illustrate that plasma of Cobalt Chloride intoxicated rats showed a significant ($P<0.05$) decrease in glutathione-S-transferase activity as compared to control group. The observed reduction may be due to the consumption of glutathione in protecting against the Cobalt Chloride –induced oxidative stress, as it helps to maintain cellular redox statues. GST level was significantly ($P<0.05$) increased in plasma of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co). Hence EDTA and Cyclam alleviated the toxic effects and oxidative damage of Cobalt Chloride (Co).

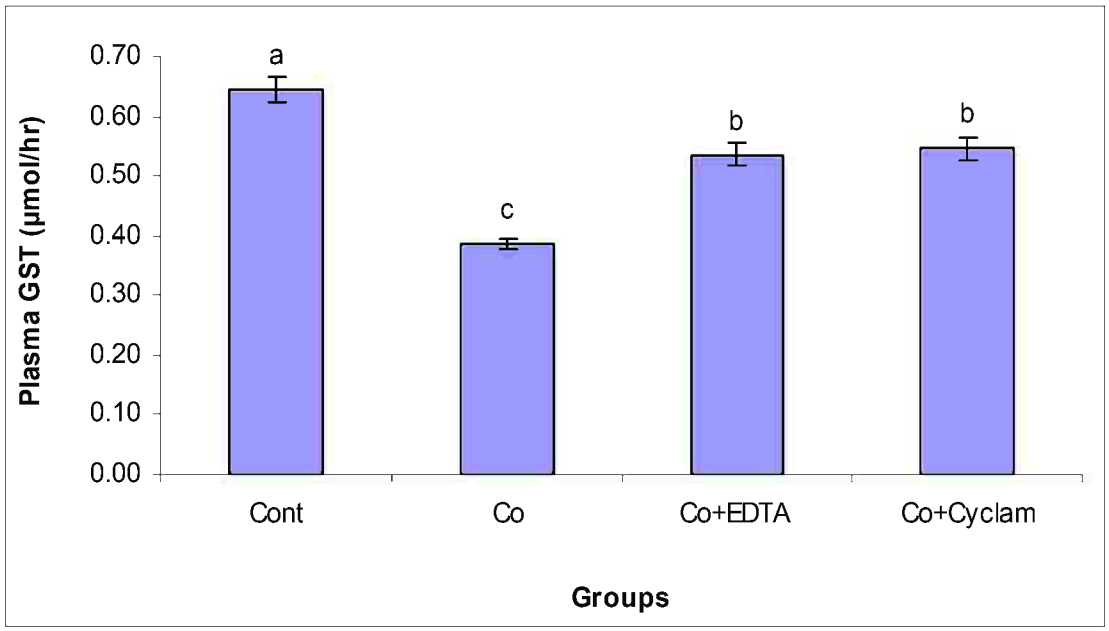

Fig.15.Changes in glutathione-S-transferase activity (GST) in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.1.6 Plasma cholesterol

Changes in lipid profile in Cobalt Chloride treated rats, a significant increase in total cholesterol, TG, LDL-C and VLDL-C levels ($P < 0.05$) were observed while the level of HDL-C was decreased. On one hand in (Co+EDTA) and (Co+Cyclam) groups showed a significant decrease in total cholesterol, TG, LDL-C and VLDL-C levels and significant increase in HDL-C as compared with Cobalt group (Table 6) and Figure(16-20).Lipids are thought to be among the most sensitive biological molecules in terms of ROS susceptibility. In particular, unsaturated fatty acids, which are located in cellular membrane, tissues and blood, are prone to ROS attack. Previous studies reported perturbations in lipid profile in serum of experimental animals and workers exposed to insecticides and heavy metals (Kalender et al., 2013). The increase in serum total cholesterol level may be attributed to the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum (Das et al., 2001) the elevation in serum triglycerides has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoprotein (Novelli et al., 1998). HDL-C 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 (Jung et al., 1983). It has been established that the decreased serum HDL-C levels are antiatherogenic (Ames et al., 1982), where as the reduced levels are associated with an increased risk for coronary artery disease (Haberland et al., 1988).

Table 6

Changes in Cholesterol, LDL, HDL, Triglyceride, VLDL in plasma of male rats treated with cobalt chloride (Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
Cholesterol(mg/dl)	143±5.23^{c}	201±8.44^{a}	171±3.57^{b}	174±5.48^{b}
LDL(mg/dl)	45.02±1.14^{c}	59.88±2.13^{a}	51.25±1.92^{b}	51.31±1.64^{b}
HDL(mg/dl)	45.92±1.30^{a}	32.29±1.10^{c}	41.50±1.43^{b}	41.34±1.63^{b}
TG(mg/dl)	112±4.54^{c}	163±5.22^{a}	134±4.99^{b}	131±4.09^{b}
VLDL(mg/dl)	22.32±0.908^{c}	32.52±1.044^{a}	26.88±0.997^{b}	26.28±0.818^{b}

Values are expressed as means ±SE; n=7 for each treatment group.
^{abc} Mean values within a row not sharing a common superscript letters were significantly different, P<0.05.

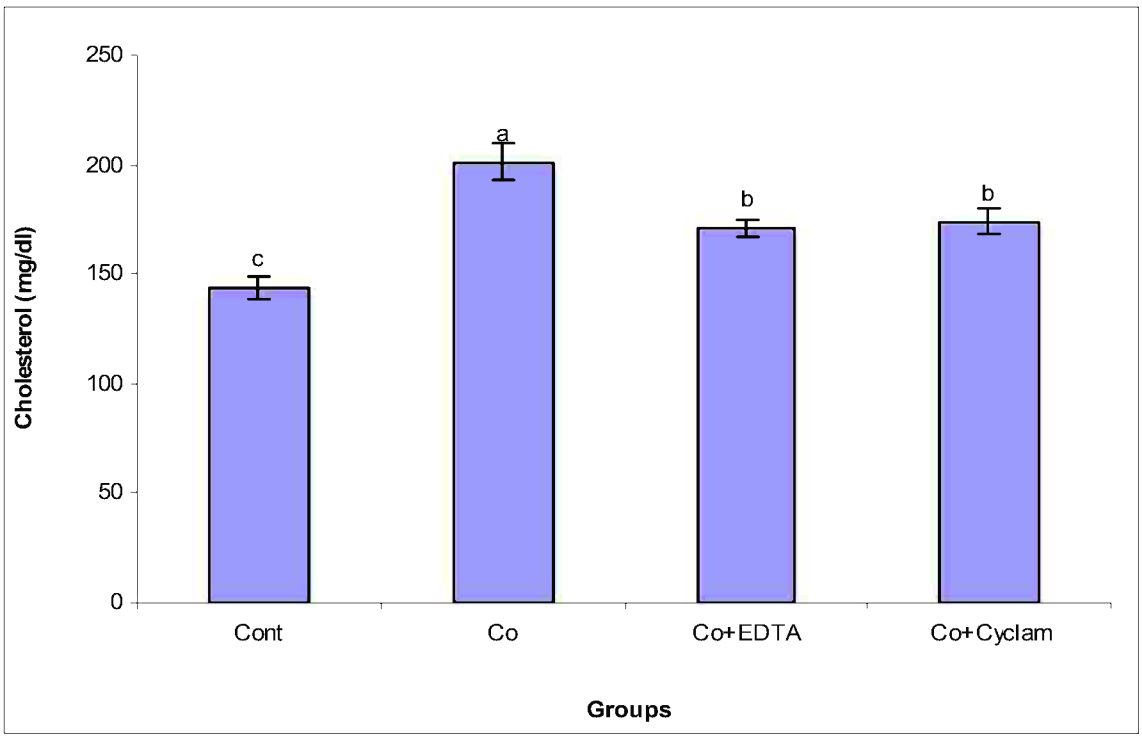

Fig.16. Changes in Cholesterol in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).^{abc} Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

