

## **5. *In Vivo* Studies on Natural and Synthetic Antioxidants**

The *in vivo* antiradical activity of selected compounds was studied by CCl<sub>4</sub> induced hepatotoxicity in a rat model. Carbon tetrachloride (CCl<sub>4</sub>) is a toxic molecule and it is used to produce liver damage. It has been suggested through a series of experimental studies that free radical species are involved in CCl<sub>4</sub> induced hepatic injury. The mechanism studied so far explains that CCl<sub>4</sub> is converted into trichloromethyl free radical ( $\cdot\text{CCl}_3$ ) by the NADPH-cytochrome P-450 system which involves the transfer of an electron from NADPH to carbon tetrachloride (Zhu and Fung, 2000). The resulting  $\cdot\text{CCl}_3$  radical may react with oxygen to form  $\text{CCl}_3\text{OO}\cdot$  (trichloromethyl peroxy radical) (Connort *et al.*, 1986). Both the free radicals,  $\text{CCl}_3\text{OO}\cdot$  and  $\cdot\text{CCl}_3$  are reactive free radicals and they may attack cellular membranes which finally results in the rupture of the structure of bio-membranes, resulting in large quantities of cytosolic enzymes released into the blood stream.

On the basis of mechanism of action of CCl<sub>4</sub>, a number of compounds have been studied for their role as anti-hepatotoxic agents. Natural antiradical compounds 2, 11, 8, 9, 23, 27, 28, 33 and 34, and synthetic antiradical compounds 45, 49, 51-56, 58, 60, 61, 63, 66, 68, 69, 86, 87, 97 and 106, were earlier evaluated as antioxidants by scavenging DPPH radicals or superoxide anions, were selected for *in vivo* study. These compounds belong to different classes and showed lower IC<sub>50</sub> values against DPPH radicals.

### **5.1. Effect of Saline and CCl<sub>4</sub> on Transaminases and Bilirubins**

The normal control received only saline solution whereas the pathological control was given an i.p. dose of CCl<sub>4</sub> (20 % in edible oil, 1 mL/100 gm body weight). The

effect of saline solution and CCl<sub>4</sub> was monitored by the estimation of serum aspartate aminotransferase (AST)/ glutamic oxaloacetic transaminase (GOT), alanine aminotransferase (ALT)/ glutamic pyruvic transaminase (GPT) and bilirubins. These are the bio-chemical parameters used for the assessment of hepatic injury by the action of free radicals generated by CCl<sub>4</sub>. There is a significant difference in the levels of hepatic transaminases and bilirubins between the pathological and normal control group, which is due to CCl<sub>4</sub> induced damage. A significant increase in the level of serum bilirubins and AST and ALT (dose of CCl<sub>4</sub>; 20 % in edible oil, 1 mL/100 gm body weight) was observed in case of pathological control.

The effects of natural and synthetic compounds were studied by pretreatment of the animals with a dose of 10 mg/kg body weight. The results of the activities by different compounds were compared with the pathological control group. The levels of AST, ALT and total, direct and indirect bilirubins in serum affected by the action of CCl<sub>4</sub> and tested compounds are summarized in Table B (Page: 194-195).

## **5.2. Effect of Natural Compounds on Transaminases and Bilirubins**

The effect of nine natural products (compounds 2, 11, 8, 9, 23, 27, 28, 33 and 34) on sAST, sALT and total, direct, and indirect bilirubins in blood samples of experimental animals, along with pathological control, and normal control is presented in Figures 5.1-5.5.

### **5.2.1. Triterpenoid and Cinnamic Acid Derivative**

A triterpenoid (compound 2), isolated from *Tamarix hispida*, and a cinnamic acid derivative (compound 11), isolated from *Lindelofia stylosa*, were administered to the

animals at a dose of 10 mg/kg weight after half an hour of CCl<sub>4</sub>-induction. Compounds 2 and 11 were found to possess significant hepatoprotective effect as apparent by the significant decrease in the levels of transaminases, sAST, sALT, and bilirubins as compared to pathological control. The *o*-dihydroxyl group is the common functionality in the structures of compounds 2 and 11. The hydroxyl groups play an important role in radical scavenging capacity. The presence of an *o*-dihydroxyl group in compounds 2 and 11 may therefore contribute to minimize the hepatotoxic effect of CCl<sub>4</sub>.

### 5.2.2. Cinnamic Acid Esters and Aurones

Cinnamic acid derivatives (compounds 8 and 9) and aurone derivatives (compounds 33 and 34) isolated from *Spatoglossum variable*, were administered to the animals with a dose of 10 mg/kg weight after half an hour of CCl<sub>4</sub>-induction and their effect on transaminases and bilirubins levels was observed. Among them compound 8 was found to possess hepatoprotective effect as reflected from various biochemical parameters which were comparable to the normal control. A different effect was observed on sAST levels in the case of compound 9 which were comparable to pathological control, whereas the sALT and bilirubins levels were comparable to propyl gallate, a standard antioxidant. Aurone derivatives (compounds 33 and 34) also exhibited significant hepatoprotective effect as evident from the lower values of sAST, sALT and bilirubins levels as compared to pathological control.

### 5.2.3. Coumarins

An isocoumarin 23, isolated from the brown algae *Spatoglossum variable*, and two furocoumarins 27 and 28, isolated from *Apium graveolens*, were administered to the

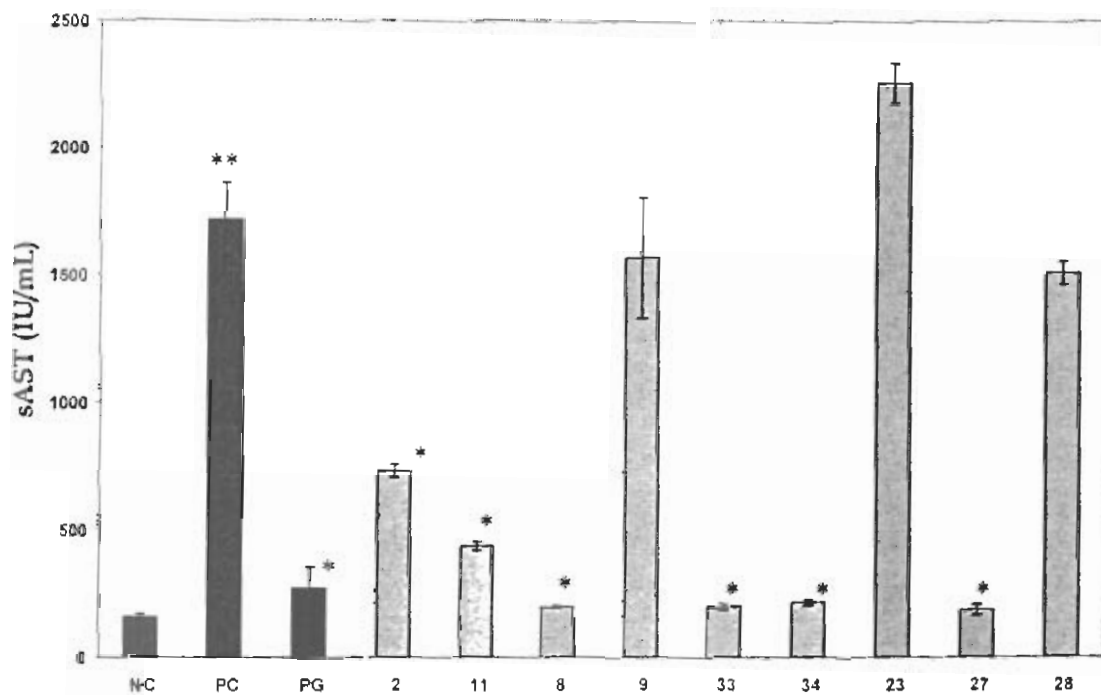
experimental animals at a dose of 10 mg/kg weight after half an hour of CCl<sub>4</sub>-induction, and their effects on transaminases and bilirubins were observed. The compounds 23, 27 and 28 showed different effects on the levels of transaminases, sAST, sALT and bilirubins as compared to the normal control. In the case of compounds 23 and 28, the sAST and sALT levels were either comparable to pathological control or even higher. However, the effect of compounds 23 and 28 on total and direct bilirubins was comparable to that of propyl gallate, while the values of indirect bilirubin were comparable to that of the normal control. In the case of compound 27, the decreased values of sAST and sALT and bilirubins were observed as compared to pathological control, while no significant decrease in bilirubins level was observed.

### **5.3. Effect of Synthetic Compounds on Transaminases and Bilirubins**

Nineteen synthetic compounds (45, 49, 51-56, 58, 60, 61, 63, 66, 68, 69, 86, 87, 97 and 102), belonging to four different classes, were studied for their hepatoprotective effects against the hepatotoxic action of CCl<sub>4</sub>. The effect of these compounds on transaminases and bilirubin levels in blood samples of experimental animals is presented in Figures 5.6-5.20.

