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Evaluation Of Hepatoprotective Activity Of *Thiourea Derivatives* On Gentamycin Induced Hepatotoxicity

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Abstract

The vital organ liver performs many of functions in the body, including the production of bile, elimination of bilirubin, cholesterol, metabolism of drugs, fats, proteins, activation of enzymes, storage of glycogen, and synthesis of plasma proteins like albumin and clotting factors. Hepatotoxicity is a definitive aspect of liver repeatedly caused by exposure to xenobiotics such as drugs, alcohols, toxins and chemicals. Thiourea and thiourea derivatives are a well-known class of synthetic organosulfur compound also known as carbamides.

The present study was designed to elucidate the scientific background for the use of *Thiourea derivatives* as hepatoprotective agent. The compound was evaluated for the hepatoprotective activity in animal model by analyzing the blood biomarkers and examining the histological changes in the liver. For this purpose, the synthetic compound thiourea derivatives were prepared by Dr Wadood Ali Shah (Assistant

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Keywords: TUD= Thio Urea Derivatives, H&E= Haematoxylin and Eosin resins, SGPT= Serum Glutamic Pyruvic Transaminase, ALT= Alanine aminotransferase, ALP= Alkaline Phosphatase, ROS= Reactive Oxygen Species, MDA= Malonaldehyde level, LPO= lipid peroxidation, CA= STO-II, CB= Psoduct-M, CC= Para-Thio-STP.

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Professor UOM). The dose of 50mg/kg and 250mg/kg from all the three compounds entitled as CA= STO-II, CB= Psoduct-M, CC= Para-Thio-STP were selected to evaluate the hepatoprotective effect. After dosing for 8 consecutive days, the blood samples were then analyzed for hepatic biomarkers such as Alanine aminotransferase (ALT) / Serum Glutamic Pyruvic Transaminase (SGPT) blood serum along with Alkaline Phosphatase ALP levels and the histology of liver. The blood serum levels of SGPT and the level of ALP were highly significantly lowered down by TUD in higher dose level of 250mg/kg, while the microscopic examination of liver histology revealed that TUD has improved clogged blood vessels, fatty changes, and inflammatory infiltrate markedly, induced by gentamicin.
Therefore, it was concluded from the findings of present study that thiourea derivatives has marked dose dependent hepatoprotective effect. As the literature review has declared that *TUD* constitutes different group of chemicals such as thiocarbonyl which possess antioxidant effect thus it might be concluded that the hepatoprotective effect of *TUD* may be due to thiocarbazone present in it.

1.0 INTRODUCTION

Liver is largest, most crucial organ of human body situated on the top of stomach, right kidney, and intestines in the upper right corner of the abdominal cavity. The liver is a dark reddish-brown, cone-shaped organ that weighs roughly three pounds [1]. Liver is site for numerous biochemical processes related to metabolism that are crucial to the health of human body. These processes include synthesis, degradation, inter-conversion, storage, and biotransformation [2].Liver performs a variety of functions in the body, including the production of bile, elimination of bilirubin, cholesterol, hormones, and drugs, metabolism of fats, proteins, carbohydrates, activation of enzymes, storage of glycogen, vitamins, and minerals, and synthesis of plasma proteins like albumin and clotting factors [3]. Furthermore, it also clarify the blood entering from digestive belt to the body. It not only detoxifies the blood but also metabolizes virulent drugs and chemicals and therefore, liver is pioneer target organ for exogenous toxicants [4]. Hepatopathy is commonly seen in diverse population of the world, irrespective of gender, age, and sex. Hepatotoxicity is a perfect aspect of liver repeatedly caused by exposure of xenobiotics such as drugs, alcohols, toxins and chemicals. Toxicity of liver is caused by both exogenous and endogenous toxicants. Drugs are the main causative agent for hepatotoxicity [5]. Drugs provoke hepatotoxicity by means of different mechanisms. General mechanisms accountable for hepatotoxicity and nephrotoxicity are rhabdomyolysis, mitochondrial permeability transition (MPT), intra-glomerular hemodynamics alteration, and bile acid-induced liver cell injury during cholestasis, tubular toxicity, crystal nephropathy, interstitial nephritis, and inflammation [6]. Majorly drugs causing hepatotoxicity are isoniazid, disulfiram, flutamide, ibuprofen, flucloxacillin, erythromycin, amoxicillin-clavulanate, and trimethoprim. Nearly 900 drugs, toxins, and herbs have been reported to cause liver injuries among these approximately 75 percent of idiosyncratic drug reactions leads to liver transplantation or death. Essentially acute liver failure, also called fulminant hepatic failure causes high risks of mortality and morbidity. By free radicals activation method gentamicin, cisplatin and acetaminophen induces hepatotoxicity [7].
Literature reports show that in the 19th century, the synthetic compounds gained much attention in every field. Literature also describes methods like reflux method, ball milling method, green method etc. for preparation of both symmetric and asymmetric thiourea [8]. Thiourea and its derivatives have shown number of pharmacological

