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Evaluation of hepatoprotective potential of selected schiff bases (SW8/SB & SW10/SB) against gentamicin-induced hepatotoxicity

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ABSTRACT: Drug-induced hepatotoxicity is a usual way that the liver can suffer harm regardless of the advantageous roles of liver. Gentamicin-induced hepatotoxicity is a significant clinical disadvantage. It is believed that gentamicin-induced hepatotoxicity is due to formation of free radicals. Like other synthetic antioxidant compounds, Schiff bases also have the ability to scavenge free radicals. This study emphasizes the hepatoprotective potential of Schiff bases (Designated as compound A and compound B) in a gentamicin-induced hepatotoxicity animal model. Thirty mices were randomly divided into six groups of five each. Group 1 was injected with 0.9% normal saline intraperitoneally (I.P.) per day and served as control while group 2 received gentamicin I.P. at a dose level of 100mg/kg/day. Group 3 received gentamicin 100mg/kg/day I.P. and compound SW8/SB at a dose level of 25mg/kg/day orally. Group 4 was injected with gentamicin 100mg/kg/day I.P. and SW8/SB at a dose level of 50mg/kg/day orally. Similarly group 5 received gentamicin 100mg/kg/day I.P. and SW10/SB at a dose level of 25mg/kg/day orally. Group 6 received 100mg/kg/day of gentamicin I.P. and 50mg/kg/day of SW10/SB orally. The said procedure lasted for eight days. Then liver function was evaluated by measurement of biomarkers of the liver, including total bilirubin, alkaline phosphatase, and alanine aminotransferase. İn addition to this, histological studies were performed to point out pathological changes in liver. Gentamicin administration elevated serum level of alanine aminotransferase, alkaline phosphatase and total bilirubin as well as gentamicin treatment also caused histopathological alterations. However, administration of Schiff bases reduced both serum level of hepatic biomarkers and histopathological changes. The 50mg/kg of compound SW10/SB showed almost normal histoarchitecture. It is concluded that Schiff bases have the ability to reduce gentamicin-induced hepatotoxicity in mice. However, further studies are still required to further determine the safety and physiological mechanisms behind this effect.

KEYWORDS: Drug induced liver injury (DILI); Schiff bases; histoarchitecture; hepatotoxicity; gentamicin; compound A/CA (SW8/SB); Compound B/CB (SW10/SB).

1. INTRODUCTION

Vital biological processes carried out by the liver include the metabolism of macronutrients (fats, carbohydrates, and proteins), removal of chemicals particularly those that lead to various health issues, secretion of bile, storage of vitamins & blood purification [1]. Since the liver metabolizes and removes majority of drugs, drug toxicity frequently affects the liver. One of the main clinical issues that causes acute liver failure and occasionally demands liver transplantation is drug-induced liver injury (DILI) [2]. Almost 1000 medications have been reported to produce risk of liver damage [3]which is the main reason behind withdrawl of these pharmaceuticals from market after post marketing surveillance [4].

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Gentamicin, member of aminoglycoside group is active against infections caused by gram negative bacteria [5]. Apart from clinical effectiveness, gentamicin also causes hepatotoxicity [6] as well as nephrotoxicity [7]. Oxidative stress, lipid peroxidation, reactive oxygen species formation, DNA damage, protein denaturation, apoptosis, necrosis, monocyte infiltration, and production of proinflammatory cytokines are among the processes that have been considered to cause gentamicin-induced hepatotoxicity. Out of these mechanisms, oxidative stress is considered the major one behind hepatotoxicity. Defects in mitochondrial function and a decrease in antioxidant enzymes like catalase are the results of these processes. Moreover, oxidative stress leads to the stimulation of transcription factor like nuclear factor-κB (NF-κB) which plays a crucial role in the regulation of genes involved in inflammatory process such as the inducible nitric oxide synthase (iNOS), cycloxygenase-2 (COX2), interleukin-1b (IL-1b) and tumor necrosis factor-α (TNF-α). Excessive intracellular reactive oxygen species (ROS) production could lead to cell apoptosis through the modulation of Bcl2 family proteins and caspases [8]. Liver injury elevates the level of certain biomarkers such as hepatic alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes. These biomarkers are extensively used for evaluation of liver function [9].

Advancement in drug discovery has not yet developed any medicine which can protect the liver from hepatotoxic effects of drugs [10]. Silymarin is a mild hepatoprotective agent which exhibits different activities such as antioxidant, lipid peroxidation reduction, glutathione level elevation, reduction of mutation risk, and hepatogenesis [11]. Schiff bases have been studied widely but only few data is available regarding its hepatoprotective ability. The compound 3-(5-Bromo-2-hydroxybenzylideneamino)-2-(5-bromo-2 hydroxyphenyl)-2,3-dihydroquinazoline-]4(1H)-one which is also known as Q-Br is a member of quinazoline Schiff base has demonstrated to lessen thioacetamide's harmful effects. [12].