#### **5.3.1. $\beta$ -N-Substituted Hydrazide Derivatives**

The eight  $\beta$ -N-substituted hydrazide derivatives (compounds 45, 49, 51-56 and 58) were selected for *in vivo* study. All  $\beta$ -N-substituted hydrazide derivatives were administered to experimental animals at a dose of 10 mg/kg weight and their effects on transaminases and bilirubin in blood samples of animals was observed.



**Figure 5.1:** Effect of natural products (compounds 2, 11, 8, 9, 23, 27, 28, 33, and 34) and standard antioxidant, propyl gallate (PG) on sAST levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). sAST values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control vs normal control, \* $P < 0.05$ : pathological control vs test sample ANOVA (Analysis of variance).

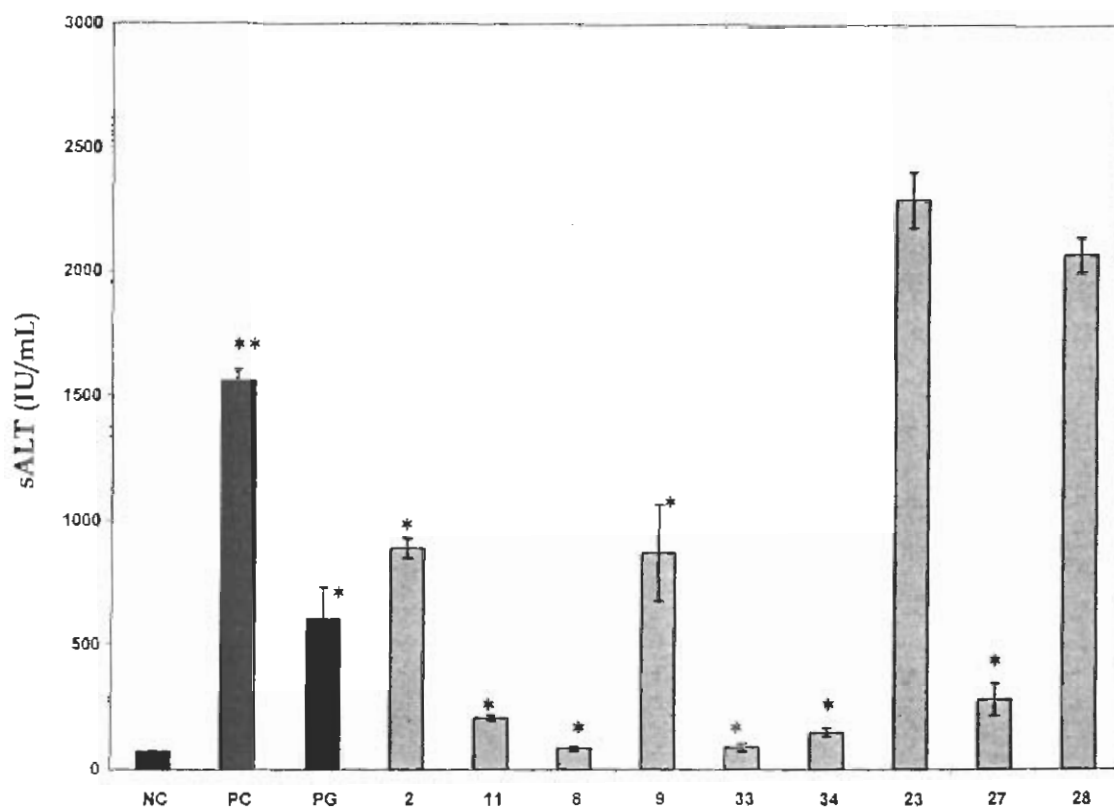


Figure 5.2: Effect of natural products (compounds 2, 11, 8, 9, 33, 34, 23, 27 and 28) and standard antioxidant, propyl gallate (PG) on sALT levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). sALT values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).

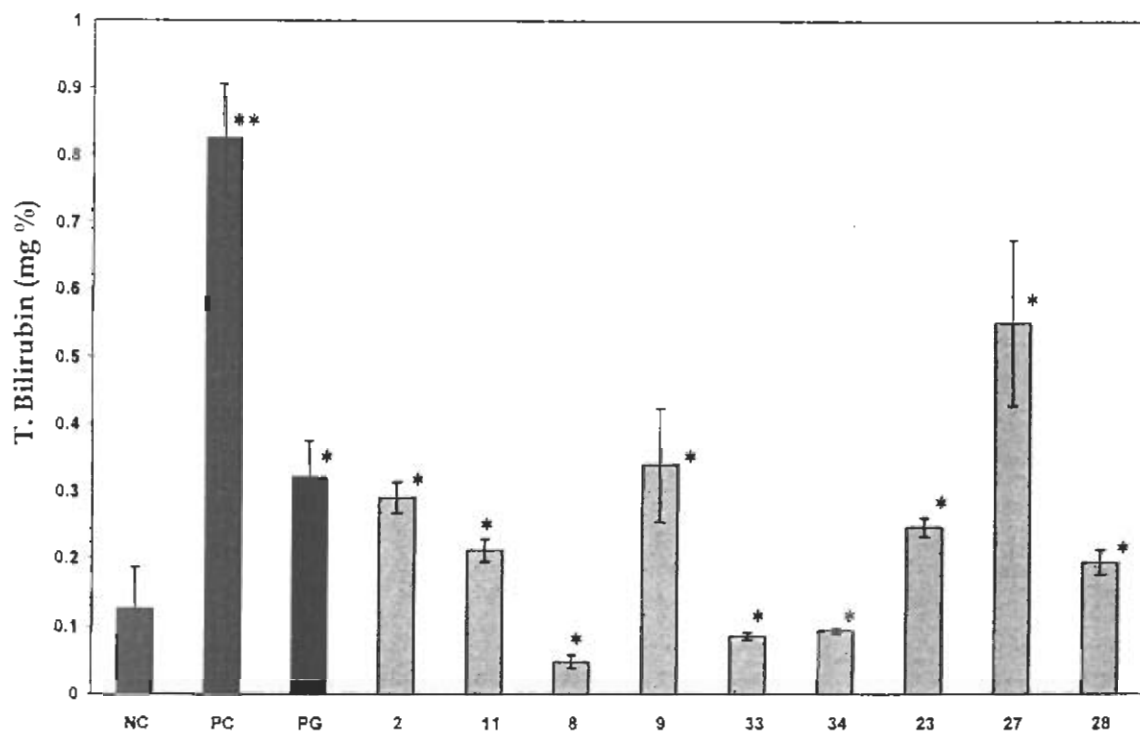


Figure 5.3: Effect of natural products (compounds 2, 11, 8, 9, 33, 34, 23, 27 and 28) and standard antioxidant, propyl gallate (PG) on serum Total bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Total bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).