activities like anti-diabetic, anti-bacterial, analgesic, anti-inflammatory, and anti-tumor [9].

$R_4SC(NH)_2$ is the general formula of Thiourea and its derivatives. Primarily they are called carbamides. Urea and carbamides have similar structures except that Sulphur in urea is replaced by oxygen and it adds various chemical and physical properties to the compound. Carbamides are water soluble and insoluble in non-polar solvents. Depending upon bonded substituents these derivatives are 1) N-Mono substituted, 2) N-Di-substituted and 3) N-Tri-substituted thioureas [10].

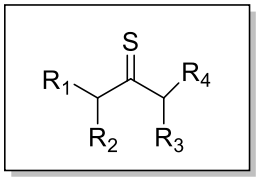


Figure 1: Thiourea (R = Aryl or Alkyl group)

As a major ligand, Thiourea by coordinating with metal center as monobasic, dibasic or neutral ligands. Donor atoms like oxygen, sulfur and nitrogen carry bonding possibilities in complex processes. For example, benzoyl thioureas bind and form mono anionic bi-dentate giving neutral homoleptic complexes with sulfur, oxygen and nitrogen [11]. Because of varying coordination thiourea, its derivatives and metal complexes also deliver in metallurgy, nanoparticles and certain other biological activities [12].

Almost in every area of chemistry thiourea derivatives are used commercially in the 19th century. Thiourea dioxide (TDO) is a potential discharge agent used in the textile industry for printing on discharged textiles [13]. During this process they let off small amount of pollutants which are environment friendly [14]. Today's synthetic organic chemistry focuses on manufacturing heterocyclic molecules with useful properties for industry. The main precursors in the realm of synthetics are derivatives of thiourea. The building blocks for the production of heterocyclic compounds like imidazolidine-2-thiones 1,3-thiazoles, and N-Arylthioureas. These substances are widely known for their antifungal, antimicrobial, and antioxidant properties [15].

2.0 MATERIALS AND METHODS

2.1 SYNTHETHIC COMPOUNDS AND CHEMICALS

To examine the hepatoprotective effect, various chemicals were used in animal model e.g., Thiourea derivatives *thiourea STO-II*, *Para-Thio-STP*, *Psoduct-M*, silymarin (Purchased from Lab Care Enterprises), normal saline, chloroform, Gentamicin purchased from Feroze Sons Nowshera.

Table 1: Chemicals list

Chemicals/ Drugs	**Source/ Manufacturer**
Gentamicin	Feroze Sons Nowshera, Pakistan
Silymarin	Purchased from Lab Care Enterprises, Pakistan
Methanol	Sigma
Chloroform	Department of Chemistry, AWKUM Mardan, Pakistan
Formalin	Department of Pharmacy, AWKUM Mardan, Pakistan
STO-II	Synthesized by Dr Wadood Ali Shah, Assistant Professor Department of Pharmacy, UOM Dir (L), Pakistan

Para-Thio-STP	Synthesized by Dr Wadood Ali Shah, Assistant Professor Department of Pharmacy, UOM Dir (L), Pakistan
Psoduct-M	Synthesized by Dr Wadood Ali Shah, Assistant Professor Department of Pharmacy, UOM Dir (L), Pakistan

2.2 ANIMALS USED

Male Swiss Albino healthy mice having weight between 24-30 grams were purchased from Capital University of Science and Technology CUST, Islamabad Pakistan (Veterinary Research Institute) for the observation of hepatoprotective activity. In the department's well-ventilated animal house, which was kept at a temperature of (37 °C), cages were utilized to house the animals. The test animals had a week of acclimatization with 12-hour cycles of darkness and light before the experiment. Test animals received fresh water and standard rodent food (pellet). The independent panel of the pharmacy department at the Garden campus of Abdul Wali Khan University (AWKUM) Mardan set criteria for the use of laboratory animals, which were followed in all experimental procedures and research.