Schiff bases, first reported by Hugo Schiff are just like aldehyde or ketone compounds with only difference is in their replacement of carbonyl functional group with imine or azomethine (–C=N–) functional group as shown in figure 1, where R_1 can be H or alkyl group and R_2 can be phenyl or substituted phenyl group. The structure of Schiff base contains carbon-nitrogen double bond where nitrogen is furthure connected with Alkyl or Aryl group (R_3) but not hydrogen [13]. Schiff bases containing aliphatic aldehydes are relatively unstable and readily polymerizable [14] where as aromatic aldehydes containing Schiff bases having effective conjugation are more stable [15]. Schiff bases posses wide range of therapeutic benefits like antiinflammatory [16], analgesic [17], antibacterial [18], antiepileptic [19], anti-tuberculosis [20], antineoplastic [21], antioxidant [22] and anthelminthic [23]. İn the past few years, metal complexes with schiff bases have gained much more importance. Therapeutic activity of Schiff bases can be boosted by designing their complex with transition metals [24]. Schiff bases have the ability of forming a monolayer on damaged surfaces for protection and in this way it acts as a corrosion inhibitor [25]. The current study focuses on the establishment of scientific validity to the hepatoprotective property of specified Schiff bases to prevent hepatotoxicity induced by gentamicin in mice.

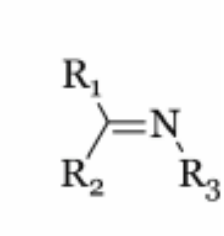

Figure 1. General Schiff Base Structure

2. RESULTS

2.1. Effect on hepatic biomarkers

2.1.1. Alanine aminotransferase (ALT) / Serum Glutamate Pyruvate Transaminase (SGPT)

Gentamicin-induced mice presented increased serum ALT level as compared to control group (blue bar). On the other hand, decreased serum ALT level was found in most groups treated with schiff bases as compared to gentamicin treated group (green bar). Administration of different doses of Schiff bases, 50 mg/kg of SW8/SB (CA) & SW10/SB (CB) reduced ALT level more significantly (** $p<0.01$) whereas 25 mg/kg of SW10/SB (CB) lowered the ALT level high significantly (*** $p<0.001$). Compound SW8/SB (CA) in

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a dose of 25mg/kg did not show any significant effect (ns: No significance). Figure 2 represents effect of different doses of Schiff bases on ALT level.

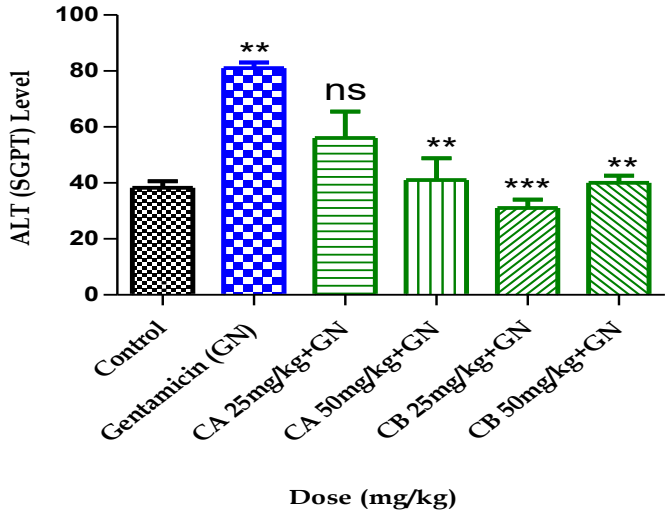

Figure 2. Graphical Representation of effect of administrtion of Gentamicin, and different doses of Schiff bases for eight days on ALT (SGPT) level. Gentamicin intoxication with 100mg/kg elevated the ALT level (Represented by blue bar with two starts **) whereas 50 mg/kg of compound A (CA) and compound B (CB) reduced the serum ALT level elevated by gentamicin more significantly (** p<0.01). 25mg/kg of compound B (CB) reduced the elevated serum ALT level high significantly (***p<0.001). 25 mg/kg of compond A (CA) did not show any significant effect on ALT level (ns).