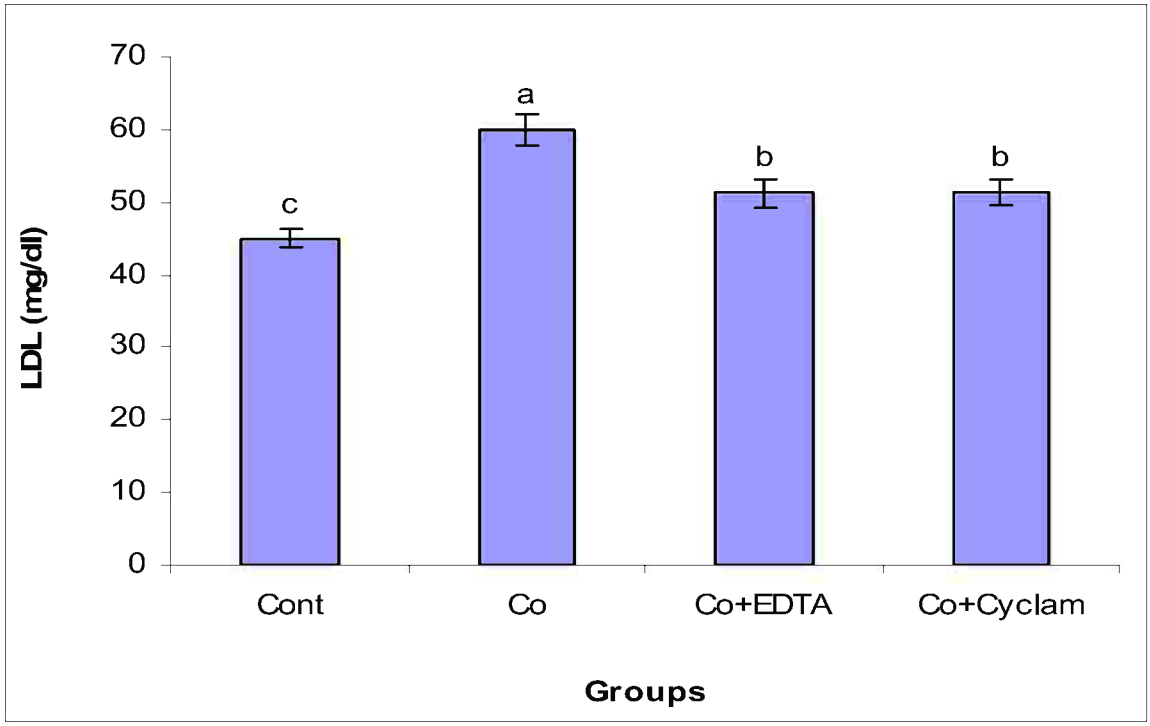

Fig.17. Changes in LDL in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

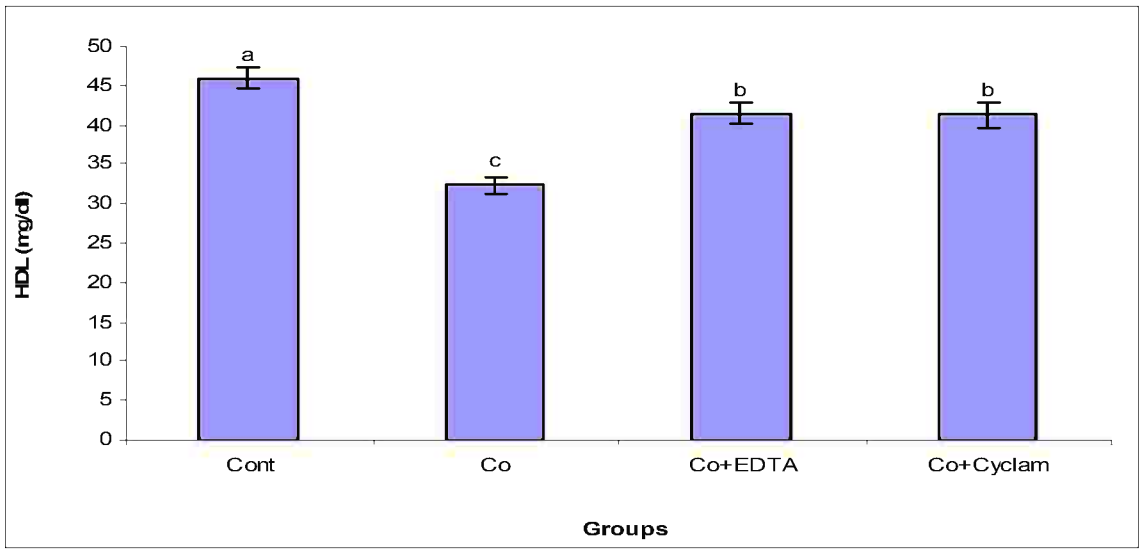

Fig.18. Changes in HDL in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

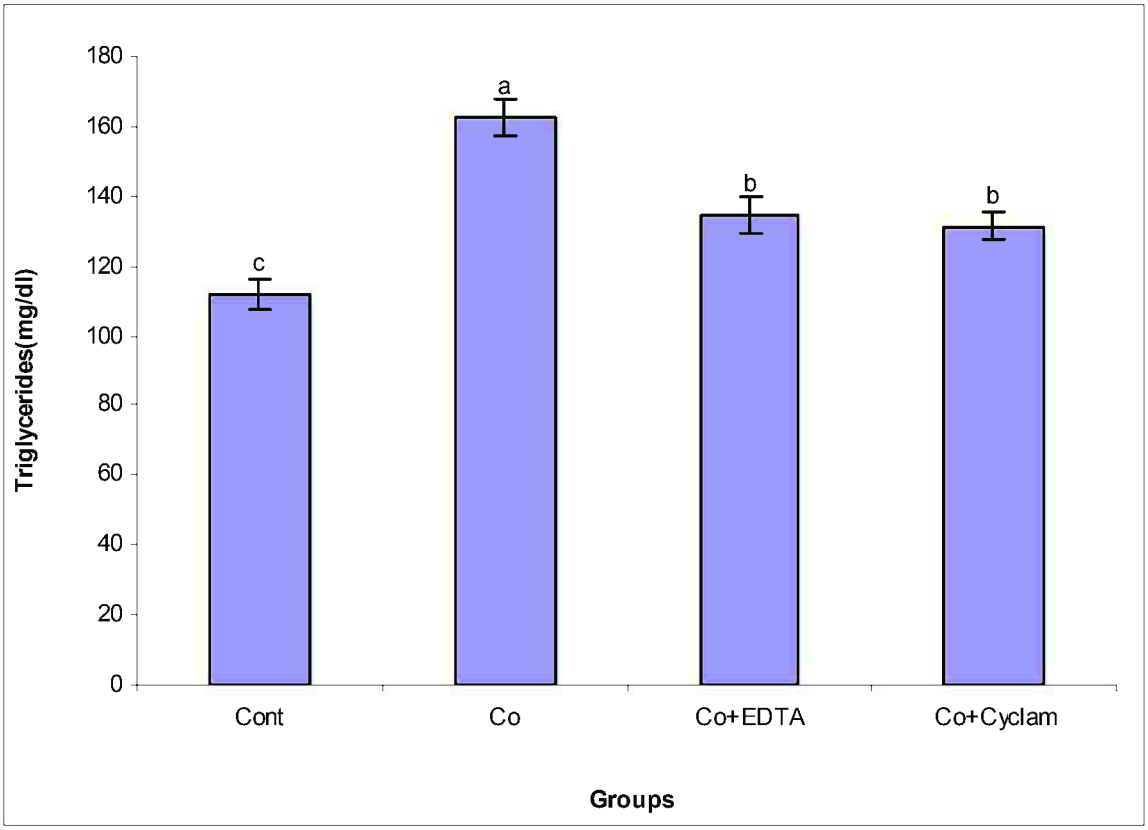

Fig (19) Changes in Triglycerides in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

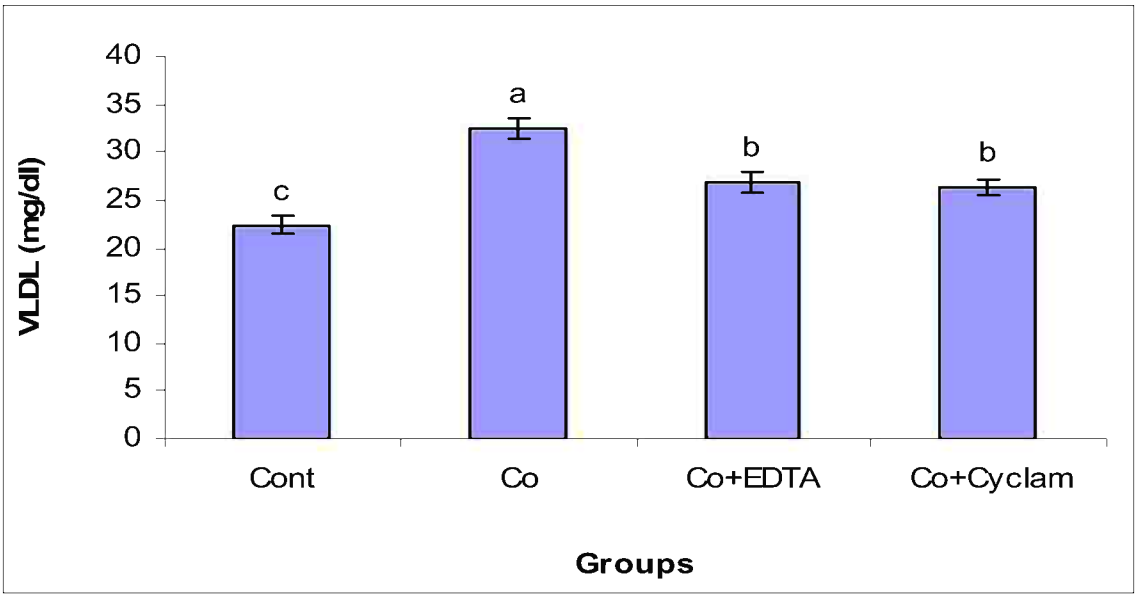

Fig.20. Changes in VLDL in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

Table 7

Changes in Creatinine, Urea and Albumin in plasma of male rats treated with cobalt chloride (Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
Creatinine(mg/dl)	0.676 ± 0.026^{c}	0.946 ± 0.029^{a}	0.798 ± 0.025^{b}	0.836 ± 0.027^{b}
Urea(mg/dl)	36.56 ± 0.88^{c}	52.84 ± 2.19^{a}	45.68 ± 1.82^{b}	46.28 ± 1.35^{b}
Albumin(mg/dl)	5.00 ± 0.152^{a}	3.88 ± 0.146^{c}	$4.72\pm0.140^{a,b}$	4.42 ± 0.186^{b}

Values are expressed as means ±SE; n=7 for each treatment group.
[Abc] Mean values within a row not sharing a common superscript letters were significantly different, P<0.05.

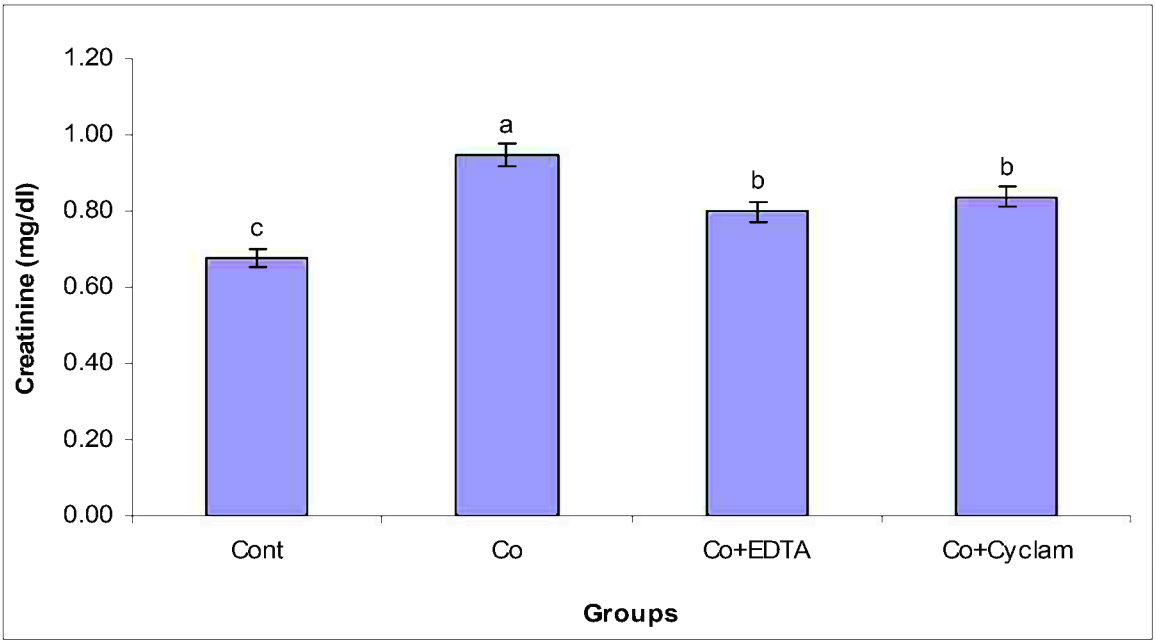

Fig.21. Changes in Creatinine in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

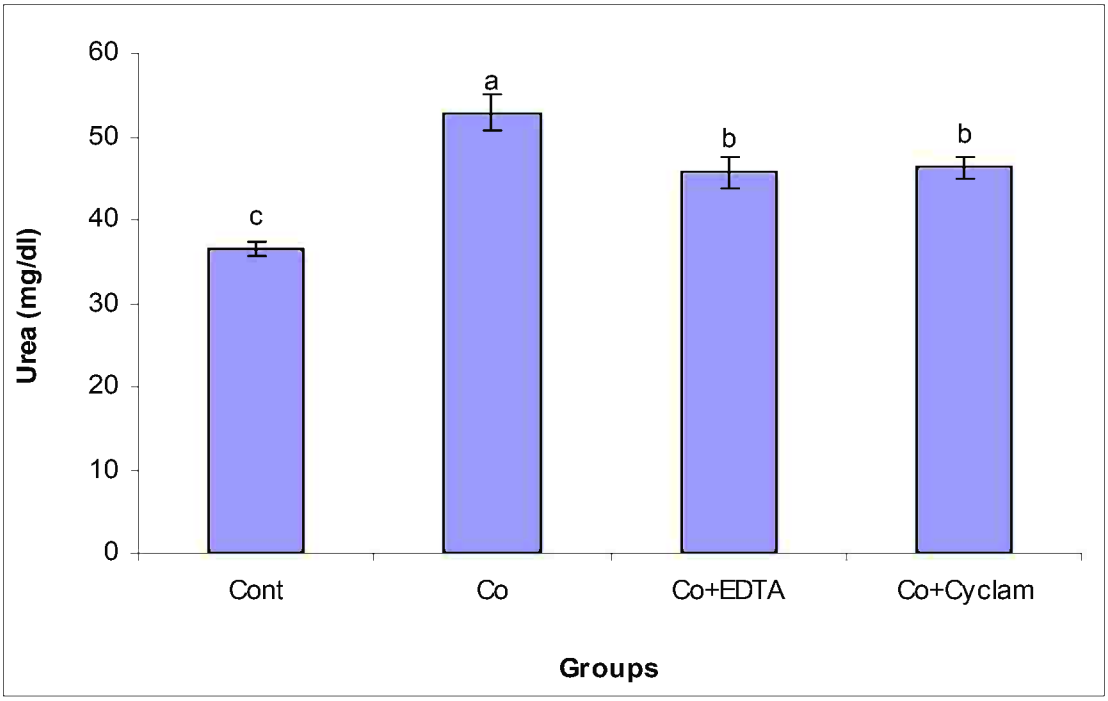

Fig.22. Changes in Urea in plasma of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