2.3 EXPERIMENTAL PROTOCOL

Nine sets of 45 Swiss albino mice (25–30 gm.) were created to test the hepatoprotective effects. Each group contained five mice (n=5).

1: The animals in this group were designated as the control group after receiving intraperitoneal dose of 0.9% normal saline for 8 days.

2: Gentamicin (100 mg/kg/day) was administered intraperitonially for 8 days to the animals in this group.

3: Intraperitoneal administration of Silymarin (50mg/kg/day) was given to the animals in this group for 8 days.

4: For 8 days, mice in this group received oral dose of STO-II (50 mg/kg/day) and Gentamicin (100 mg/kg/day).

5: STO-II (250mg/kg/day) oral dose followed by Gentamicin (100mg/kg/day) intraperitoneal dose were administered for 8 days.

6: Para-Thio-STP (50mg/kg/day) oral and intraperitoneal dose of Gentamicin (100mg/kg/day) were injected to animals of this group for 8 days.

7: Para-Thio-STP (250mg/kg/day) was given orally followed by 8 days intraperitoneal dose of Gentamicin (100mg/kg/day) to this group.

8: This group was given Psoduct-M (50mg/kg/day) orally along with Gentamicin (100mg/kg/day) intraperitonially for 8 days.

9: Psoduct-M (250mg/kg/day) orally followed by Gentamicin (100mg/kg/day) intraperitonially were administered to the animals of this group.

2.4 BLOOD SAMPLE COLLECTION

On 9^{th} day of experiment, blood samples were collected from mice. Before sample collection, the animals were kept starving from night. The animals were treated with chloroform and then sacrificed. Blood collection was carried out directly by cardiac puncture method and soon moved to Gel tubes. At room temperature the Gel tubes were kept for 15 minutes to clot. Centrifugation of the Gel tubes at 4000 rpm for 5 minutes is done. By using a micropipette, serum was isolated from the Gel tube and then poured into eppendrof tubes or serum cups. Following that, the serum was stored at 2 to 8 °C for biochemical tests. Using an auto analyzer and various commercial diagnostic kits, the collected blood samples were then examined for Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and cholesterol.

2.5 HISTOPATHOLOGICAL ASSESSMENT

The liver was also subjected to a histopathological examination. The animals were dissected after the blood samples were taken, and the mice's liver was promptly extracted and rinsed side wise by normal saline. To preserve isolated livers, 10% neutrally buffered formalin (NBF) was used and after that it was kept in paraffin for 24 hours. The liver was cut into pieces of 4-5µm, hydrated and then de-paraffinized. Eosin and Hematoxylin stains were used for staining purposes. For the examination of slides, Light microscope was used for the clear marking of the infected areas in liver [16].

2.6 STATISTICAL ANALYSIS

The findings were mentioned as mean ± SEM and statistical significance between groups administered with drug or compound and a control group was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for the multiple comparisons using Graph Pad Prism 5 software. The values for $p<0.05$ were reviewed significant [17].

3.0 RESULT AND DISCUSSION

3.1 INVESTIGATION OF HEPATIC BIO MARKERS

3.1.1 Alanine aminotransferase (ALT) / Serum Glutamic Pyruvic Transaminase (SGPT)

The results of our experiment showed that, in comparison to normal control, gentamicin considerably raised the serum level of SGPT and induced hepatotoxicity. With the administration of TUD, the serum SGPT level has been markedly decreased. Figure 2 shows the various TUD dosages and their given response. The findings of our investigation demonstrated that all the three doses of concentration 250mg/kg of CA250 (STO-II), CB250 (Psoduct-M) and CC250 (Para-Thio-STP) showed the hepatoprotective activity as compared to dose concentration of 50mg/kg of the same three derived compounds. CB250 (Psoduct-M) and CC250 (Para-Thio-STP) with dose concentration of 250mg/kg considerably protected the hepatocytes up to ($P<0.05$) but the third isomer entitled as CA250 (STO-II) of concentration 250mg/kg ($P< 0.01$) substantially reduced SGPT. This contrasted with the gentamicin-treated group, which has significantly raised level of serum SGPT.