2.1.2. Alkaline Phosphatases (ALP)

Gentamicin-induced mice showed increased serum ALP level than control group (blue bar). However, among administrations of various doses of Schiff bases i.e. 25mg/kg & 50mg/kg of SW8/SB (CA) and 25mg/kg & 50mg/kg of SW10/SB (CB), only 50mg/kg of SW8/SB (CA) decreased the ALP level more significantly (** p<0.01). No significant effect was found on serum ALP level (ns) with other doses of schiff bases. Figure 3 represents effect of Schiff bases on alkaline phosphatase (ALP) level.

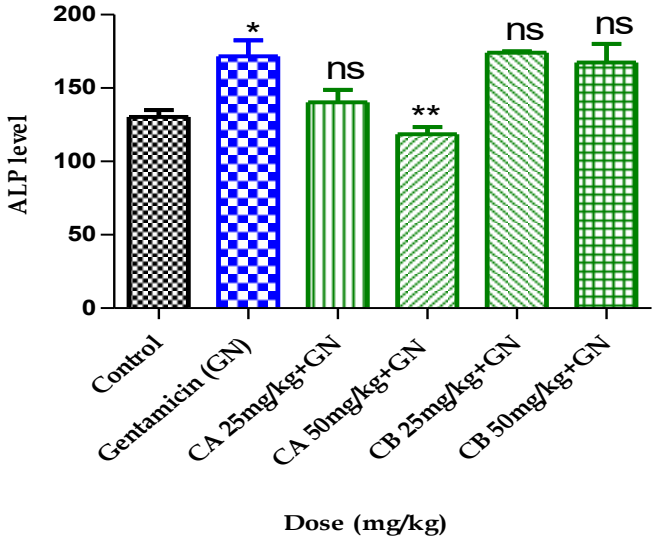

Figure 3. Graphical representation of effect of Gentamicin and Schiff bases administration for eight days on ALP level. Genatmicin intoxication with 100mg/kg elevated the serum ALP level (Blue bar with one star *). Among doses of Schiff bases only 50 mg/kg of compound A (CA) reduced the elevated serum ALP level more significantly (** p<0.01). Remaining doses of compounds didn't show any significant effect on ALP level (ns).

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2.1.3. Total bilirubin

Administration of gentamicin in mice raised the bilirubin level as compared to control group (blue bar). However, treatment with Schiff bases lowered this increased bilirubin level. As represented in figure 4, only 25mg/kg & and 50mg/kg of SW10/SB (CB) significantly (* $p<0.05$) reduced the bilirubin level whereas both doses of SW8/SB (CA) did not show any considerable effect on bilirubin level (ns).

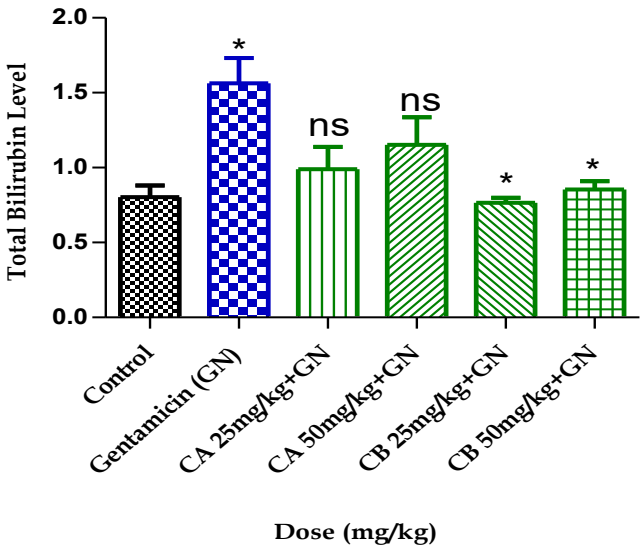

Figure 4. Graphical representation of effect of gentamicin and Schiff bases administration for 8 days on Total Bilirubin level. Intoxication with 100 mg/kg of gentamicin elevated the serum total bilirubin level (blue bar with one stars*). 25 mg/kg & 50 mg/kg of compound B (CB) reduced the elevated serum total bilirubin level significantly (* $p<0.05$) whereas, both doses of compound A (CA) didnt not show any significant effect on total bilirubin level (ns).

2.2. Effects on Liver Morphology

According to histopathological findings, almost normal hepatic architecture of group 1 (Control group) was found in which hepatocytes are present around central vein with blood sinosoids (Figure 5A). Liver sections of group 2 in which gentamicin was injected showed significant pathological alterations characterized by spotty necrosis, ballooning degeneration, pyknotic nuclei, and sinosoidal dilatation (Figure 5B & 5C). Liver section of group 3 administered with gentamicin 100mg/kg/day and Schiff base SW8/SB 25mg/kg/day revealed ameliorative effect but still there is present some degree of ballooning degenaration, portal inflammation, spotty necrosis, pyknotic nuclei, and sinosoidal dilatation (Figure 5D).