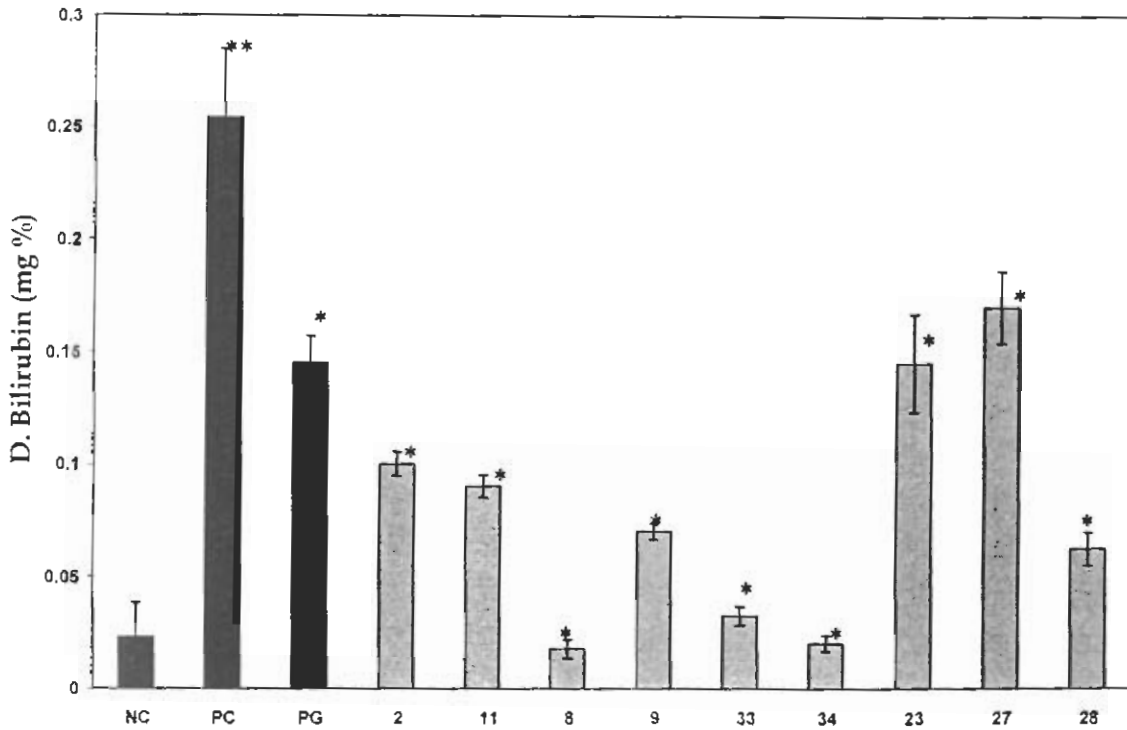


Figure 5.4: Effect of natural products (compounds 2, 11, 8, 9, 33, 34, 23, 27 and 28) and standard antioxidant, propyl gallate (PG) on serum Direct bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Direct bilirubin values are the  $\pm$  SEM of six rats. \*\*\**P* < 0.05: pathological control *vs* normal control, \**P* < 0.05: pathological control *vs* test sample ANOVA (Analysis of variance).

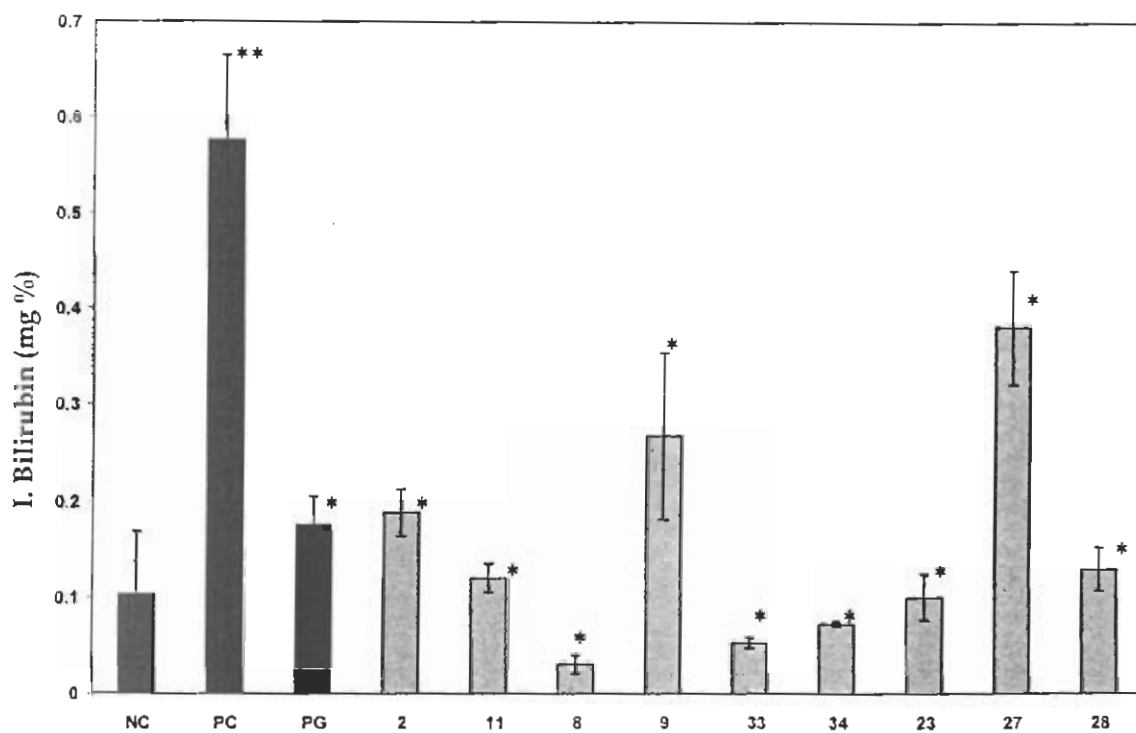


Figure 5.5: Effect of natural products (compounds 2, 11, 8, 9, 33, 34, 23, 27 and 28) and standard antioxidant, propyl gallate (PG) on serum Indirect bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100g body weight). Indirect bilirubin values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control *vs* normal control, \*P < 0.05: pathological control *vs* test sample ANOVA (Analysis of variance).

The effect of all tested hydrazide derivatives on hepatic enzymes, sAST, sALT and bilirubin levels in the serum of tested animals is presented in Figures 5.6-5.10. The pretreatment of the experimental animals with substituted acyl hydrazides significantly minimize the effect of CCl<sub>4</sub>-induced toxicity. Among all the acyl hydrazide derivatives, compounds 51-56 and 58 were found to be the most protective against CCl<sub>4</sub> liver damage, apparent from the lower values of sAST, sALT and bilirubins as compared to pathological control. Compounds 45 and 49 were also found to be hepatoprotective when compared with the pathological control.

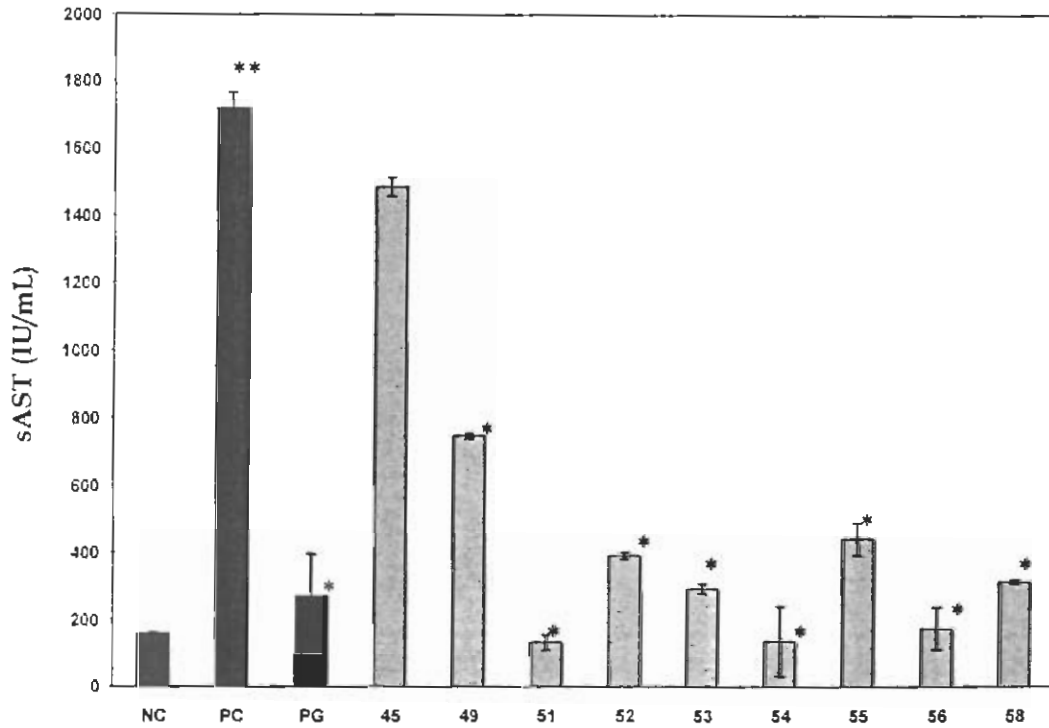


Figure 5.6: Effect of synthetic compounds 45, 49, 51-56 and 58 and standard antioxidant, propyl gallate (PG) on sAST levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). sAST values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).