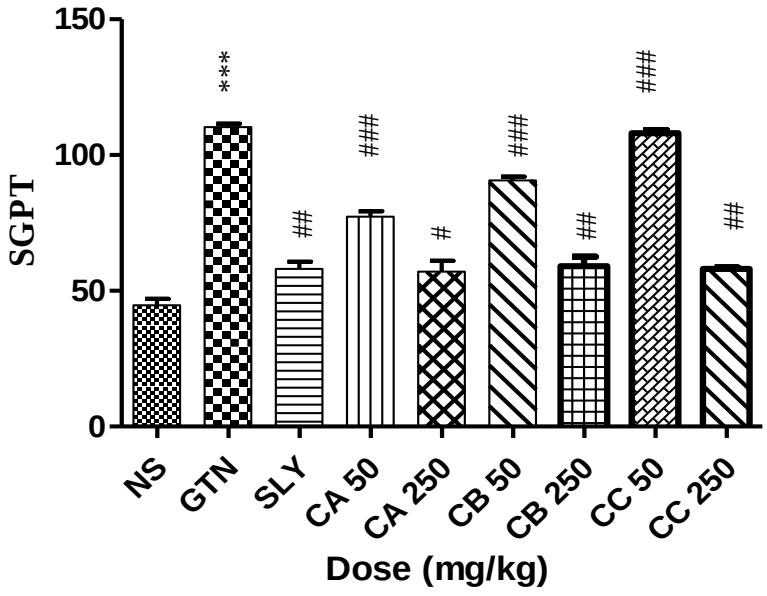


Figure 2: TUD response on SGPT level

Data is described as mean ± SEM (n=6); $^{***}P<0.001$ (in comparison to the group containing normal saline); $^{\#}P< 0.01$, $^{\#\#}P< 0.05$, $^{\#\#\#}P<0.001$ (in comparison to

gentamicin). TUD= Thio Urea Derivatives, NS= Normal saline, SLY= Silymarin 50mg/kg, GTN= gentamicin 100mg/kg, CA50 (STO-II) = 50mg/kg + gentamicin 100mg/kg, CA250 (STO-II)= 250mg/kg + gentamicin 100mg/kg, CB50 (Psoduct-M)= 50mg/kg + gentamicin 100mg/kg, CB250 (Psoduct-M)= 250mg/kg + gentamicin 100mg/kg, CC50 (Para-Thio-STP)= 50mg/kg + gentamicin 100mg/kg, CC250 (Para-Thio-STP)= 250mg/kg + gentamicin 100mg/kg

3.1.2 Alkaline Phosphatase (ALP)

Figure 3 shows the results of the TUD treatment on the ALP level. When compared to the control group, the test animals' serum ALP levels were ($P<0.001$) considerably enhanced after receiving 100 mg/kg gentamicin intra-peritoneally for 8 days. This suggests that gentamicin has severely injured the liver cells and produced hepatotoxicity. As silymarin did, it was seen that co-administration of TUD to several groups of experimental animals at various doses considerably reduced the serum ALP level. When compared to gentamicin-induced hepatotoxic groups of dose concentration 50mg/kg and 250mg/kg, the level of serum ALP with dose concentration of 250mg/kg was considerably ($P<0.05$) decreased in CA250 (STO-II), and CB250 (Psoduct-M) while highly ($P<0.01$) decreased in the CC250 (Para-Thio-STP) group but the dose of concentration 50mg/kg didn't showed any significant effect.