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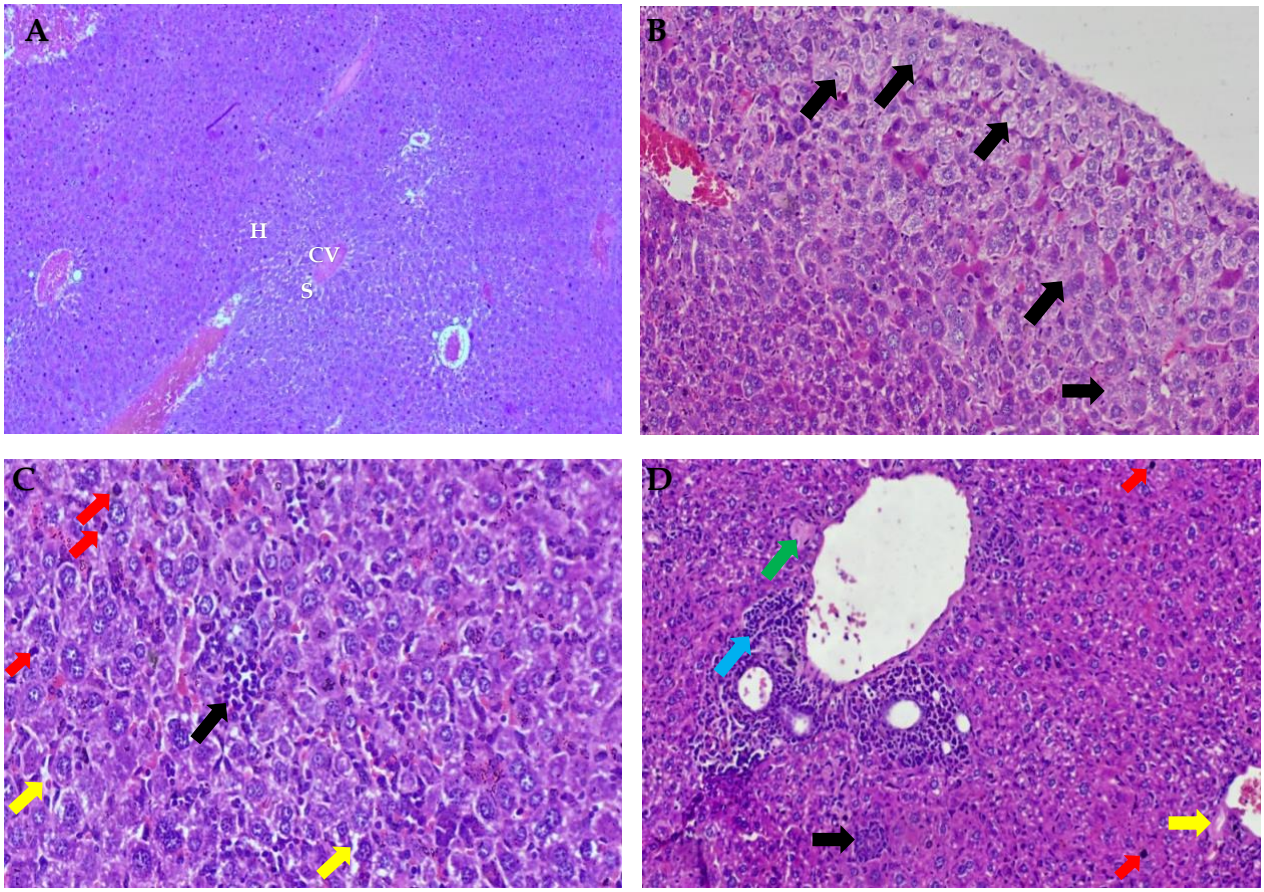

Figure 5. A. Histological section of liver of control group (Microscopic illustration of normal saline-induced liver exhibits almost normal histoarchitecture. CV: Central vein, H: Hepatocytes, S: Sinosoids). **B**. Histological section of liver of gentamicin-induced group (Black arrows represent marked ballooning degenaration). **C**. Histological section of liver of gentamicin-induced group (Red arrows show marked pyknotic nuclei, Yellow arrows represent mild sinosoidal dilatation and black arrow represents moderate spotty necrosis). **D.** Histological section of liver of gentamicin + SW8/SB 25mg/kg induced group (Green arrow represents mild ballooning degenaration, Blue arrow represents moderate portal inflammation, Black arrow represents mild spotty necrosis, Red arrows represent mild pyknotic nuclei & Yellow arrow shows mild sinosoidal dilatation).