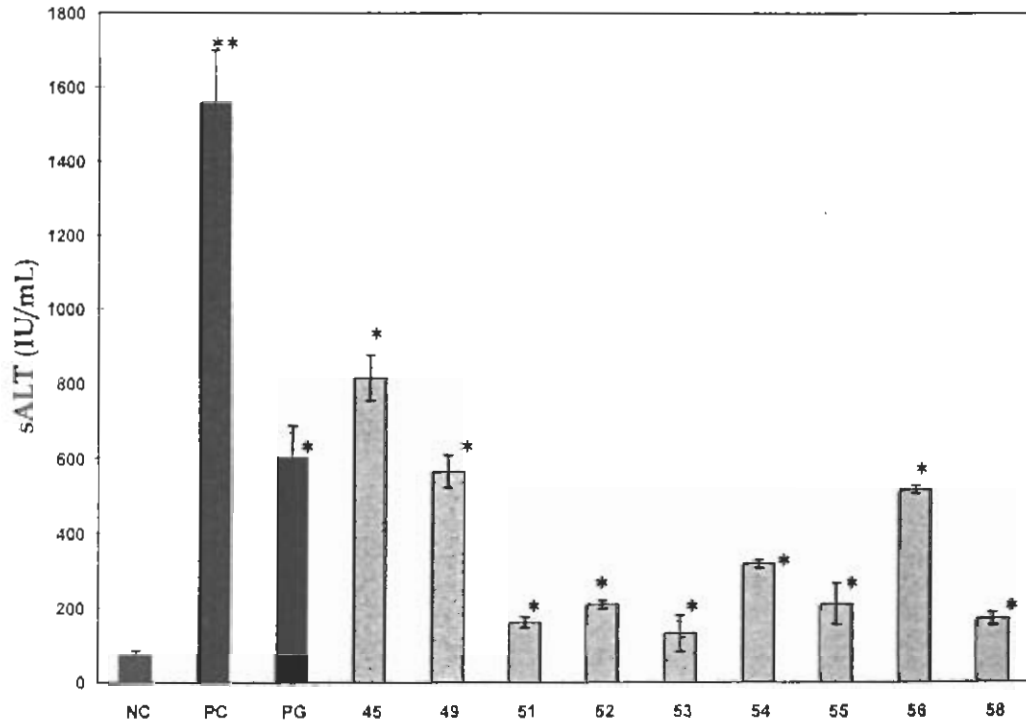


Figure 5.7: Effect of synthetic compounds 45, 49, 51-56 and 58 and standard antioxidant, propyl gallate (PG) on sALT levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). sALT values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control vs normal control, \*P < 0.05: pathological control vs test sample (Analysis of variance) ANOVA.

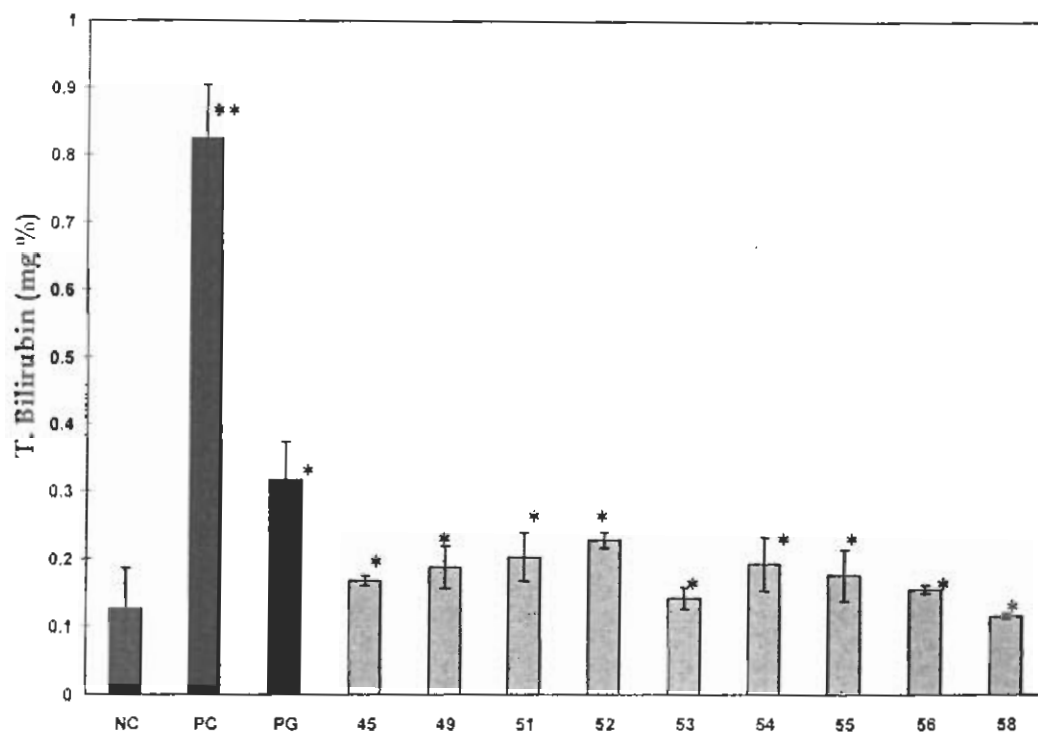


Figure 5.8: Effect of synthetic compounds 45, 49, 51-56 and 58 and standard antioxidant, propyl gallate (PG) on Total bilirubin levels in rats after 48 hrs of CCL<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCL<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Total bilirubin values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control vs normal control, \*P < 0.05: pathological control vs test sample ANOVA (Analysis of variance).

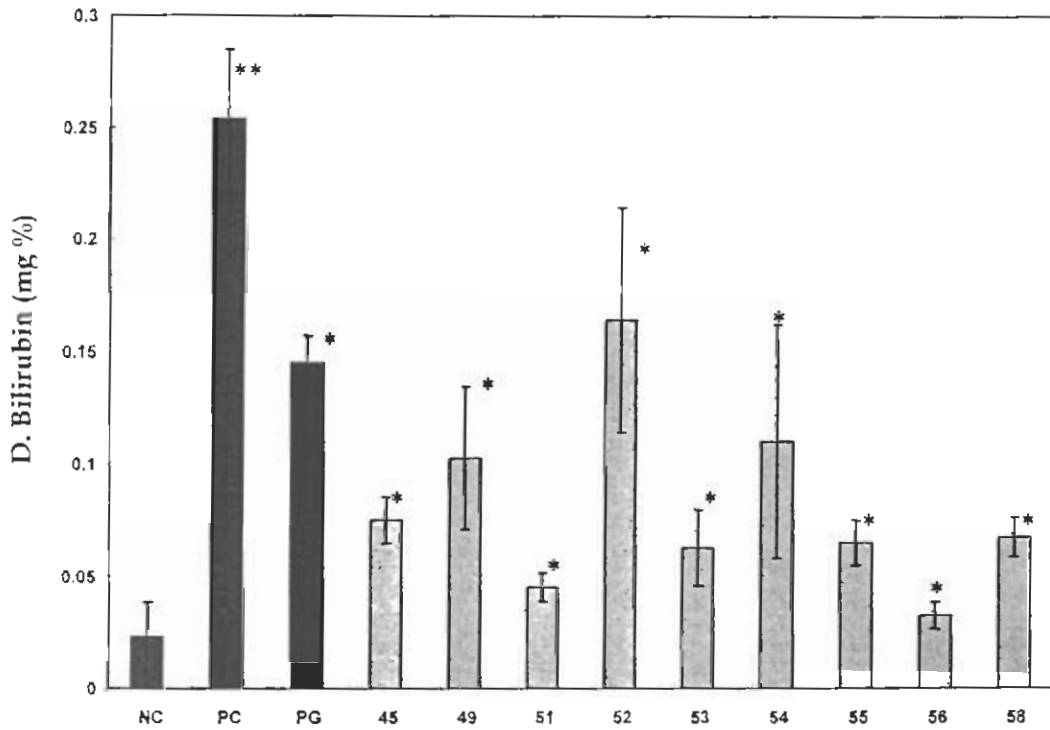


Figure 5.9: Effect of synthetic compounds 45, 49, 51-56 and 58 and standard antioxidant, propyl gallate (PG) on Direct bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Direct bilirubin values are the  $\pm$  SEM of six rats. \*\*P < 0.05; pathological control vs normal control, \*P < 0.05; pathological control vs test sample ANOVA (Analysis of variance).