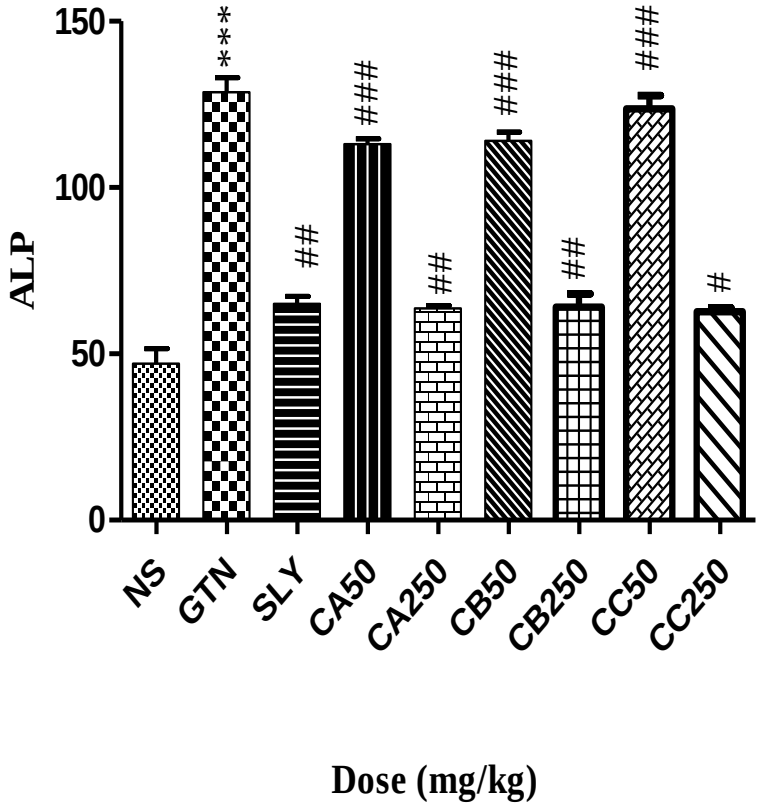


Figure 3: Effect of TUD on ALP level of mice

Data is described by mean ± SEM where (n=6); and $^{***}P<0.001$ (in comparison with the group containing normal saline); $^{\#}P< 0.01$, $^{\#\#}P< 0.05$, $^{\#\#\#}P<0.001$ (in comparison with gentamicin). TUD= Thio Urea Derivatives, GTN= gentamicin 100mg/kg, NS= Normal saline, SLY= Silymarin 50mg/kg, CA50 (STO-II)= 50mg/kg + gentamicin 100mg/kg, CA250 (STO-II)= 250mg/kg + gentamicin 100mg/kg, CB50 (Psoduct-M)= 50mg/kg + gentamicin 100mg/kg, CB250 (Psoduct-M)= 250mg/kg + gentamicin 100mg/kg, CC50 (Para-Thio-STP)= 50mg/kg + gentamicin 100mg/kg, CC250 (Para-Thio-STP)= 250mg/kg + gentamicin 100mg/kg

3.2 EFFECT ON LIVER HISTOPATHOLOGY

Table 2 lists the findings of our study. Our findings showed that the histological evaluations of the liver of the control group were confirmed to be normal (figure 4A). While the gentamicin-induced group (figure 4B) displayed mild histological alterations, including clogged blood vessels, fatty changes, and inflammatory infiltrate. While inflammatory infiltration was undetectable in the animals which were given dose of silymarin, displayed modest fatty alterations and clogged blood vessels (figure 5A). Severe fatty alterations, moderately clogged blood vessels, and inflammatory filtrate were all present in the CA250 and CB250 treated group (figure 5B&6A). While the CC250-treated group's histological analysis showed improvement in morphological abnormalities, such as minimally clogged blood vessels, no additional histological changes were found (figure 6B).

Table 2: TUD's impact on the histopathology of the experimental animals' livers

Groups	Histological Measures		
	Fatty Change	Inflammatory Infiltrate	Congested Blood vessels
N/Saline	-	-	-
Genta	++	++	++
Silymarin	+	-	+
CA250	+++	+	++
CB250	+++	+	++
CC250	-	-	++