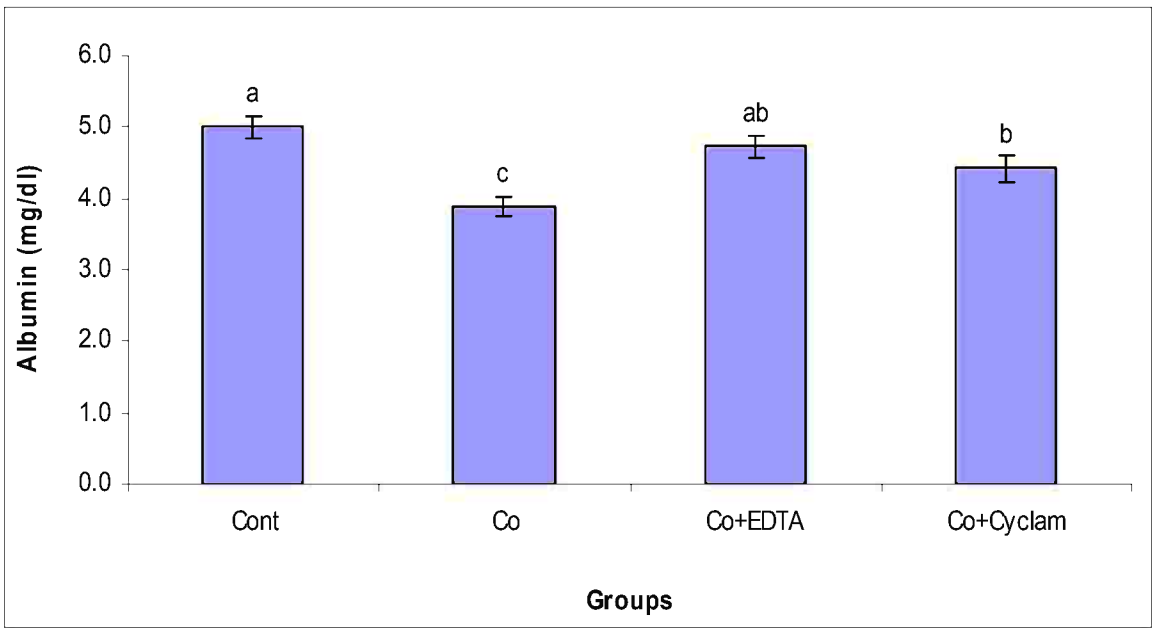

Fig.23. Changes in Albumin in plasma of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

5.2.1. Effects of cobalt chloride, ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane on lipid peroxidation in rat liver

Table 8 and Figure 24 show the changes in the levels of lipid peroxidation product in control and experimental animals. In cobalt chloride treated rats the levels of thiobarbituric acid reactive substances were significantly ($P < 0.05$) increased, when compared with control rats. But treatment of cobalt chloride with EDTA significantly ($P < 0.05$) decreased the levels of thiobarbituric acid reactive substances. Also, treatment of cobalt chloride with Cyclam significantly ($P < 0.05$) decreased the levels of thiobarbituric acid reactive substances when compared with cobalt rats. Gonzales et al. (2005) reported that, 1 h after a single dose of cobalt exposure, TBARS levels were strongly increased in rats. Enhanced peroxidation of lipids in liver tissue, observed in our study, resulted in the increased generation of different radical species includingO_2 and OH. These potentially deleterious free radicals were controlled by an enzymatic and non-enzymatic antioxidant defense system, which eliminate pro-oxidants and scavenge free radicals (Di Mascio et al. (1991)). Chelation therapy is thought to be not only remove contaminating metals but also to decrease free radical production (Lamas And Ackermann, 2000), so EDTA and Cyclam decreased the levels of thiobarbituric acid reactive substance in the liver of cobalt chloride treated group.

Table 8

Thiobarbituric acid reactive substances concentration(TBARS), glutathione content(GSH), superoxide dismutase(SOD), Catalase(CAT) and Glutathione S-Transferase(GST) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
TBARS(nmol/g tissue)	37.76 ± 1.60^{c}	51.27 ± 0.61^{a}	42.73 ± 0.98^{b}	42.49 ± 0.93^{b}
GSH(mmol/mg protein)	2.05 ± 0.078^{a}	1.26 ± 0.034^{c}	1.70 ± 0.039^{b}	1.67 ± 0.029^{b}
SOD(U/mg protein)	80.50 ± 3.58^{a}	49.28 ± 1.83^{c}	64.96 ± 1.46^{b}	63.14 ± 1.84^{b}
CAT(U/mg protein)	48.26 ± 0.97^{a}	33.08 ± 0.79^{c}	41.83 ± 1.21^{b}	43.11 ± 1.08^{b}
GST(Umol/h/mg protein)	1.09 ± 0.03^{a}	0.72 ± 0.01^{c}	0.93 ± 0.04^{b}	0.91 ± 0.03^{b}

Values are expressed as means ±SE; n=7 for each treatment group.

^{abc} Mean values within a row not sharing a common superscript letters were significantly different, $P<0.05$

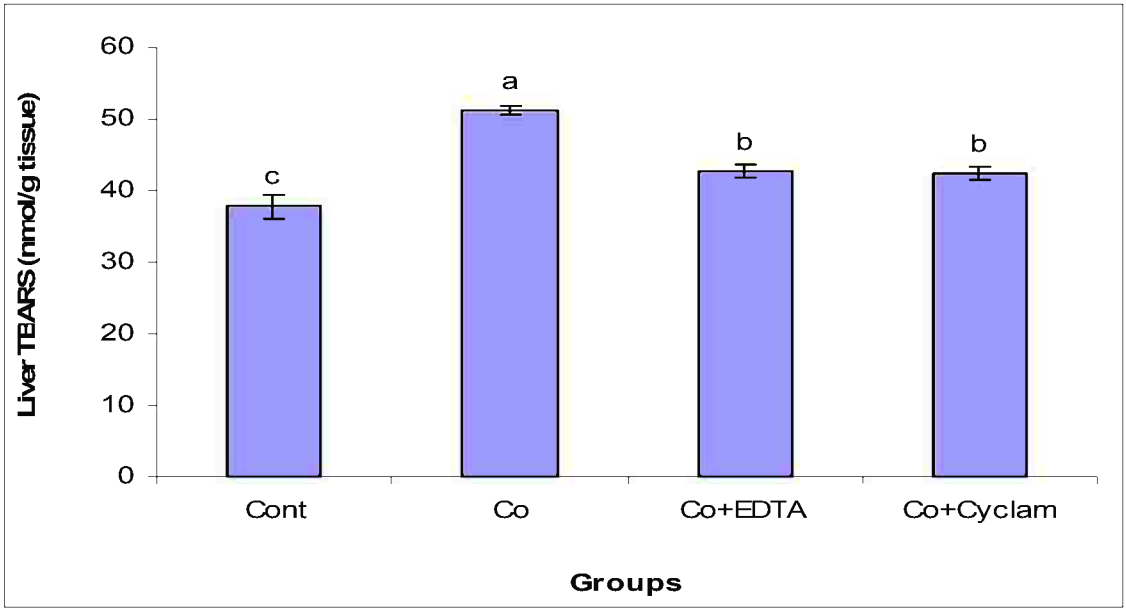

Fig.24. Changes in Thiobarbituric acid reactive substances(TBARS) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.2.2. Effects of Cobalt Chloride on non-enzymatic antioxidant

Table 8 and figure25 shows the changes in the levels of hepatic non-enzymatic antioxidant namely reduced glutathione in the liver of control and experimental rats. A significant ($P < 0.05$) decrease in the levels of non-enzymatic antioxidants were noticed in rats treated with Cobalt Chloride when compared to control rats. It seemed that Co^{2+} bound to thiol groups (–SH) and inhibits heme synthesis in the liver leading to cell damages. Moreover, this metal could bind to sulphydryl groups of proteins and various enzymes and interfered with metabolism of GSH which served as an essential antioxidant molecule responsible for metabolism and excretion of xenobiotics (Hultberg et al., 2001). GSH level was significantly ($P<0.05$) increased in liver of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co). Hence EDTA and Cyclam alleviated the toxic effects and oxidative damage of Cobalt Chloride (Co).

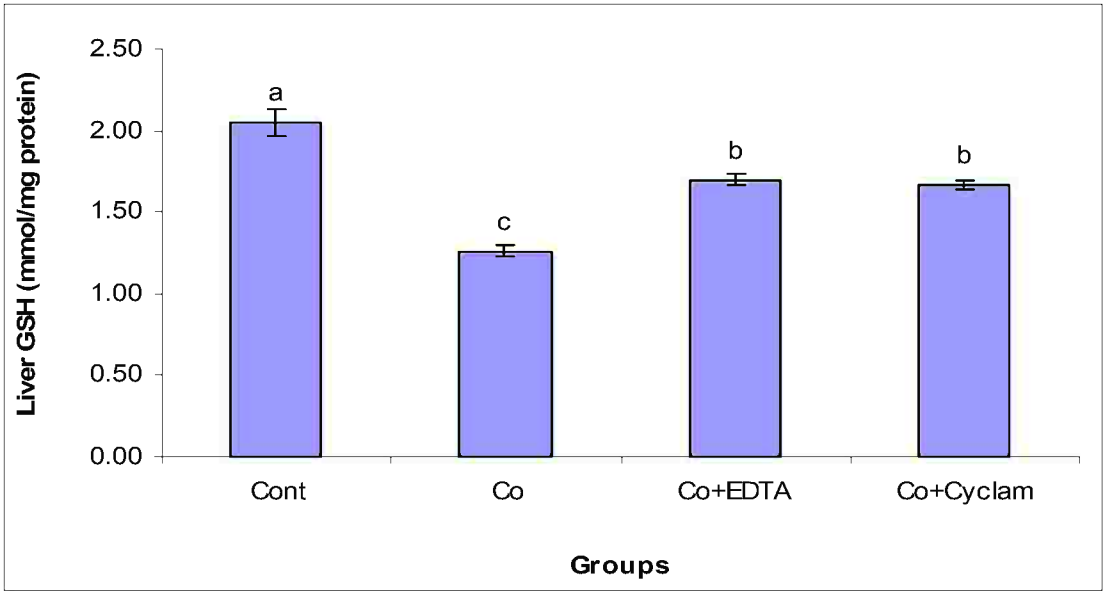

Fig.25. Changes in reduced glutathione content (GSH) in liver of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.2.3. Effect of Cobalt Chloride on enzymatic antioxidants

Table 8 and Figures 26, 27, 28, illustrate the activities of enzymatic antioxidants namely superoxide dismutase, catalase and glutathione-S-transferase in liver of control and experimental rats. A significant ($P < 0.05$) depletion in the activities of enzymatic antioxidants in Cobalt Chloride treated rats was observed. It has been also reported that cobalt bound stochiometrically at the zinc and copper sites in SOD protein had low enzymatic activity (Banci et al., 1999; Lyons et al., 2000). Cobalt can cause DNA fragmentation (Zou et al., 2001), activation of caspases (Zou et al., 2002) and increases production of reactive oxygen species (ROS) leading to oxidative stress (Olivieri et al., 2001). These free radicals may lead to cellular damage when the rate of their generation overcomes the rate of their decomposition by antioxidant defense systems, such as (SOD), (CAT), or GSH (Di Mascio et al., 1991; Mates et al., 1999; Datta et al., 2000). SOD, CAT, GST levels were significantly ($P<0.05$) increased in liver of male rats treated with (Co+EDTA) and (Co+Cyclam) compared to rats plasma treated with Cobalt Chloride (Co) due to their free radical scavenging and chelating properties.