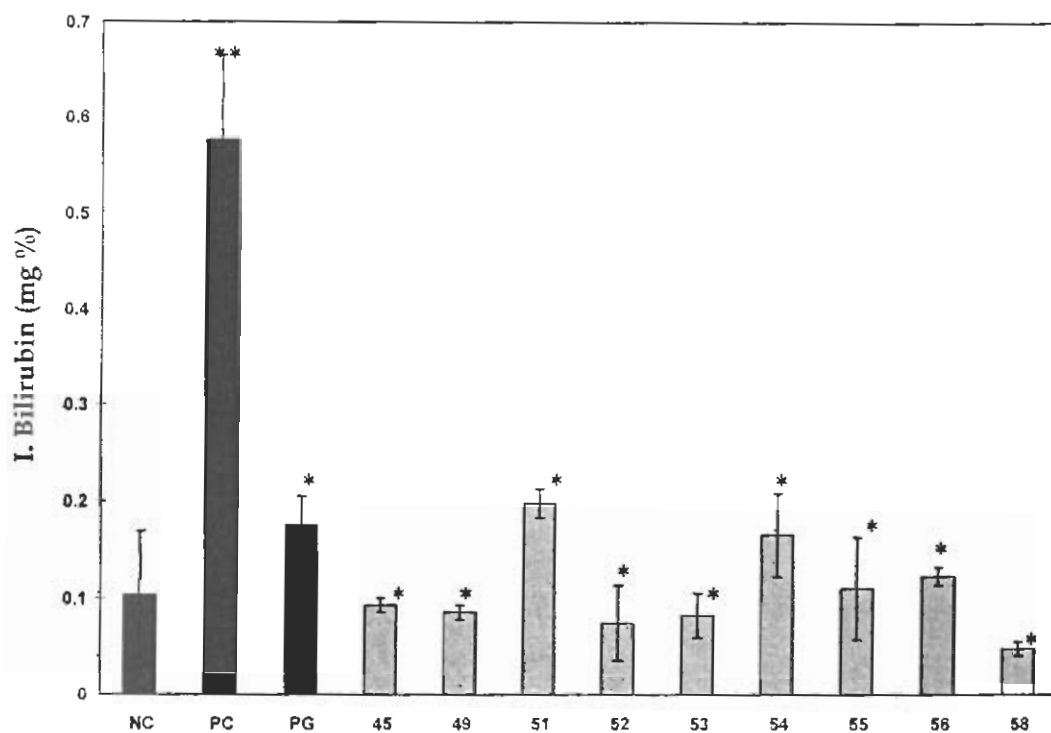
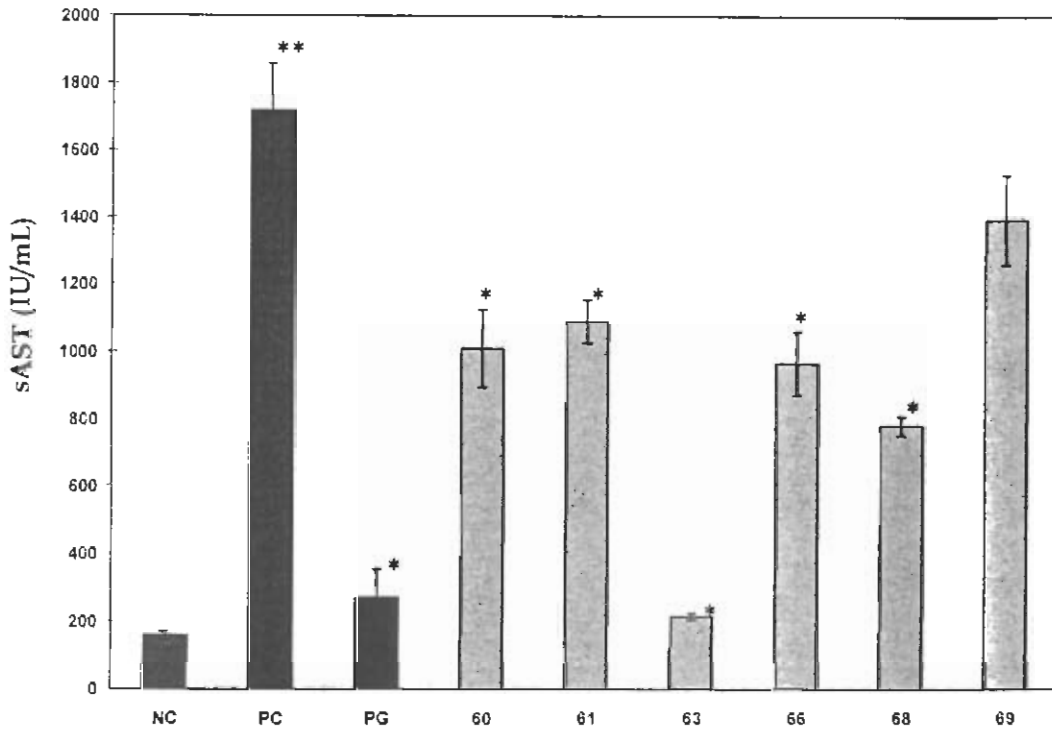


Figure 5.10: Effect of synthetic compounds 45, 49, 51-56 and 58 and standard antioxidant, propyl gallate (PG) on Indirect bilirubin levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). Indirect bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control vs normal control, \* $P < 0.05$ : pathological control vs test sample ANOVA (Analysis of variance).

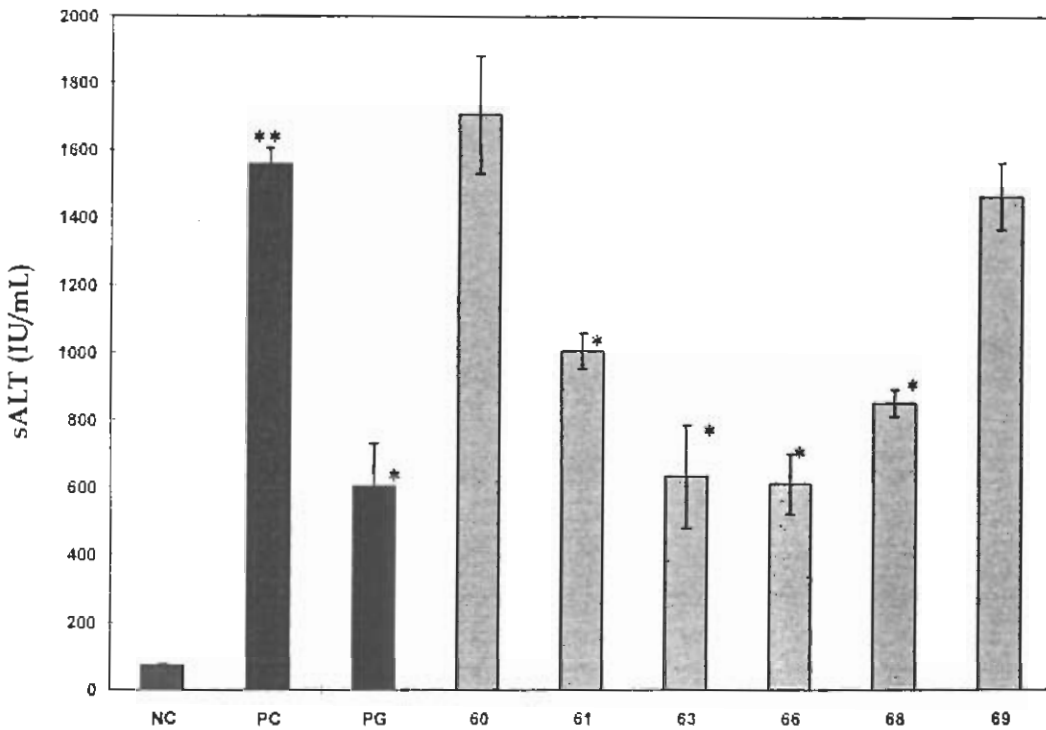
### 5.3.2. Oxadiazole Thione Derivatives

The six 1,3,4-oxadiazole-2-(3*H*)-thione derivatives (compounds, 60, 61, 63, 66, 68 and 69) were selected for *in vivo* study. All derivatives were administered to the experimental animals at a dose of 10 mg/kg body weight and their effects on transaminases and bilirubins in blood samples of treated animals were observed. The effects of pretreatment of the experimental animals with oxadiazole thione derivatives on the levels of bilirubins and transaminases in CCl<sub>4</sub>-injected animals are depicted in Figures 5.11-5.15. These compounds caused significant variations in the values of sAST, sALT and bilirubins as compared to pathological control. The most active compound was compound 63, as inferred from the lowest levels of transaminases and bilirubins. The other compounds 61, 66 and 68 were also found to be successful in maintaining the levels of enzymes and bilirubins when compared with pathological control. The effect of compound 69 on the levels of sAST and sALT was comparable to pathological control while the values of bilirubins were comparable with normal control.

An increase in the levels of sAST and total and direct bilirubins, as compared to pathological control, was observed in the case of compound 60, which shows that the compound did not protect hepatic cells against the damaging effect of CCl<sub>4</sub>.



**Figure 5.11:** Effect of synthetic compounds 60, 61, 63, 66, 68 and 69 and standard antioxidant, propyl gallate (PG) on sAST levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). sALT values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control vs normal control, \*P < 0.05: pathological control vs test sample ANOVA (Analysis of variance).



**Figure 5.12:** Effect of synthetic compounds 60, 61, 63, 66, 68 and 69 and standard antioxidant, propyl gallate (PG) on sALT levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). sALT values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control vs normal control, \*P < 0.05: pathological control vs test sample ANOVA (Analysis of variance).