In the histologic section of group 4 treated with gentamicin 100mg/kg/day and SW8/SB 50mg/kg/day, there is found mild ballooning degeneration, pyknotic nuclei & portal inflammation. However, sinosoidal dilatation is almost absent (Figure 6A). The liver sections of group 5 administered with gentamicin 100mg/kg/day and SW10/SB 25 mg/kg/day showed almost same degree of pathological changes as observed in liver section of group 3 (Figure 6B & 6C). Liver section of group 6 treated with gentamicin 100mg/kg/day and SW10/SB 50mg/kg/day exhibited almost normal hepatic architecture. However, mild degree of pyknotic nuclei and ballooning degenaration is still present (Figure 6D).

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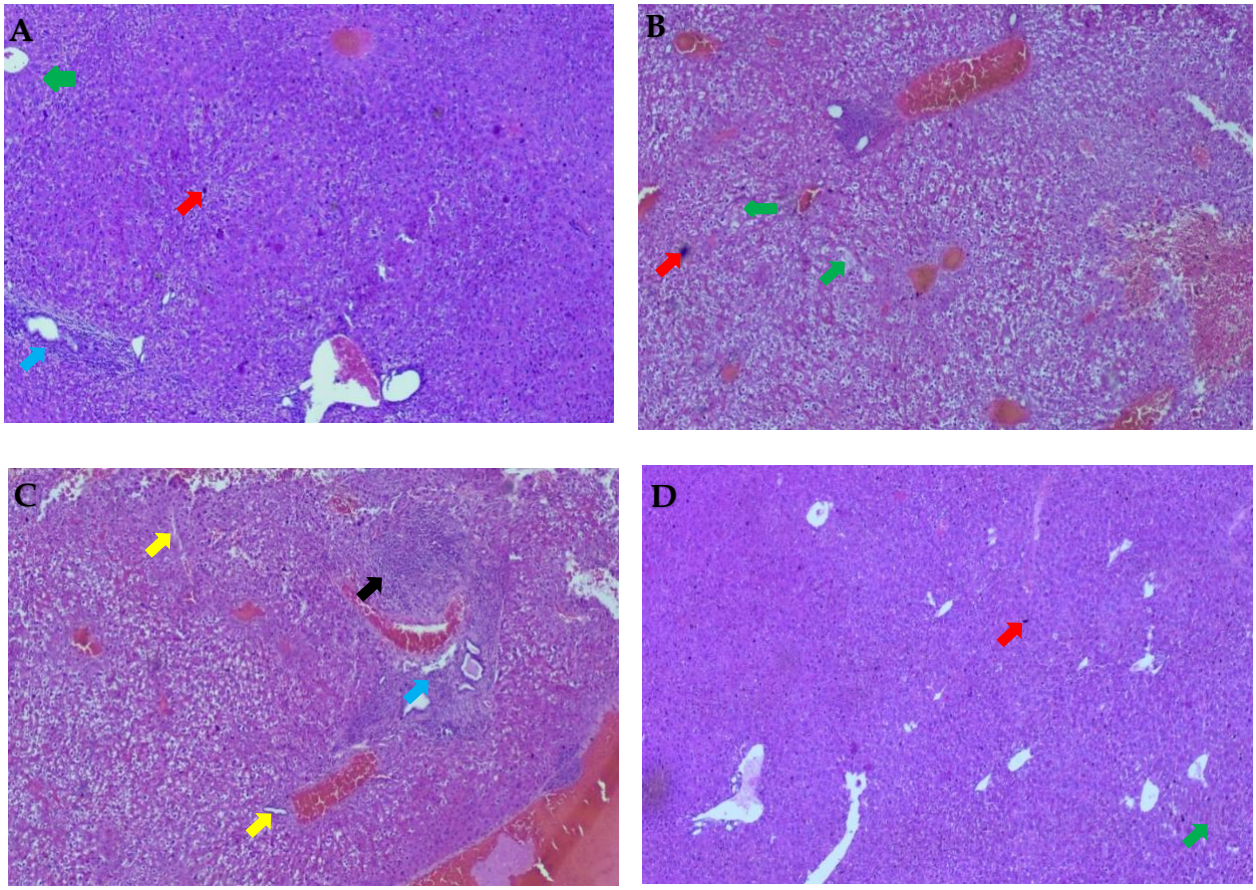