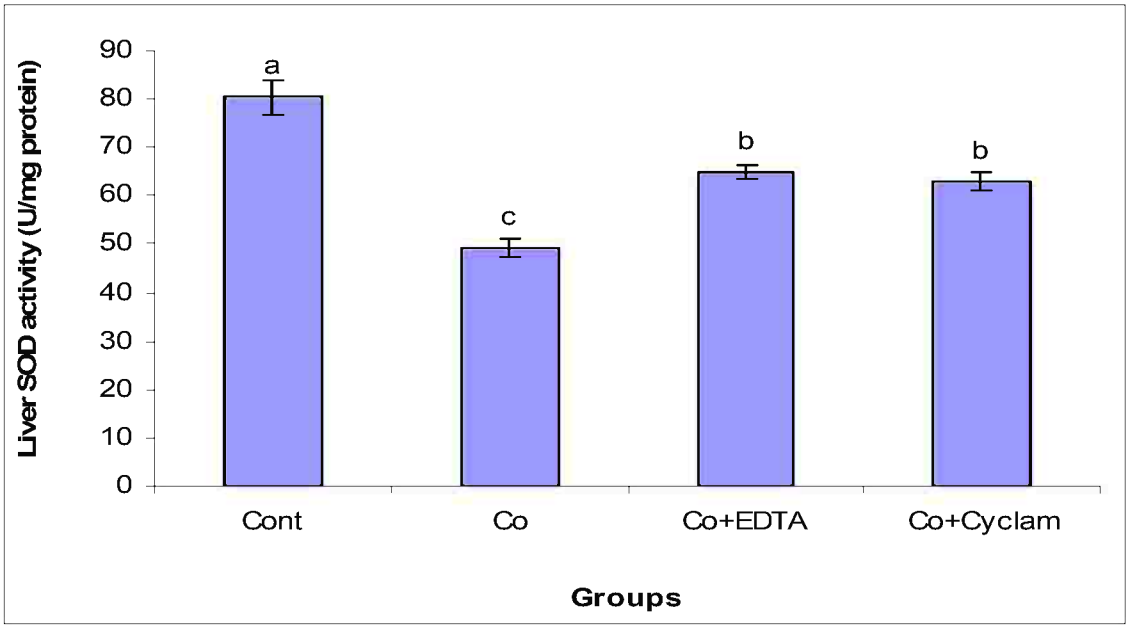

Fig.26. Changes in superoxide dismutase activity (SOD) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

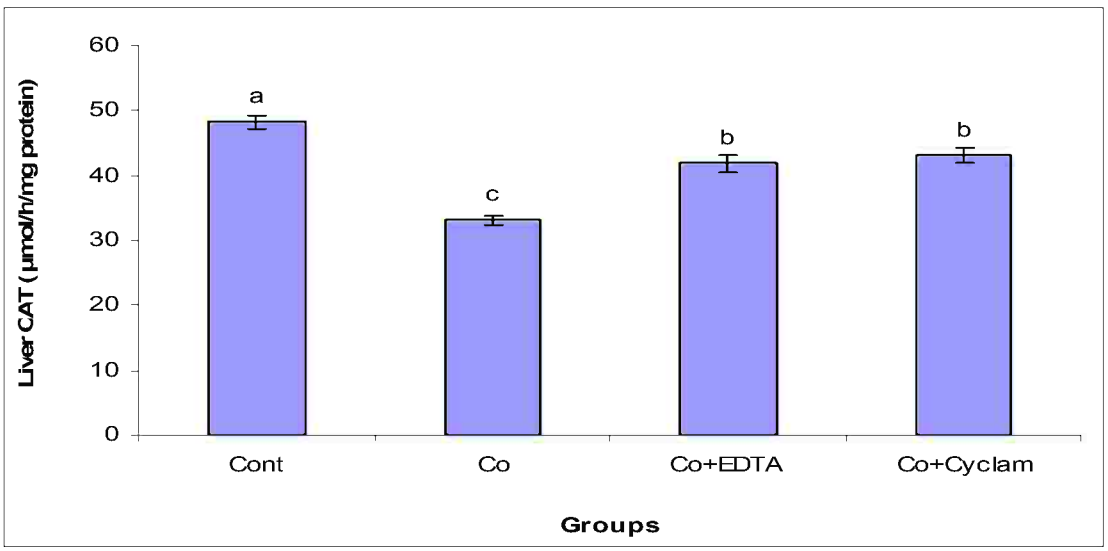

Fig.27. Changes in Catalase activity (CAT) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

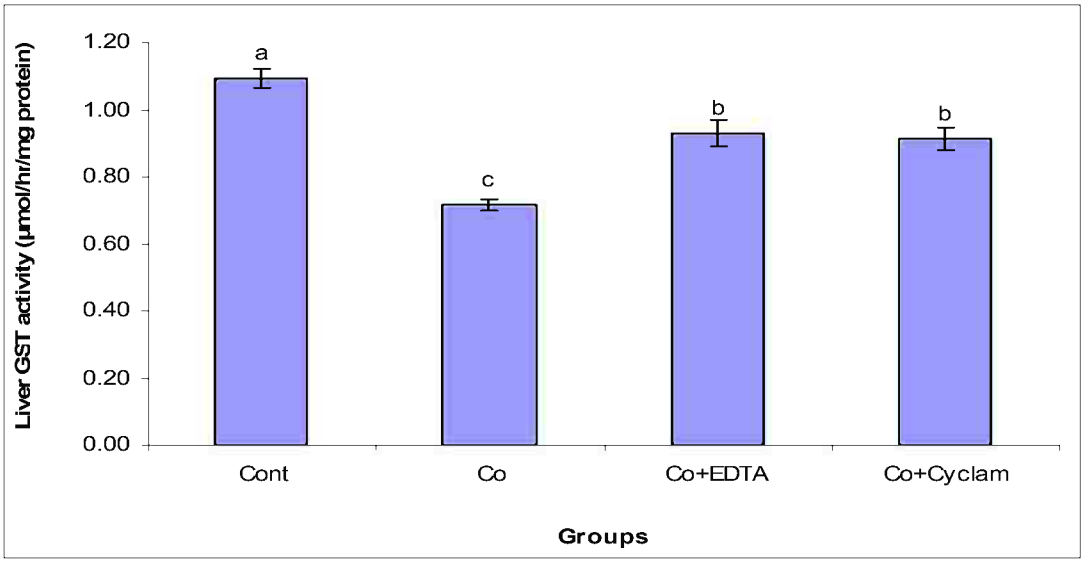

Fig.28. Changes in Glutathione S- Transferase activity(GST) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.2.4. Effects of cobalt chloride, ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane on biochemical changes in rat liver

For the determination of liver damage induced by Cobalt Chloride and the protective effect of EDTA and Cyclam, the activities of AST, ALT AlP and LDH and protein content of liver, and plasma total protein and albumin were used as hepatotoxic biomarkers. Following treatment period of Cobalt Chloride administration, changes of enzyme activities indicating the occurrence of hepatic injury were observed(**Table 9** and **Figures (29-33**) ALT and AST activities decreased significantly in rat liver treated with Cobalt Chloride as compared to control group ($P < 0.05$) also total protein content were significantly decreased. Nevertheless all measured parameters showed a significant increase with (Co+EDTA) and (Co+Cyclam) as compared with Cobalt Chloride (Co) treated group.

Table 9

Aspartate aminotransferase(AST), Alanine aminotransferase(ALT), alkaline phosphatase(ALP), Lactate dehydrogenase(LDH), protein content in liver of male rats treated with cobalt chloride (Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
AST(U/mg protein)	119±3.16[a]	82±2.55[c]	103±3.8[b]	102±1.91[b]
ALT(U/mg protein)	165±4.53[a]	113±2.44[c]	140±2.11[b]	142±4.84[b]
ALP(U/mg protein)	360±14.03[a]	224±6.03[c]	319±11.03[b]	319±9.46[b]
LDH(U/mg protein)	1197±46[c]	1652±39[a]	1342±59[b]	1340±39[b]
Protein content (mg protein/g tissue)	196±3.35[a]	141±2.08[c]	171±5.72[b]	169±3.74[b]

Values are expressed as means ±SE; n=7 for each treatment group.

[abc] Mean values within a row not sharing a common superscript letters were significantly different, P<0.05.

In the present study, rats treated with Cobalt Chloride showed perturbations in ALT and AST activities as compared to control (**Table 9**). These enzymes are secreted into the blood after hepatocellular injury. Lipid peroxidation is known to disturb the integrity of cellular membranes, leading to the leakage of cytoplasmic enzymes. Activities of many enzymes reported to be changed by xenobiotics in different tissue organs. This perturbations in liver enzyme may be due to liver damage or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases (El-Shenawy et al ., 2010, Kalender et al .,2010).