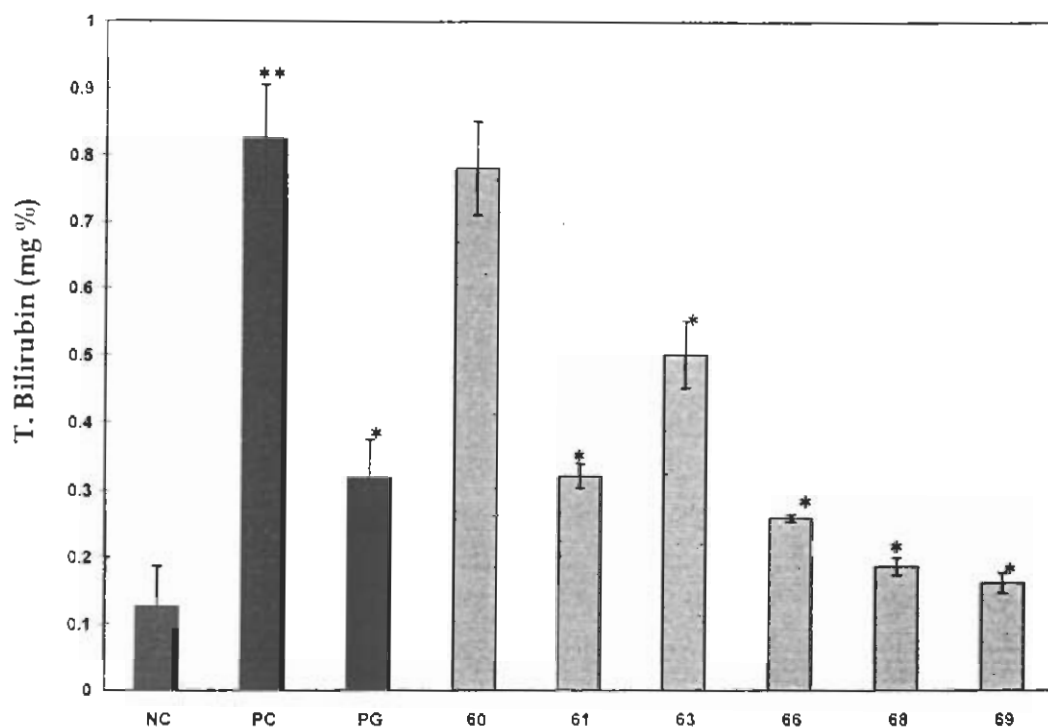
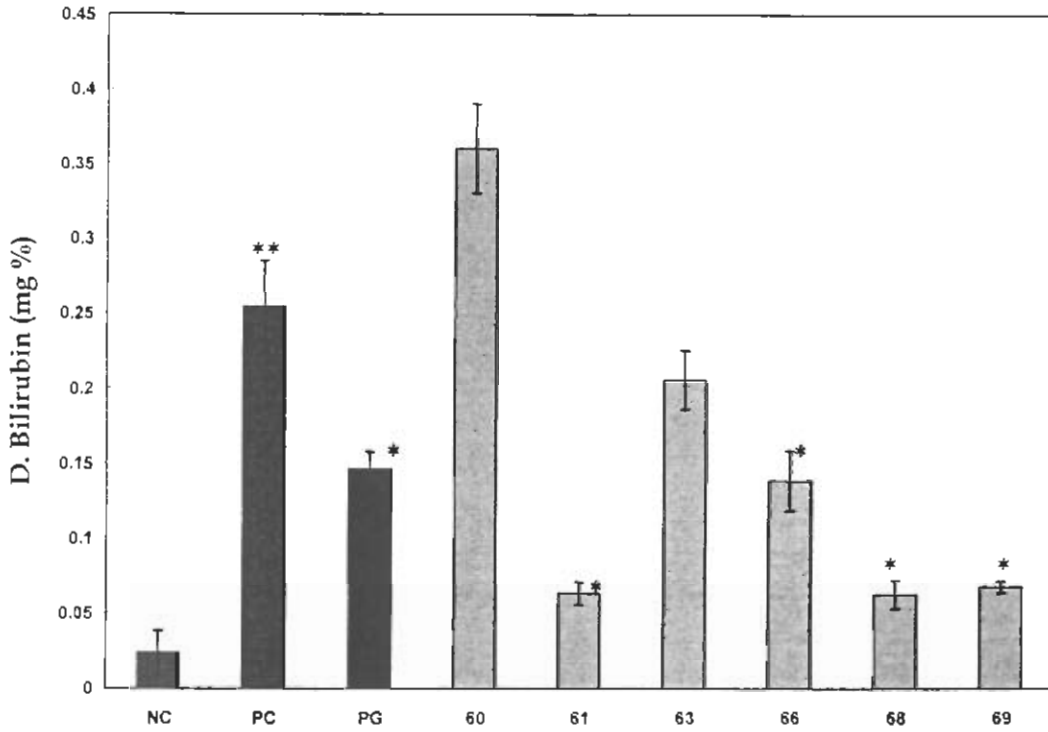
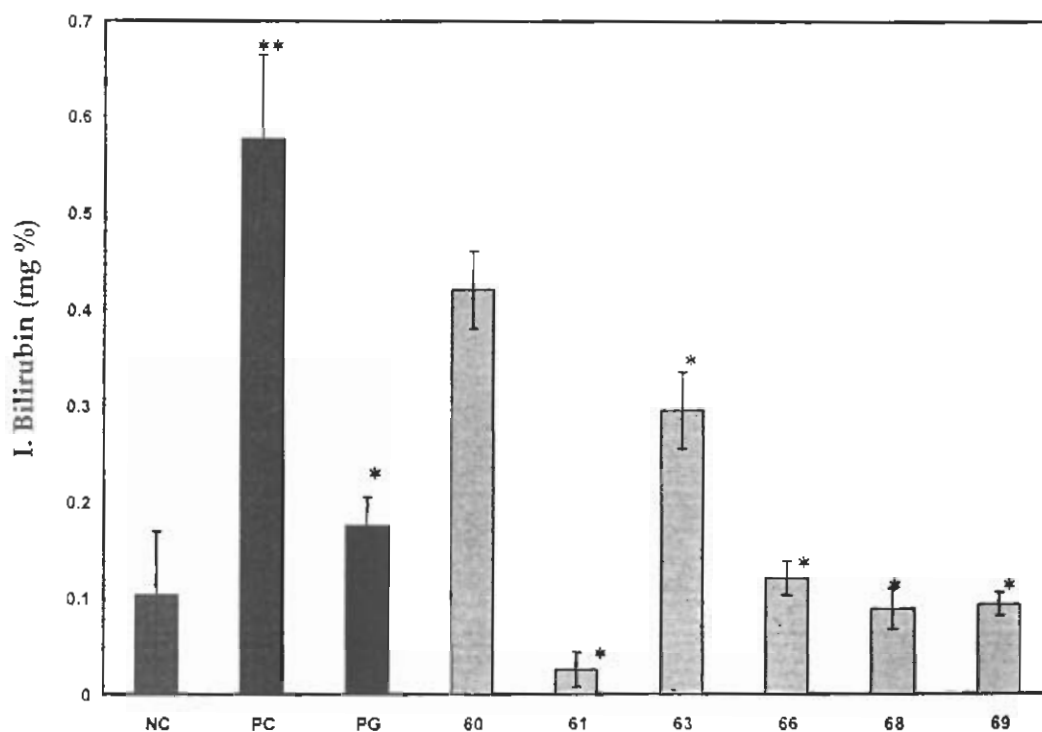


Figure 5.13: Effect of synthetic compounds 60, 61, 63, 66, 68 and 69 and standard antioxidant, propyl gallate (PG) on serum Total bilirubin levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). Total bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).



**Figure 5.14:** Effect of synthetic compounds 60, 61, 63, 66, 68 and 69 and standard antioxidant, propyl gallate (PG) on serum Direct bilirubin levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). Direct bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).



**Figure 5.15:** Effect of synthetic compounds 60, 61, 63, 66, 68 and 69 and standard antioxidant, propyl gallate (PG) on serum Indirect bilirubin levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). Indirect bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).

### 5.3.3. Coumarins and Benzothiazepine Derivatives

The selected coumarin and benzothiazepine derivatives were administered to the experimental animals at a dose of 10 mg/kg body weight after half an hour of CCl<sub>4</sub>-induction and their effect on transaminases and bilirubins was observed. The hepatoprotective effects of two coumarins 86 and 87, a biscoumarin 97 and a benzothiazepine derivative compound 102, along with pathological and normal controls, are presented in Figures 5.16-5.20. The bilirubin levels in the blood samples of experimental animals (Figures 5.18-5.20) were found to be lower in the case of compounds 86 and 87 as compared to the pathological control. However an elevated level of sAST and sALT was observed as compared to the pathological control. A different effect was observed in the case of compound 97, as apparent from the lower values of sAST and sALT. The benzothiazepine derivative 102 was found to have much better protective effect on hepatic cells as compared to coumarin derivatives (compounds 86 and 87) as is evident from significantly lower levels of transaminases and bilirubins.