Keys; +: mild, ++: moderate, +++: severe, - negative

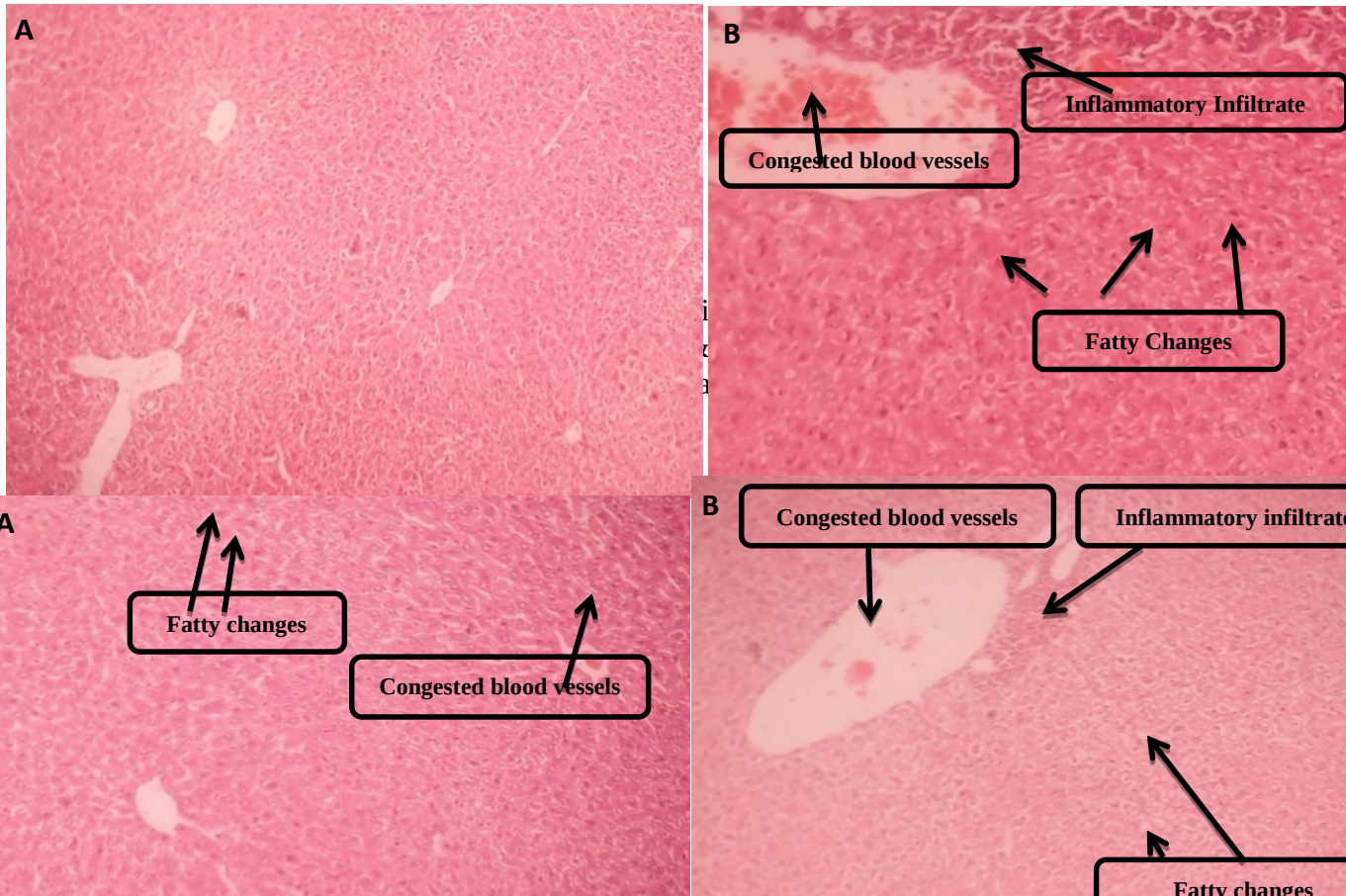
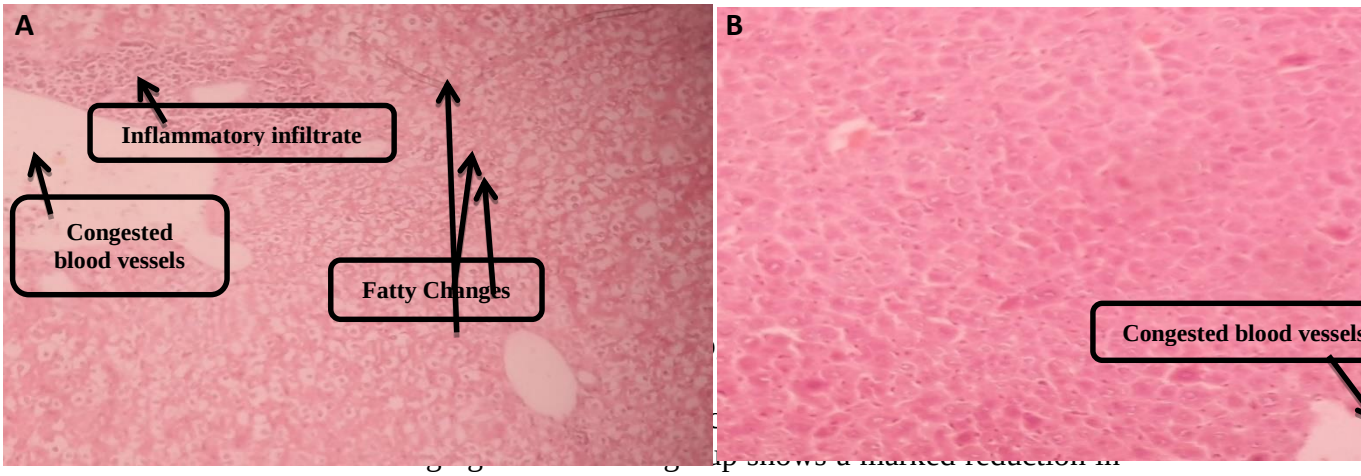