Figure 6.A. Histological section of liver of gentamicin + SW8/SB 50mg/kg induced group (Green arrow shows mild ballooning degenaration, Red arrow represents mild pyknotic nuclei & blue arrow points out mild portal inflammation). **B.** Histological section of liver of gentamicin + SW10/SB 25mg/kg induced group (Red arrow represents mild pyknotic nuclei & Green arrows represent mild ballooning degenaration). **C.** Histological section of liver of gentamicin + SW10/SB 25mg/kg induced group (Black arrow represents mild spotty necrosis, Blue arrow represents moderate portal inflammation & Yellow arrows show mild sinosoidal dilatation). **D.** Histological section of liver of gentamicin + SW10/SB 50mg/kg induced group (Red arrow points out mild pyknotic nuclei & Green arrow represents mild ballooning degenaration)

It should be noted that some kind of portal inflammation was found in groups (Group 3, 4 & 5) administered with Schiff bases (Figure 5D, 6A, 6B & 6C). This finding may be associated with adverse effect of Schiff bases.

3. DISCUSSION

Various physiological functions are carried out by liver, including metabolism, detoxification, and bile secretion [1]. Hepatotoxicity by drug is a major clinical problem that may result in acute liver failure and, in severe cases, lead to transplantation [2]. Many drugs have been associated with the risk of liver damage which is the main reason behind withdrawal of some pharmaceuticals from market after post-marketing surveillance [3, 4].

Gentamicin belongs to aminoglycoside antibiotic group and is effective against gram-negative bacterial infections [5]. Gentamicin is known for its hepatotoxic and nephrotoxic effects [6, 7]. The precise mechanisms of liver damage by gentamicin are not fully recognized, but lipid peroxidation and the production of reactive oxygen species are believed to play a role [8]. Anti-inflammatory and antioxidant are the two mechanisms of Schiff bases that may be associated with its protective effect against gentamicin-induced hepatotoxicity [16, 22].

To investigate the hepatoprotective effect of Schiff bases, we conducted experiments on Swiss Albino Mice, treating them with combinations of gentamicin 100mg/kg and different doses (25mg/kg & 50mg/kg) of Schiff bases.

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We measured serum biomarkers such as Alanine aminotransferase (ALT), alkaline phosphatase (ALP), and Total bilirubin to assess hepatic function. Alanine aminotransferase (ALT)/serum glutamate pyruvate transaminase (SGPT) is common and accurate indicator of Hepatocytes necrosis. The enzyme is predominantly found in the liver [26]. Alkaline phosphatase is present on the sinusoidal surface of hepatocytes and in the liver's bile canaliculi [27]. Liver or bones are the main source of circulating alkaline phosphatase [28]. Damaged liver lost the ability to eliminate ALP from liver, bone and intestine [29]. Hemoglobin catabolism produces bilirubin [30]. Liver lesions cause hepatocytes reduction that may lead to hyperbilirubinemias [31]. Serum analysis of bilirubin is frequently used as a diagnostic tool for liver problems but bilirubin is not considered a particular marker of hepatic function so other authentic diagnostic procedures are required for careful monitoring of hepatic function [32]. Mice intoxicated with gentamicin 100 mg/kg showed elevated levels of these biomarkers, indicating liver damage. However, supplementation with Schiff bases (SW8/SB & SW10/SB) brought about a significant reduction in ALT, ALP, and total bilirubin levels, especially at specific doses of the compounds. This suggests that Schiff bases have hepatoprotective properties, as they helped restore normal liver function.

Histopathological examination of liver tissues provided further awareness. The gentamicin-induced group expressed marked pathological changes, including spotty necrosis, pyknotic nuclei, ballooning degeneration, and sinosoidal dilatation (Figure 5B & 5C). However, the group supplemented with Schiff bases, particularly at certain doses, showed milder histopathological alterations. Furthermore the group supplemented with SW10/SB (CB) at a dose level of 50mg/kg represented nearly normal histoarchitecture (Figure 6D). The mild to moderate histopathological changes in liver of groups supplemented with low dose Schiff bases (25mg/kg/day) indicate a dose-dependent response (Figure 5D, 6B & 6C). These findings validate the biochemical results and support the idea that Schiff bases can protect the liver from gentamicin-induced damage.

The correlation of biochemical and histopathological results provides a brief picture of liver function recovery. However, relying solely on these parameters may not be enough and can be benefitted from more precise biomarkers such as gamma-glutamyl transpeptidase (GGT), HMGB1, cytokeratin 18 (K18), glutathione-S-transferase-α (GST-α), glutamate dehydrogenase (GLDH), malate dehydrogenase (MDH), oxidative stress (e.g., malondialdehyde, glutathione levels) or inflammation (e.g., TNF-α, IL-6)and microRNAs (miRNA) etc. As observed in histopathological findings that 50 mg/kg dose of Schiff bases represented almost normal histology, doses beyond 50 mg/kg raises questions whether higher doses would be beneficial or toxic.