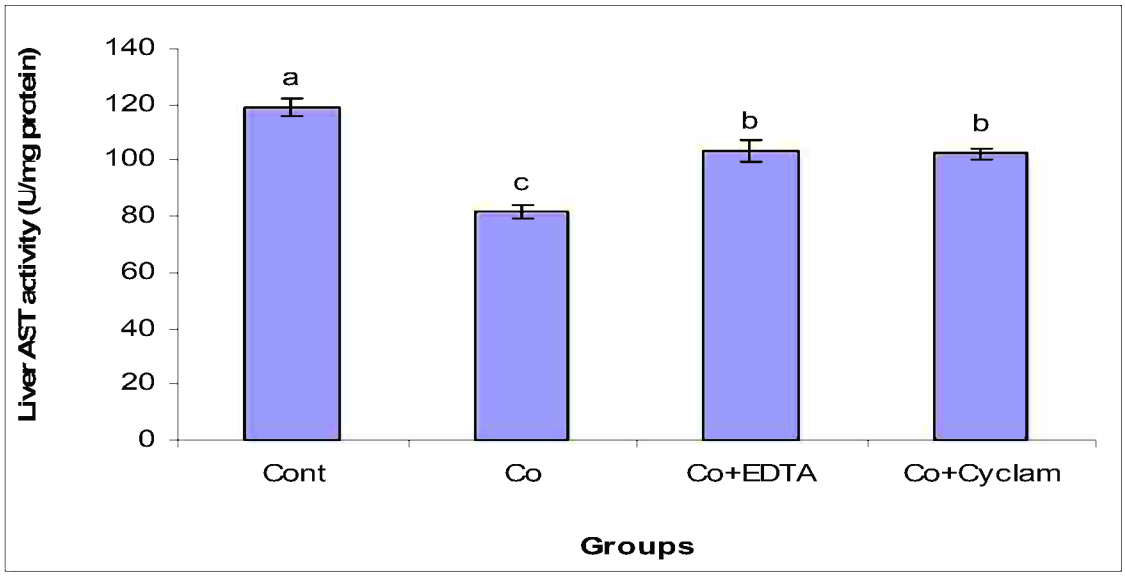

Fig.29. Changes in aspartate aminotransferase activity (AST) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

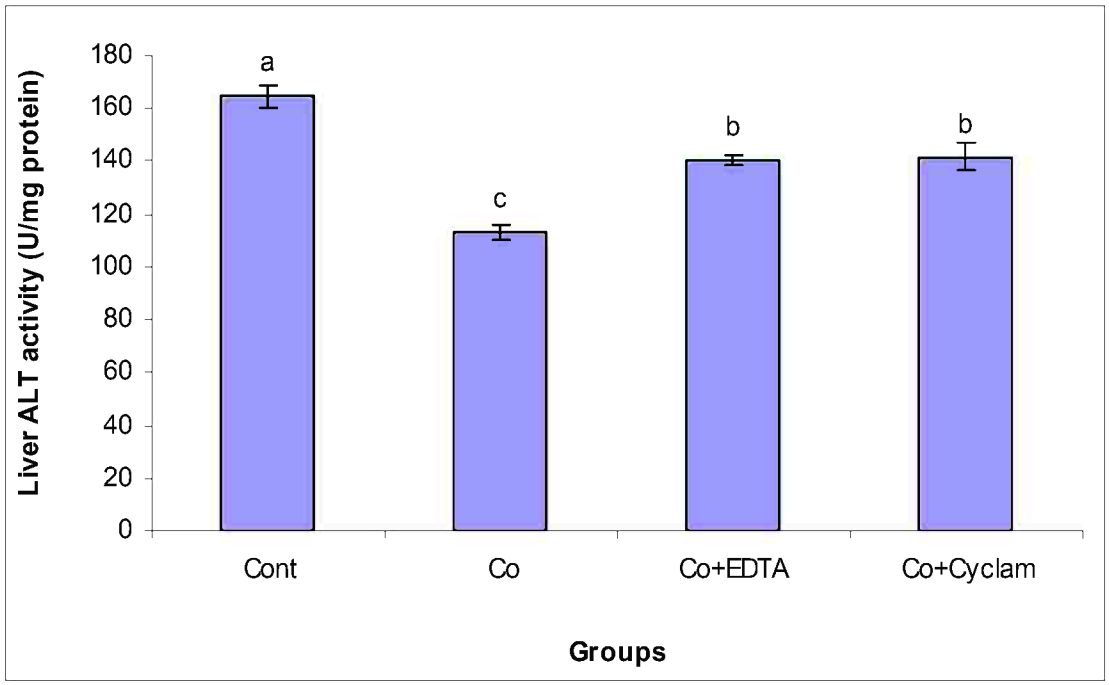

Fig.30. Changes in alanine aminotransferase activity (ALT) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

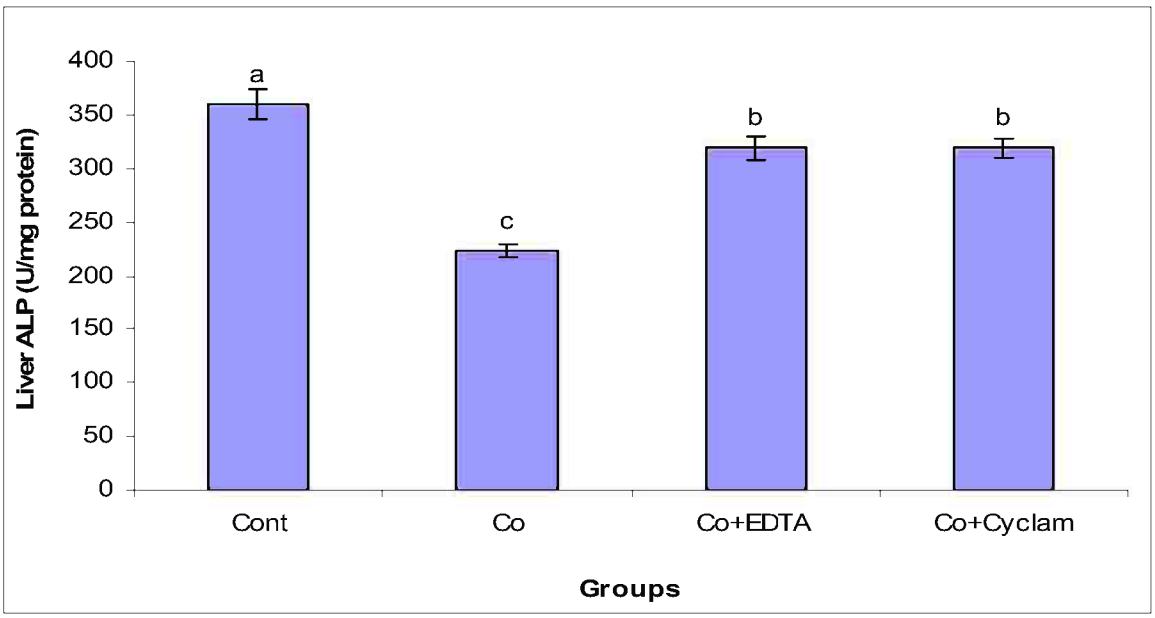

Fig.31. Changes in alkaline phosphatase activity(ALP) in liver of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

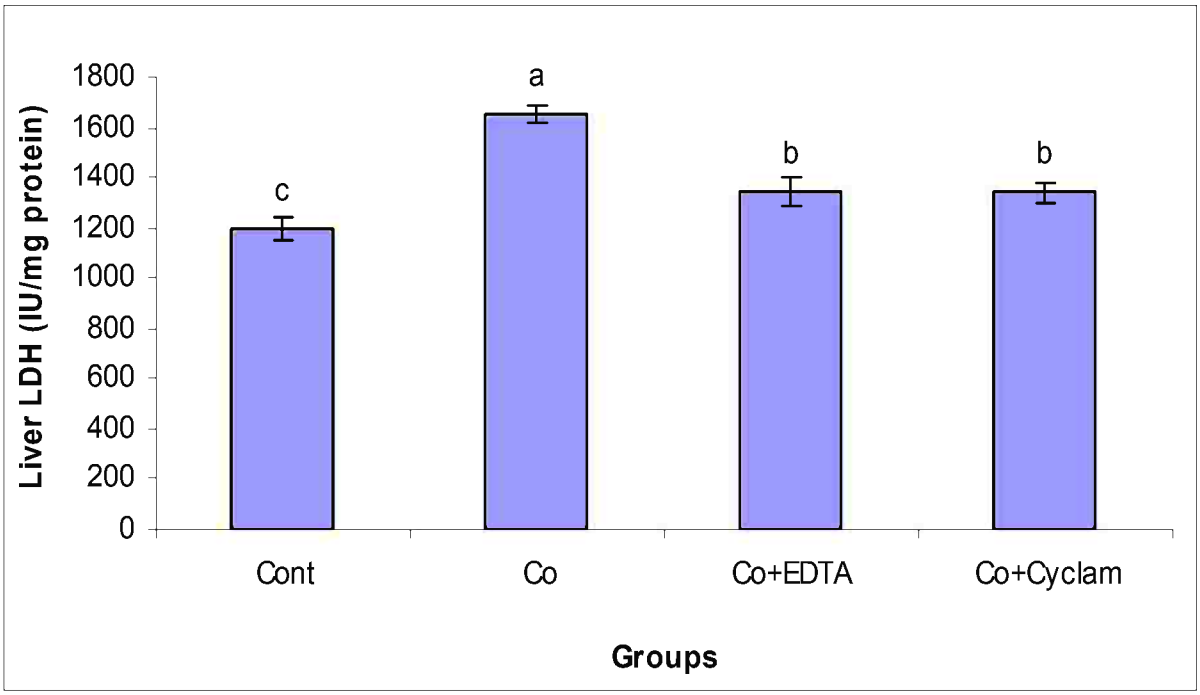

Fig.32. Changes in lactate dehydrogenase activity (LDH) in liver of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

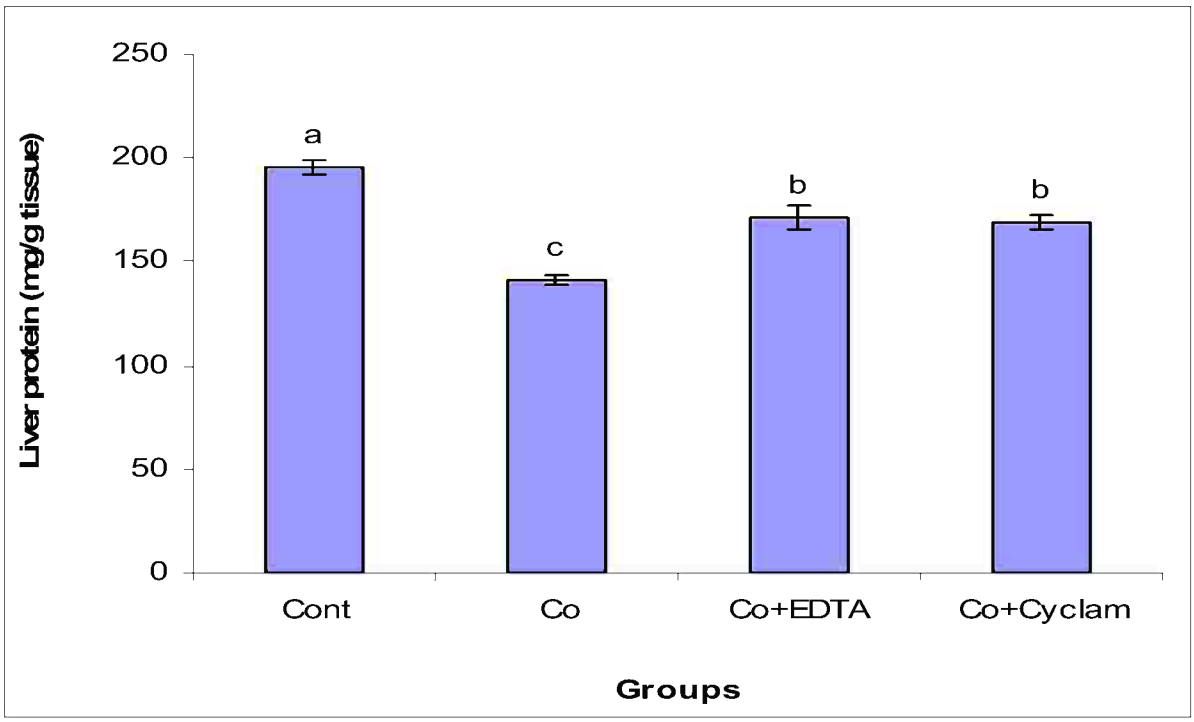

Fig.33. Changes in protein content in liver of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

5.3. Kidney

The kidney is a complex organ consisting of well-defined components that function in a highly coordinate fashion. A number of drugs, chemicals, heavy metals have been shown to alter its structure and function. The extent of renal damage by heavy metals depends upon the nature, the dose, route and duration of exposure. Both acute and chronic intoxication have been demonstrated to cause nephropathies with various levels of severity ranging from tubular dysfunction to acute renal failure (Barbier et al., 2005). cobalt toxicity leading to the generation of reactive oxygen species (ROS), which in turn cause lipid peroxidation and protein oxidation in several tissues including the kidney (Ahmed and Siddiqui, 2007).