In conclusion, a number of natural and synthetic compounds were investigated for their hepatoprotective effects *in vivo* in CCl<sub>4</sub>-induced hepatotoxicity assay. Some of them have caused interesting effects on the levels of hepatic enzymes and bilirubins. The results of this *in vivo* study indicated that the levels of sAST, sALT and total, direct, and indirect bilirubins were largely unchanged ( $p < 0.05$ ) after the pretreatment of compounds 2, 11, 8, 33, 34, 45, 49, 51-56, 58, 66, 68, and 102. Among the natural compounds, the most hepatoprotective were compounds, 8, 2, and 11 which belong to cinnamic acid ester, triterpene and cinnamide classes of

compounds, respectively. In the case of synthetic compounds, the acyl hydrazide derivatives 45, 49, 51-56, 58, were found to be the most effective against the hepatotoxic action of CCl<sub>4</sub> as compared to other classes. Interestingly, a benzothiazepine derivative 102 provided much better hepatoprotection.

As all these compounds were found to possess antiradical potential by *in vitro* assay model, it could be safely deduced that these compounds may provide hepatoprotection by acting as free radical scavengers. This contraction in the elevation of cytosolic enzymes is probably due to the interaction of CCl<sub>4</sub>-generated free radicals with hepatic cells. The mechanism of CCl<sub>4</sub> interaction with hepatic cells is discussed in section 8.3.1 (Page: 160). However, further investigations are needed to understand the exact mechanism of action of hepatoprotective activity of these compounds.

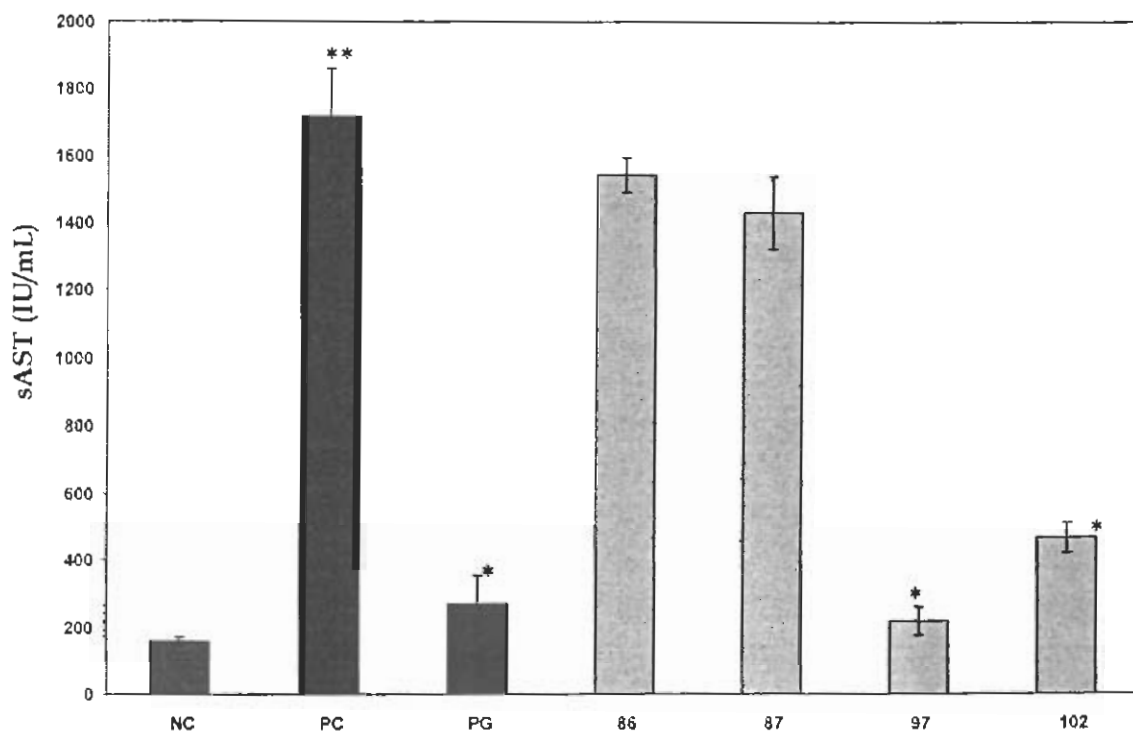
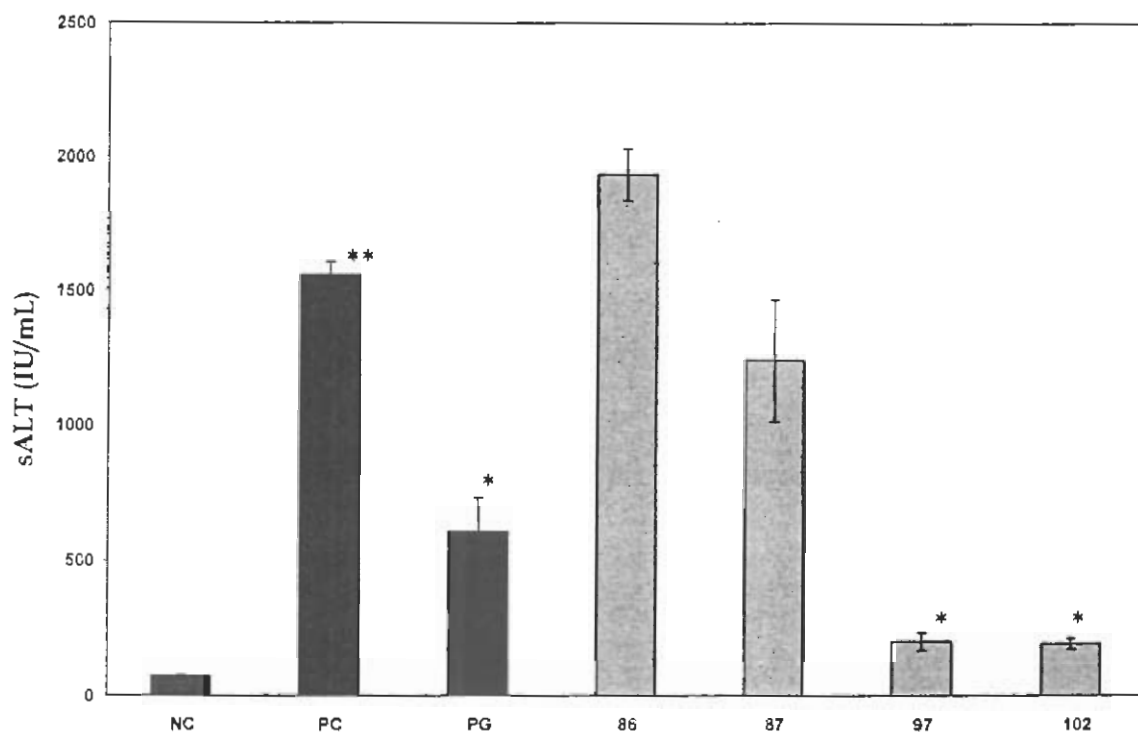
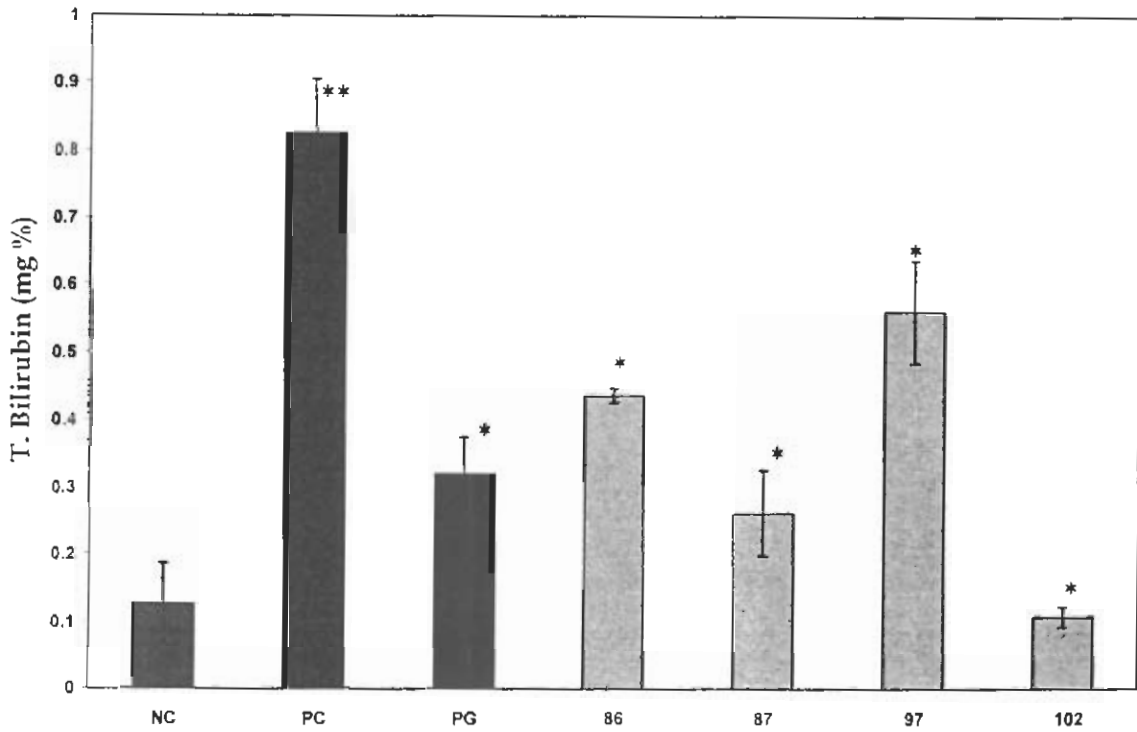