The study does not elaborate the actual mechanism of Schiff bases whether it is anti-inflammatory or antioxidant activity in reversing the gentamicin-induced hepatotoxicity. Moreover, the study shows short-term results and does not address long-term effects of Schiff bases. It is unclear whether the protective effect is sustained on liver or damage may recur when treatment with Schiff bases is stopped. The study just uses gentamicin as a hepatotoxic drug that limits the general applicability of findings. The research would provide broader understanding if conducted on different liver injury model such as alcohol-induced, paracetamol-induced, thioacetamide-induced and carbon tetrachloride (CCl_4)-induced model etc.

Our study is basically contribution to the hepatoprotective effect of schiff bases since few publication are available pointing out hepatoprotection by schiff bases. One of the studies focused to explore the hepatoprotective effects of a ruthenium(II) Schiff base complex in a diet-induced pre-diabetic rat model. ruthenium(II) Schiff base complex was administered at a dose level of 15 mg/kg, both with and without dietary intervention, over 12 weeks. Results indicated that the treatment restored liver and body weights and reduced biomarkers of liver damage, including liver enzymes, bilirubin, and sterol regulatory element binding protein 1c (SREBP-1c) levels. The findings suggest that the ruthenium(II) complex may prevent diabetes-related liver dysfunction while avoiding hepatotoxicity, though further research is required to illustrate the underlying mechanisms [33]. While silymarin is a well-known hepatoprotective agent [36], our study aimed to evaluate the potential of Schiff bases as alternatives. The results indicate that some Schiff bases, especially at specific doses, have hepatoprotective effects comparable to silymarin. This is significant because it suggests that synthetic compounds like Schiff bases could be explored as potential treatments or preventive agents for liver diseases.

This study provides scientific evidence supporting the hepatoprotective potential of selected Schiff bases against gentamicin-induced hepatotoxicity in mice. These findings open approaches for further research into the use of Schiff bases as therapeutic agents for liver diseases, especially drug-induced liver injury.

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4. CONCLUSIONS

The findings of the study support hepatoprotective effect of Schiff bases against gentamicin-induced hepatotoxicity. Schiff bases was effective in returning the liver's biomarker level to normal. Similarly, Schiff bases also recovered gentamicin-induced histopathological alterations. Therefore, Schiff bases can be used for creation of novel medication or supplement that protect the liver. In order to bring these compounds in final clinical use, additional research is needed to reach its various aspects such as mechanism of action, pharmacokinetics, pharmacodynamics and safety profile etc. Moreover, as mentioned earlier that complexes of Schiff bases with transition metals have better therapeutic activity. Therefore, hepatoprotection by Schiff bases may be furthure enhanced if additional study is conducted on complexes of SW8/SB & SW10/SB with transition metals.

5. MATERİALS AND METHODS

5.1. Preparation of Schiff bases

Schiff bases (SW8/SB & SW10/SB) were prepared by Assistant Professor Dr. Wadood Ali Shah of Malakand University. Synthesis of Schiff base involves two steps. In the first step there is condensation of aldehyde or ketone compounds with amine which gives rise to intermediate compound known as carbinolamine and in the second step dehydration of intermediate molecule occurs as shown in figure 7. Dean Stalk apparatus is employed for this reaction. Schiff base reaction is reversible, and the intermediate compound known as carbinolamine formed during this reaction is dehydrated by using acid or base catalyst or upon heating. After completion of reaction, molecular sieve is used to remove water completely. Removal of the final product or dehydration moves the process towards completion [34].

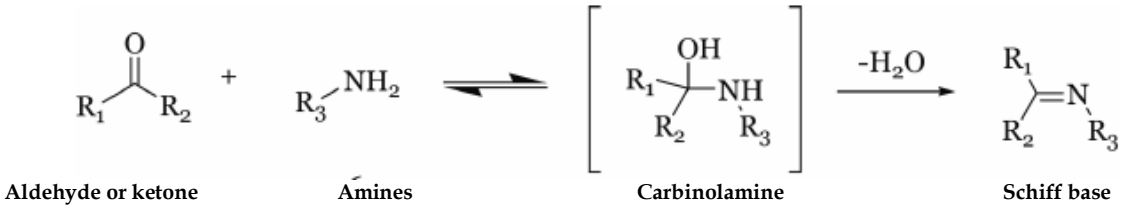
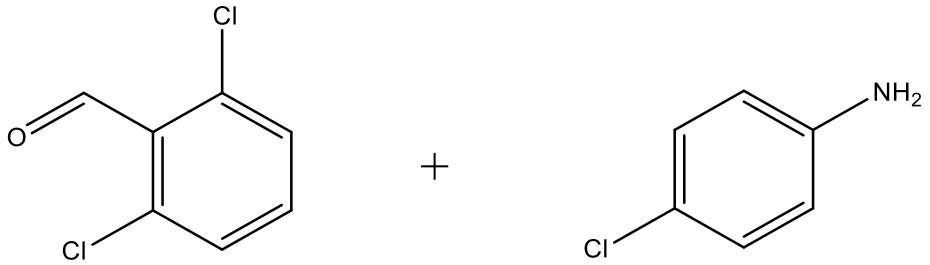