5.3.1. Effects of cobalt chloride, ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane on kidney biomarkers

Data presented in table.10. Showed that rats treated with Co caused significant increase ($p < 0.05$) in plasma urea and creatinine while protein content in kidney was significantly decreased as compared with control. Nevertheless protein content showed a significant increase with Cobalt Chloride plus ethylene diamine tetraacetic acid (Co+EDTA) and Cobalt Chloride plus 1, 4, 8, 11-tetraazacyclotetradecane (Co+Cyclam) as compared with Cobalt Chloride treated group. It is known that the kidney is the main site of biotransformation and elimination of several metals and metalloids (Matos et al., 2009). Co^{2+} inhibit the incorporation of amino acid into protein causing an increase in urea and creatinine levels which are the major nitrogen-containing metabolic products of protein metabolism (Cuzzocrea et al., 2002; Atessahin et al., 2005). In the present study, the increase in plasma creatinine and urea levels and the decreased creatinine clearance reflect the diagnosis of renal failure after cobalt treatment and by others in humans and rats exposed to other pollutants (Donadio et al., 1997; Fetoui et al., 2010). 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. The elevation in plasma urea and creatinine levels in Cobalt treated rats are considered as a significant marker of renal dysfunction and 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 (Rudenco et al., 1998).In this context, the present study also confirmed that using EDTA and Cyclam reversed the kidney function against the toxic effect of cobalt.

Table 10

Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), protein content in kidney of male rats treated with cobalt chloride (Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
ALP(U/mg protein)	198±3.33^{a}	125±7.06^{c}	179±5.35^{b}	176±3.82^{b}
LDH(U/mg protein)	953±31^{b}	1297±44^{a}	1049±26^{b}	1067±48^{b}
Protein content (mg protein/g tissue)	67.92±2.00^{a}	46.68±2.00^{c}	56.04±1.22^{b}	60.36±2.45^{b}

Values are expressed as means ±SE; n=7 for each treatment group.

^{abc} Mean values within a row not sharing a common superscript letters were significantly different, P<0.05.

In the present study, we noticed impairment in glomerular filtration and renal damage in cobalt treated rats. Oxidative stress occurs when the redox environment is disrupted and this causes cellular death in a variety of circumstances (Schafer and Buettner, 2001; Valko et al., 2007). Cobalt exposure enhances intracellular ROS production and increases lipid peroxidation, which eventually leads to cell damage and leakage of lactate dehydrogenase. In fact, increased extracellular activity of LDH, which is an intracellular enzyme, is the result of disrupted cell membrane integrity, which occurs during the lipid peroxidation, under the influence of free radicals and in the conditions of oxidative stress (Zou et al., 2001). EDTA and Cyclam significantly decreased the levels of plasma urea and creatinine indicating that EDTA and Cyclam chelate the Co ion and prevented the renal functions from Co induced damage.

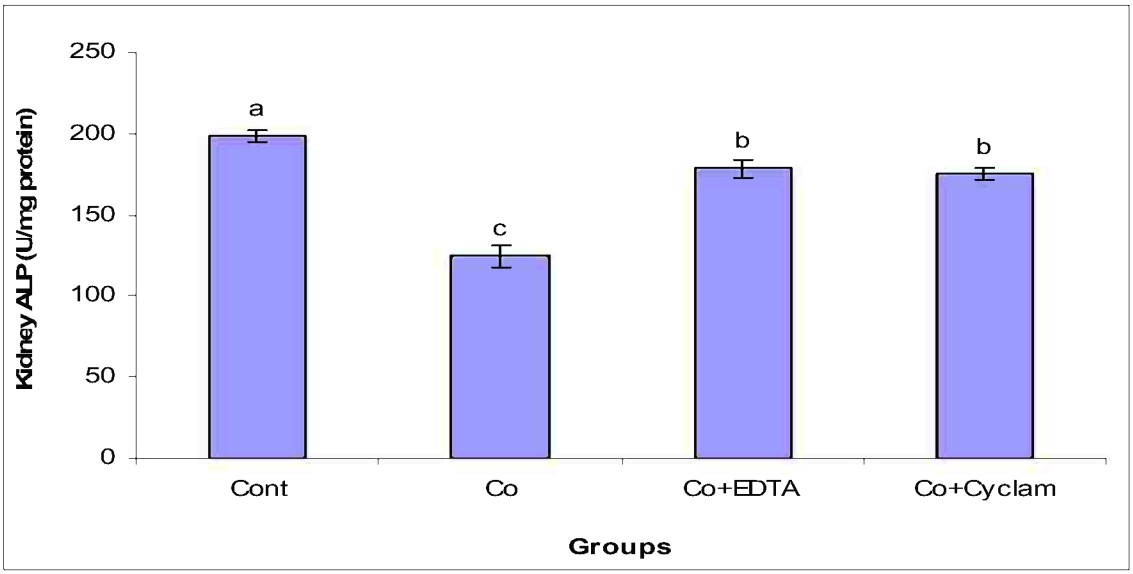

Fig.34. changes in alkaline phosphatase(ALP) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).^{abc} Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

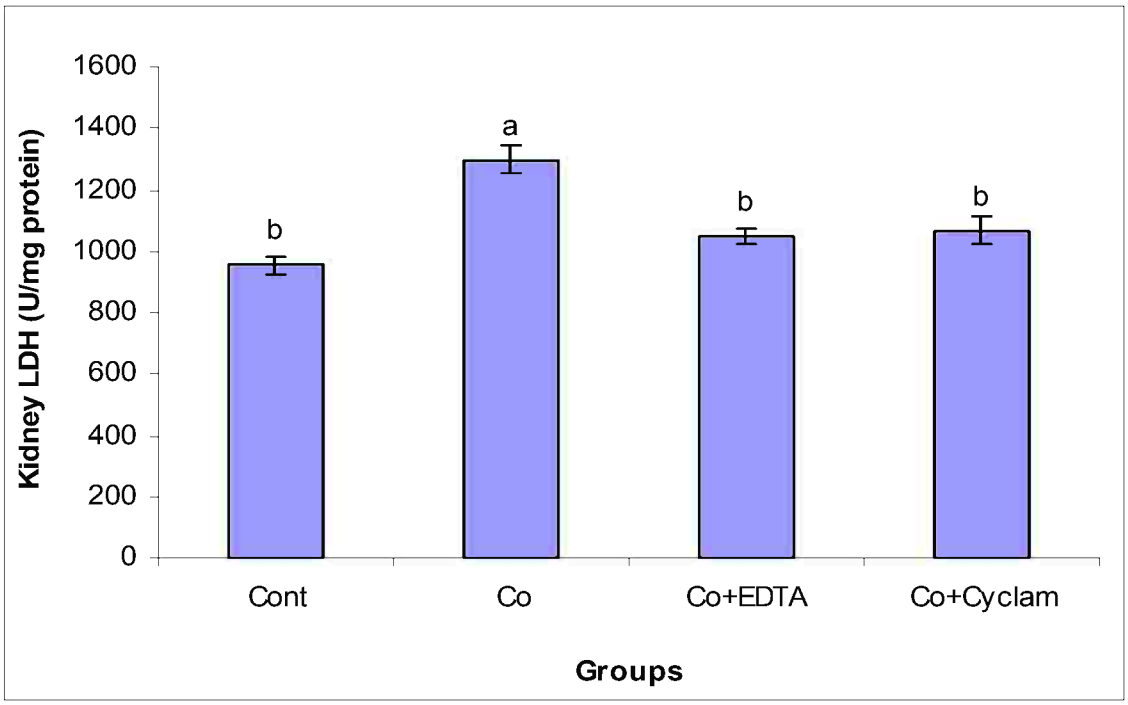

Fig.35. changes in lactate dehydrogenase activity (LDH) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

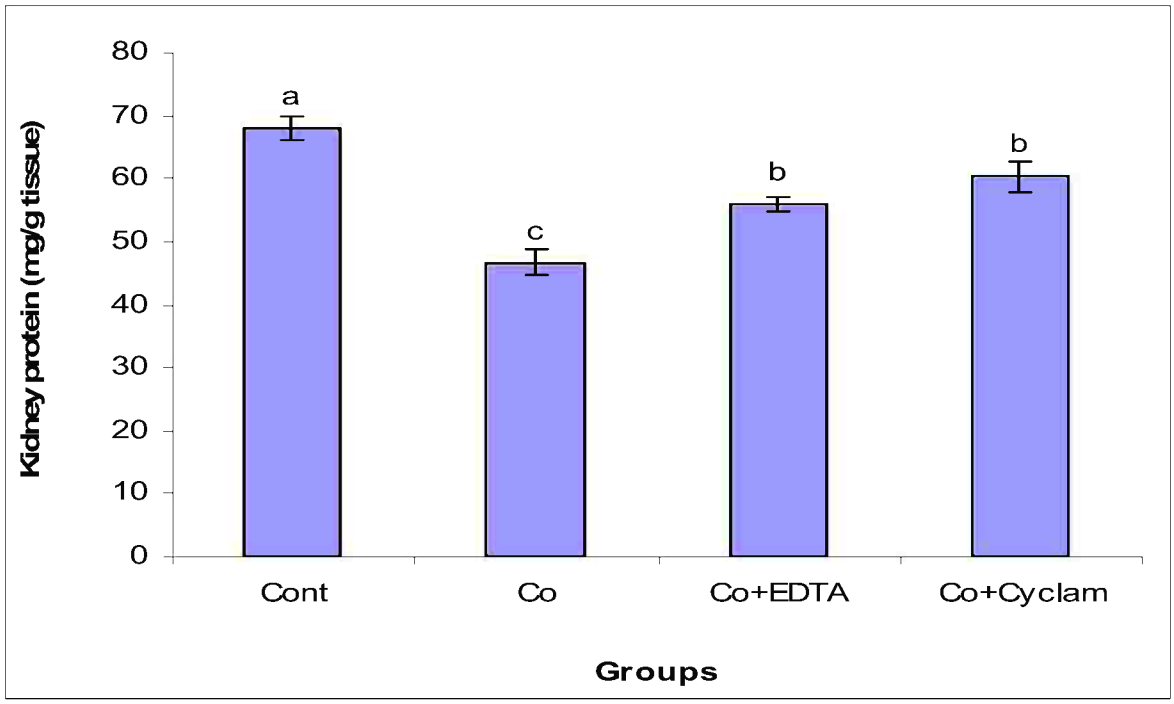

Fig.36. changes in protein content in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

Table11

Thiobarbituric acid reactive substances concentration(TBARS), glutathione content(GSH), superoxide dismutase(SOD), Catalase(CAT), Glutathione S-Transferase(GST) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).