Figure 5. A. Microscopic image of H&E liver histology from the group receiving silymarin treatment. **B.** Microscopic view of liver H&E slides receiving CA dose 250mg/kg of TUD with marked Fatty changes, inflammatory infiltrate and congested blood vessels.



fatty alterations, inflammatory infiltration, and clogged blood vessels.

3.3 DISCUSSION

Although aminoglycosides have long been utilized therapeutically, their availability is currently limited due to hepatotoxicity [18]. Various mechanisms including mitochondrial dysfunction have been proposed to explain drug-induced Hepatotoxicity. These drugs alter the hepatic mitochondrial processes leading to necrosis and cytolytic hepatitis, which may cause liver failure [19].

Aminoglycosides interfere with intracellular signal pathways and are known to increase cellular permeability [20]. According to Bellés et al., aminoglycosides alter the activity of antioxidant enzymes glutathione peroxidase, superoxide dismutase, and glutathione reductase in various organs [21]. Lietz and Brya reported that aminoglycosides disrupt the activity of hepatic glycogen phosphorylase which is responsible to lower the glycogen level in the liver [22].

Certain biological biomarkers have been used to assess liver functions including serum SGPT and ALP [23]. In the present study, the elevated blood levels of SGPT and ALP along with histological inspection, confirmed that gentamicin at a dose of 100 mg/kg caused significant hepatotoxicity. Histological analysis conducted in the present study confirmed that silymarin significantly reversed fatty alterations, inflammatory infiltration, and clogging of blood vessels, along with other gentamicin induced liver problems.

To evaluate TUD's hepatoprotective effect, several doses of its constituents, including CA50 mg/kg, CB50 mg/kg, and CC50 mg/kg, as well as CA250 mg/kg, CB250 mg/kg, and CC250 mg/kg, have been administered. TUD compounds at lower doses of 50 mg/kg has not shown much hepatoprotective effects, but CA, CB, and CC at higher doses of 250 mg/kg each, significantly improving the recovery of fatty alterations, inflammatory infiltration, and congestion of blood vessels.

In conclusion, TUD may exhibit hepatoprotective effects, but further studies are required to elucidate the exact mechanism by which TUD exhibit hepatoprotective effects.

4.0 CONCLUSION

The objective of the present study was to establish the scientific basis for the hepatoprotective potential of thiourea derivatives. The drug's potential to protect the liver in experimental animals was evaluated by assessing biochemical parameters and histological analysis of the liver tissues.

The results of the current studies, including hepatic biomarkers (SGPT and ALP) and histological findings have demonstrated that TUD has a significant hepatoprotective effect. Based on the present study, it can be concluded that TUD has a hepatoprotective effect, but further studies will be required to fully elucidate the underlying mechanisms. Although the present study has established the initial scientific validation of TUD's hepatoprotective potential, exact mechanism responsible for this effect remains unclear. Therefore, these findings have opened new areas for future research aimed at identifying the exact mechanisms and techniques for isolation of thiourea derivatives with potential hepatoprotective properties.

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