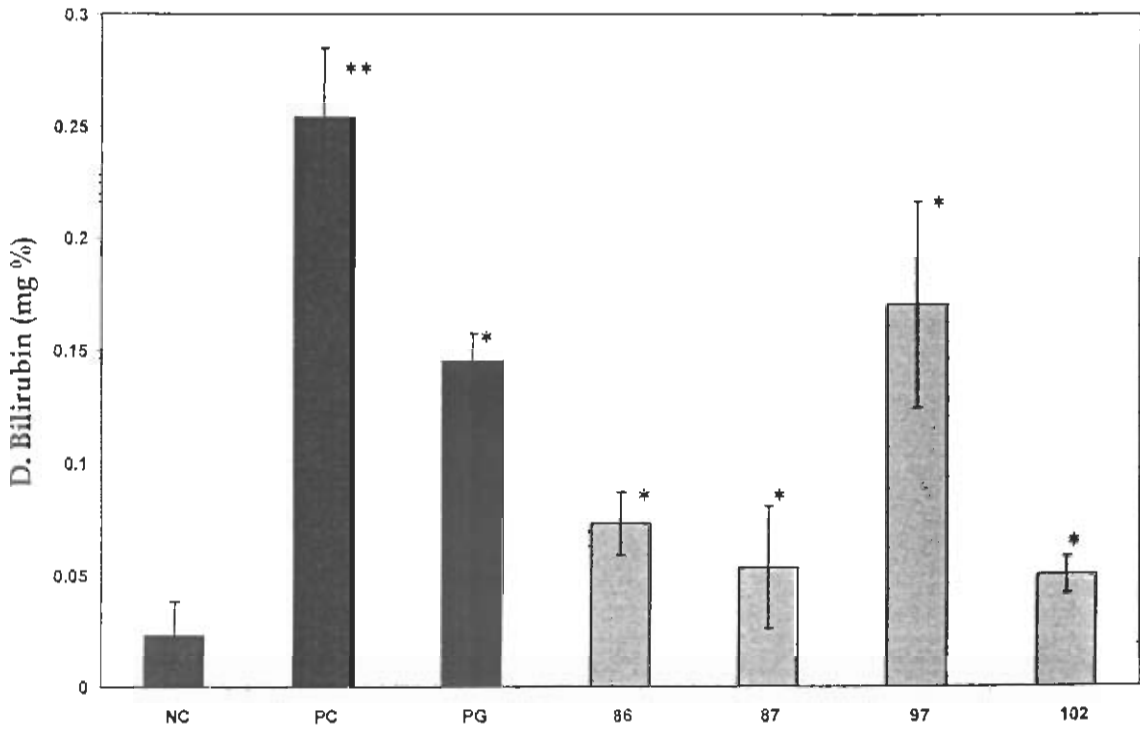
Figure 5.16: Effect of synthetic compounds 86, 87, 97 and 102 and standard antioxidant, propyl gallate (PG) on sAST levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). sAST values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).



**Figure 5.17:** Effect of synthetic compounds 86, 87, 97 and 102 and standard antioxidant, propyl gallate (PG) on sALT levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). sALT values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control vs normal control, \* $P < 0.05$ : pathological control vs test sample ANOVA (Analysis of variance).



**Figure 5.18:** Effect of synthetic compounds 86, 87, 97 and 102 and standard antioxidant, propyl gallate (PG) on serum Total bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Total bilirubin values are the  $\pm$  SEM of six rats. \*\*P < 0.05: pathological control *vs* normal control, \*P < 0.05: pathological control *vs* test sample ANOVA (Analysis of variance).



**Figure 5.19:** Effect of synthetic compounds 86, 87, 97 and 102 and standard antioxidant, propyl gallate (PG) on serum Direct bilirubin levels in rats after 48 hrs of CCl<sub>4</sub> induction. NC: Normal control, saline solution; PC: Pathological control, CCl<sub>4</sub>/edible oil (1.0 mL/100 g body weight). Direct bilirubin values are the  $\pm$  SEM of six rats. \*\**P* < 0.05: pathological control *vs* normal control, \**P* < 0.05: pathological control *vs* test sample ANOVA (Analysis of variance).

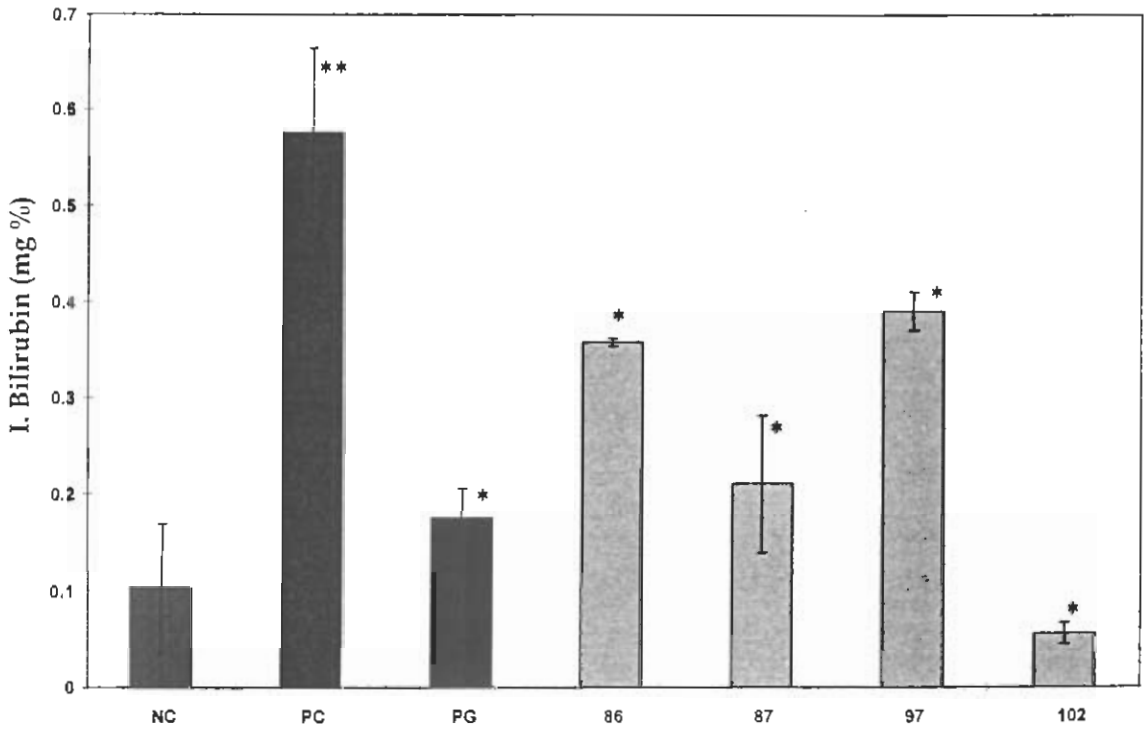


Figure 5.20: Effect of synthetic compounds 86, 87, 97 and 102 and standard antioxidant, propyl gallate (PG) on serum Indirect bilirubin levels in rats after 48 hrs of  $\text{CCl}_4$  induction. NC: Normal control, saline solution; PC: Pathological control,  $\text{CCl}_4$ /edible oil (1.0 mL/100 g body weight). Indirect bilirubin values are the  $\pm$  SEM of six rats. \*\* $P < 0.05$ : pathological control *vs* normal control, \* $P < 0.05$ : pathological control *vs* test sample ANOVA (Analysis of variance).