Parameters	Control	Co	Co+EDTA	Co+Cyclam
TBARS(nmol/g tissue)	27.28 ± 0.87^{c}	37.40 ± 0.99^{a}	30.18 ± 0.48^{b}	30.38 ± 0.87^{b}
GSH(mmol/ mg protein)	2.43 ± 0.079^{a}	1.58 ± 0.044^{c}	2.08 ± 0.063^{b}	2.06 ± 0.049^{b}
SOD(U/mg protein)	73.59 ± 2.67^{a}	48.29 ± 1.66^{c}	60.17 ± 1.59^{b}	59.73 ± 2.48^{b}
CAT(U/ml)	56.76 ± 1.21^{a}	37.93 ± 1.34^{c}	48.99 ± 1.55^{b}	50.37 ± 1.55^{b}
GST(U mol/h/mg protein)	0.531 ± 0.016^{a}	0.345 ± 0.012^{c}	0.428 ± 0.013^{b}	0.439 ± 0.014^{b}

Values are expressed as means ±SE; n=7 for each treatment group.
^{Abc} Mean values within a row not sharing a common superscript letters were significantly different, $P<0.05$.

Xenobiotics and environmental pollutants such as heavy metals are known to induce a broad spectrum of toxicological effects and biochemical dysfunctions constituting serious hazards to health. Oxidative damage is considered a likely cause of tissue injuries through reactive oxygen species (ROS) generation which destroy proteins, lipids and DNA by oxidation (Halliwell and Gutteridge, 1989). ROS attack the cell membrane and lead to destabilization and disintegration of cell membrane as a result of lipid peroxidation (Stajn et al., 1997). The increased lipid peroxidation, and decreased GSH contents observed in our study after Cobalt exposure implicated the oxidative stress in Co-induced Nephrotoxicity. Glutathione and other thiol containing proteins play a crucial key role in cellular defense against xenobiotics toxicity (Halliwell and Gutteridge, 1989). The significant decrease of GSH content in Co-treated group could lead to increased susceptibility of the renal tissue to free radical damage. The scavenging property and the ability of EDTA and Cyclam to inhibit the radical generation could further reduce the oxidative threat caused by Cobalt Chloride.

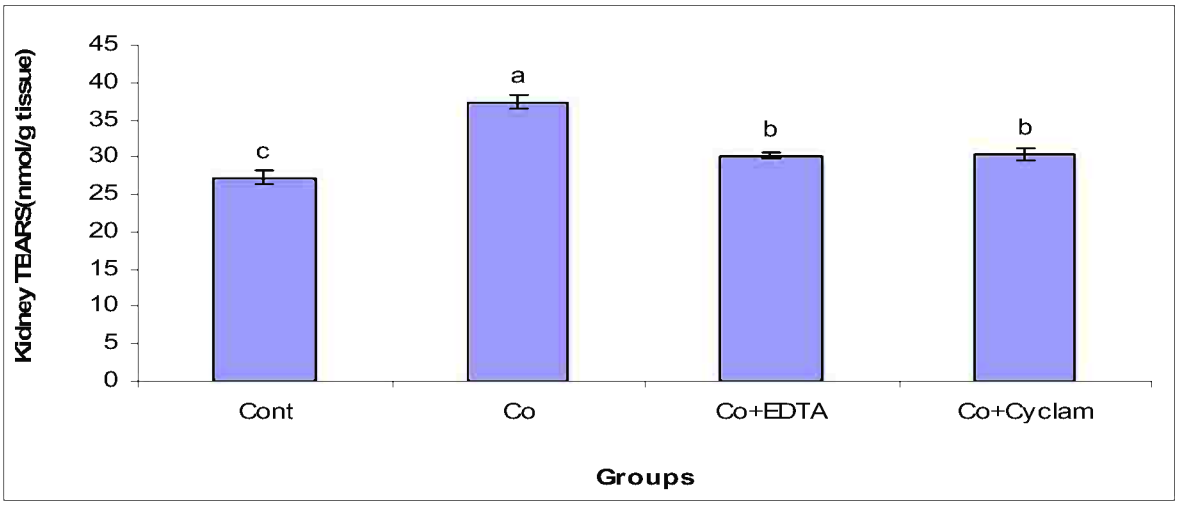

Fig.37. changes in thiobarbituric acid-reactive substances(TBARS) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

Oxidative stress caused by increased generation of reactive oxygen species (ROS) has been suggested to play the major role. EDTA and Cyclam can prevent the uncontrolled formation of free radicals and activated oxygen species or inhibit their reaction with biological structures. The destruction of most free radicals and activated oxygen species rely on the oxidation of endogenous antioxidant mainly scavenging and reducing molecules .in this context, the present study confirms that EDTA and Cyclam reversed the kidney function against the toxic effects of Cobalt. Lipid peroxidation is often discussed as a cause of metal induced toxicity (Ames et al., 1982). Lipids are constituents of biological membrane. Peroxidation of membrane lipids disrupts its structural organization. In addition, aldehydes produced from the decomposition of polyunsaturated fatty acids are capable of forming cross linking in proteins and nucleic acid which leads to inactivation of many cellular constituents, membrane and enzymes (Haberland et al., 1988). The present study documented a significant increase in the level of (TBARS) in Co toxicity is generally thought to be the consequence of increased lipid peroxidation, which may be due to increased free radical generation induced by Co. In addition to cellular lipids, studies have shown that cellular proteins may also be affected by free radical accumulation. The formation of carbonyl derivatives of proteins is suggested to be a useful measure of oxidative damage to proteins (Sundari et al., 1997).EDTA and Cyclam decreased the level of TBARS which may be resulting from the scavenging of free radicals. It clearly demonstrated the ability of EDTA and Cyclam to directly interact with reactive oxygen species that may initiate lipid peroxidation and its potentiality in reducing Cobalt accumulation in kidney. GSH is a low molecular weight tripeptide. It forms the first line of defense against oxidative insult by acting as nonenzymic antioxidants by direct interaction of its sulfhydryl groups of GSH leading to its inactivation which plays an important role in cellular defense against oxidative stress by reducing protein disulfides and other cellular molecule (Rahman et al., 2004). In the present study, because of the chelating effect of EDTA and Cyclam ,it decreased oxidative stress and maintained the levels of non-enzymic antioxidant in Co treated rats and decreased their toxic accumulation in tissue.

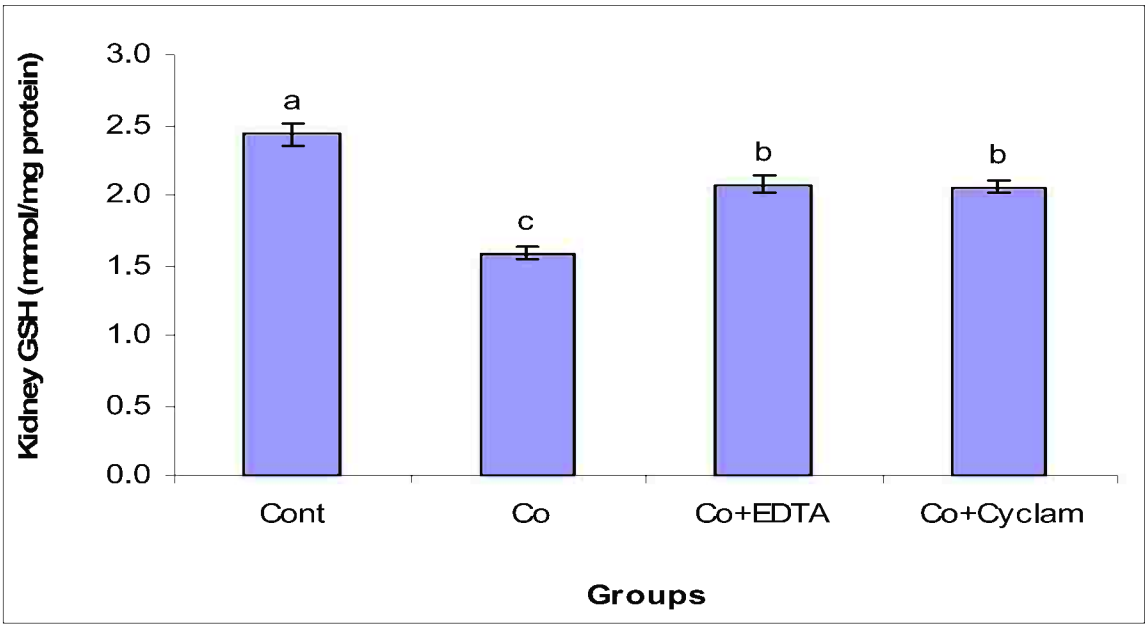

Fig.38. changes in reduced glutathione content (GSH) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant (P <0.05) difference between groups. Values are mean ± SE of seven rats in each group.

5.3.3. Effects of cobalt chloride, ethylene diamine tetraacetic acid and the 1, 4, 8, 11-tetraazacyclotetradecane on kidney Antioxidant enzymes

Data concerning kidney antioxidant enzyme activities (SOD, CAT, and GST) are presented in **Table 11** A significant (P < 0.05) reduction in the antioxidant enzymes activity was observed in Co treated rats as compared to control. However, rats treated with (Co+EDTA) and (Co+Cyclam), antioxidant enzymes activity showed a significant recovery as compared to Co treated group.

The decreased activities of antioxidant enzymes (SOD, CAT and GST) observed in the renal tissue indicated the failure of antioxidant defense system to overcome the influx of ROS induced by Co exposure. ROS generated in tissues are normally scavenged by enzymatic and non-enzymatic antioxidants (Sener et al., 2005). In fact, GST and CAT which act as preventive antioxidants and SOD, a chain-breaking antioxidant, play an important role in protection against the deleterious effect of lipid peroxidation (Ray and Husain, 2002). Depletion in the activities of SOD, CAT and GST in kidney of Co-treated rats may be due to the decreased synthesis of enzymes or oxidative inactivation of enzyme protein and generation of various free radicals like hydroxyl radical, superoxide radical and nitrogen species. Administration of (Co+EDTA) and (Co+Cyclam) increased antioxidant enzyme activities in Co-treated rats. The chelation property of EDTA and Cyclam to react with free radicals or with highly reactive byproducts of lipid peroxidation as well as enhancement of tissue thiol pools might be responsible for the reduction of oxidative modification for enzymes and are versal of the activities of antioxidants and glutathione metabolizing enzymes.

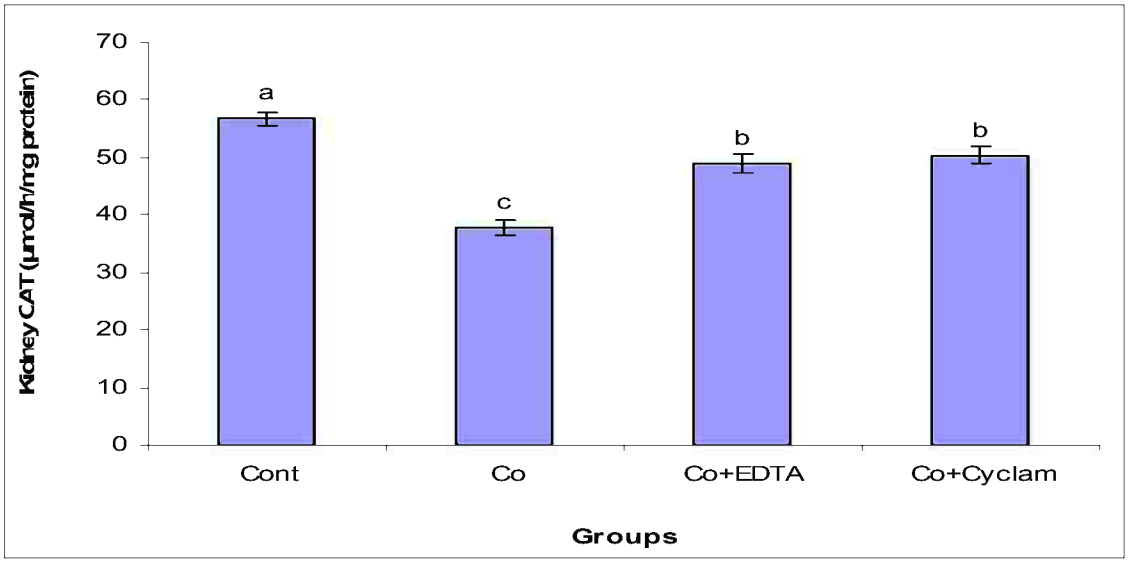

Fig.39. Changes in Catalase activity (CAT) in kidney of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

Superoxide dismutase, catalase and glutathione peroxidase constitute mutually a supportive team of defense against reactive oxygen species. $CoCl_2$ induce toxicity might result in significantly decreased activities of antioxidant enzymes which include superoxide dismutase, catalase and glutathione peroxidase. Such decreases in enzymes activity can result from either inhibition of protein synthesis or impairment of enzymatic activity. Administration of EDTA and Cyclam increased antioxidant enzyme activities in Co-treated rats which might be due to its ability to reduce the accumulation of free radical generation.

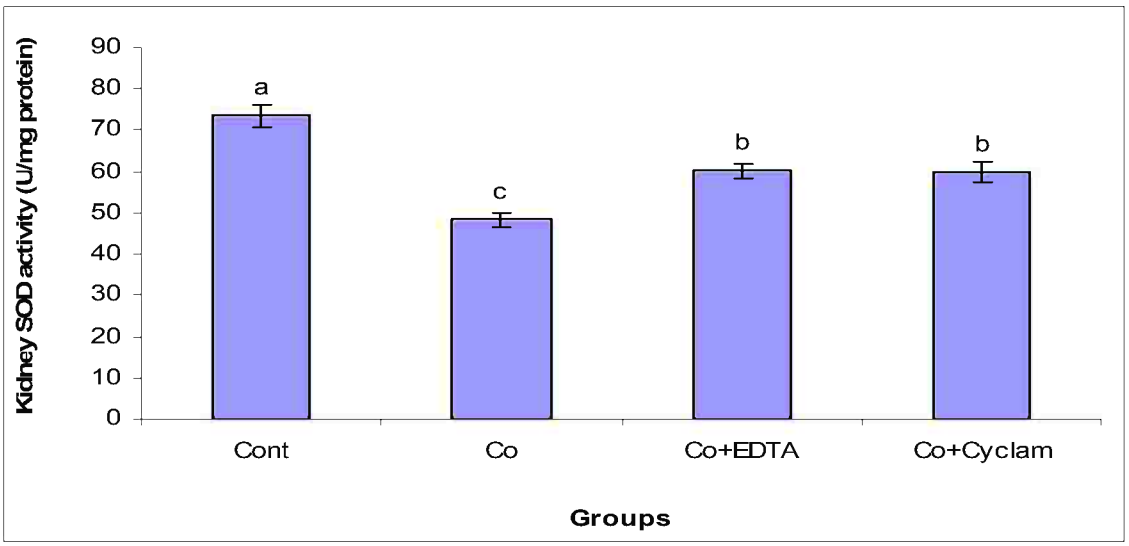

Fig.40. Changes in superoxide dismutase activity (SOD) in kidney of male rats treated with Cobalt Chloride (Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

Glutathione-S-transferase activity was found to be decreased in Co treated rats. This might be due to many SH groups which form complex with the metal and might lead to a decrease in the activities of GST. Administration of EDTA and Cyclam chelation therapy in Co treated rats showed significantly increase in the activity of GST to near normal, due to the chelation property of EDTA and Cyclam which block the reactive sites of the trace metal ions thus inhabiting their normal action so they enhance the elimination of Co from the renal tissue. In addition to that, the capability of EDTA and Cyclam to react with free radicals or with highly reactive byproducts of lipid peroxidation as well as enhancement of tissue thiol pools may be responsible for the reduction of oxidative modification of enzymes and prevented the activities of antioxidant and glutathione metabolizing enzymes in Co treated rats.

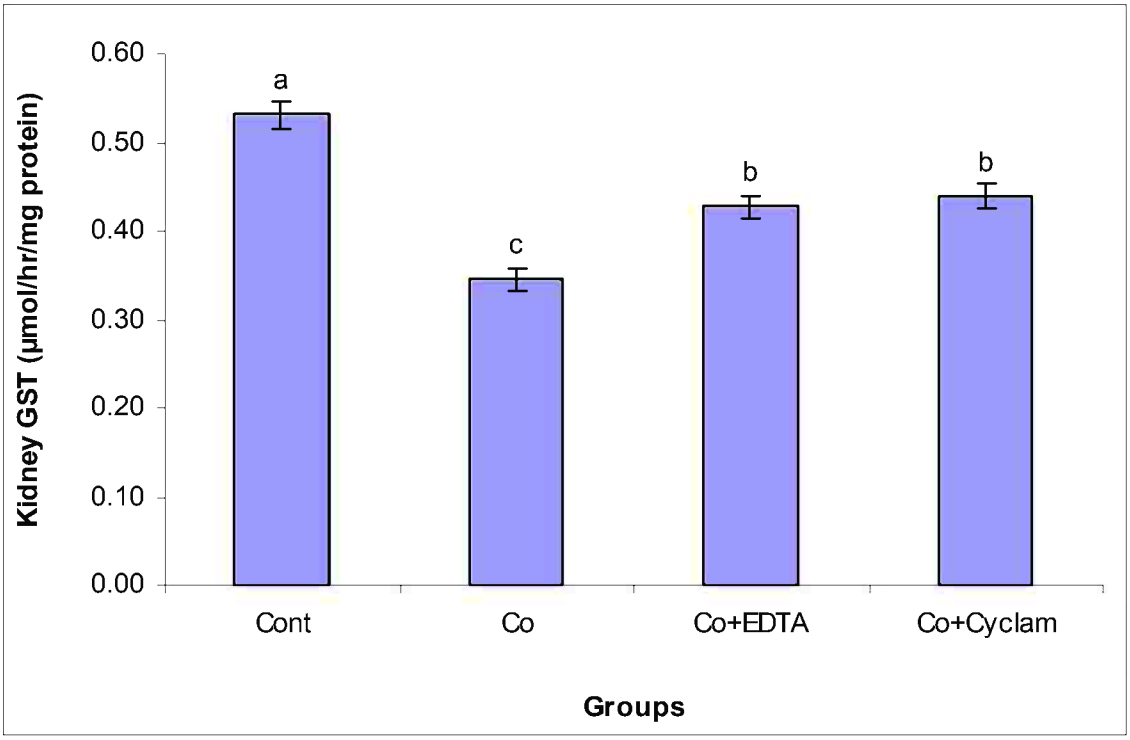

Fig.41. Changes in Glutathione S-Transferase (GST) in kidney of male rats treated with Cobalt Chloride(Co), (Co+EDTA) and (Co+Cyclam).[abc] Different letters indicate significant ($P < 0.05$) difference between groups. Values are mean ± SE of seven rats in each group.

6. SUMMARY AND CONCLUSION

The present study was carried out in the Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University. The aim of this study was to determine the effect of Cobalt chloride ($CoCl_2$) on biochemical characteristics, the levels of free radicals (TBARS) and enzyme activities of male Rats and the role of ethylenediamine tetraacetic acid(EDTA) and The 1, 4, 8, 11-tetraazacyclotetradecane (cyclam) in alleviating its toxic effects. Twenty eight male Rats (each weighing 107-143g) were used .animals were divided into four groups of 7 Rats each. The first group was used as control, the second group was used to study the effect of cobalt chloride (35mg/kg BW/LD50), the third group was used to study the effect of ethylenediamine tetraacetic acid EDTA (55mg/kg BW/24hours) and cobalt chloride ($CoCl_2$), while the fourth group was used to study the effect of The 1, 4, 8, 11-tetraazacyclotetradecane cyclam (30 mg/kg BW/24hours) and cobalt chloride(($CoCl_2$).the tested doses of $CoCl_2$,EDTA and cyclam were administered inter-peritoneally for 24hours. At the end of the experimental period all animals were killed and blood samples and different organs were collected and the tested parameters were carried out.

The results obtained can be summarized as follows:

1- Treatment with $CoCl_2$ increased liver and kidney thiobarbituric acid reactive substances and decreased the activities of superoxide dismutase, catalase and glutathione S-Transferase, and reduced glutathione content of liver and kidney. On the other hand, Rats treated with both $CoCl_2$ and EDTA alleviated the harmful effect of $CoCl_2$ because the results show significant decrease in TBARS levels and increase in the activities of antioxidant enzymes in liver and kidney as compared to the $CoCl_2$ group. Moreover, Rats treated with both $CoCl_2$ and cyclam also showed significant alleviation as compared to the $CoCl_2$ group.

2- Protein content, were decreased in Rats treated with $CoCl_2$ .this decrease may be due to excessive loss through nephrosis and may be due to reduced protein synthesis. Also, treatment with $CoCl_2$ caused significant increase in urea and creatinine. The increase in urea and creatinine concentration is considered as significant markers of renal dysfunction. On the other hand, Rats treated with both $CoCl_2$ and EDTA alleviated the harmful effect of $CoCl_2$ because the results show significant decrease in urea and creatinine levels. Moreover, rats treated with both $CoCl_2$ and cyclam also showed significant alleviation for the toxic effect of $CoCl_2$.

3- Results indicated that treatment with $CoCl_2$ caused significant decrease in plasma total protein and Albumin .in addition EDTA and Cyclam alleviated the harmful effect of $CoCl_2$ on both parameters.

4- Treatment with $CoCl_2$ caused significant decrease in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rat liver homogenates. Treatment With Cobalt Chloride in combination with EDTA and Cyclam showed protective effect against $CoCl_2$.

In conclusion, it is clear that $CoCl_2$ pronounced hazardous effects on liver and kidney biomarkers in addition to the antioxidant defense system. Estimation of lipid peroxidation,

enzymatic, non- enzymatic antioxidants in addition to biochemical parameters could be used as biomarkers for the harmful effect of $CoCl_2$ exposure. Using EDTA and Cyclam in combination with $CoCl_2$ minimized and alleviated its hazardous effects on most of the tested parameters and this may be attributed to vital role of EDTA and Cyclam as chelation therapy.

Finally, it can be recommended that:

Care must be taken into account to avoid exposure to Cobalt Also, chelation therapy with EDTA and Cyclam can be used to remove the toxic effect of cobalt.