Figure 7: Reaction involved in synthesis of Schiff Bases

5.2. Chemicals

Muhammad Zai medical store, peshawar, pakistan provided gentamicin (Manufactured by Abbott Laboratories, Pakistan), silymarin (Manufactured by Abbott Laboratories, Pakistan) and normal saline (Manufactured by Marions Laboratories Pakistan). 10% Neutral Buffered Formalin (Manufactured by Sigma-Aldrich, USA) and chloroform (Manufactured by Sigma Aldrich, USA) was provided by the pharmaceutical chemistry lab of pharmacy department, AWKUM. Synthetic compounds (SW8/SB & SW10/SB) were dissolved in Di Methyl Sulphoxide (Manufactured by Akkshat Pure Chem, India) in saline. Chemical structures of both Schiff bases under study, SW8/SB & SW10/SB are illustrated in figure 8 & 9 respectively.



2,6-dichlorobenzaldehyde 4-chloroaniline

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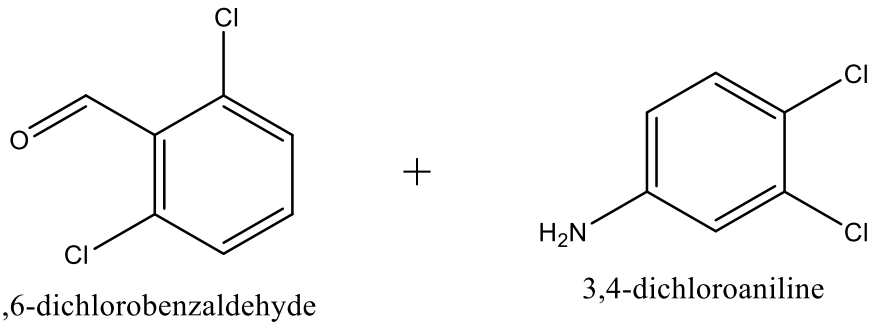
Figure 8. Chemical Structure of SW8/SB or Compound A (CA) or 2, 6-dichlorobenzaldehyde + 4-chloroaniline



2,6-dichlorobenzaldehyde

3,4-dichloroaniline

Figure 9. Chemical Structure of SW10/SB or Compound B (CB) or 2, 6-dichlorobenzaldehyde + 3, 4-chloroaniline

5.3. Experimental animals and protocol

Mice (Swiss Albino) of both genders weighing 25-35 grams were provided by the veterinary research institute (VRI) in Comsats University of Science and Information technology, Abbottabad Pakistan. The mice were kept in cages within an animal house having proper ventillation facility of Pharmacy department of Abdul Wali Khan University, Mardan, Pakistan. Animal house temperature was maintained at 24±2°C. The mice were given 7 days to adjust with the environment of light and dark cycle of 12 hours duration each before procedure. The mice were provided with fresh water and a standard rodent food (pellets) throughout the research. All protocoals were fulfilled according to the standards set by an independent panel of the pharmacy department of Abdul Wali Khan University Mardan (AWKUM). 30 mice were divided in to 6 groups and each group consist of five mice. Mice of each group received normal saline, gentamicin, and synthetic compounds for 8 days in the following manner:

Group 1 (n=5): This group, also know as control group was injected with vehicle/normal saline only.
Group 2 (n=5): Mice of this category were injected with the hepatotoxic dose of gentamicin i.e. 100mg/kg/day through intraperitoneal route.
Group 3 (n=5): These mice were injected with gentamicin (100mg/kg/day) intraperitoneally as in group 2 along with oral administration of SW8/SB (25mg/kg/day).
Group 4 (n=5): This category received intraperitoneal administration of gentamicin (100mg/kg/day) as in group 2 along with oral administration of SW8/SB (50mg/kg/day).
Group 5 (n=5): These mice received intraperitoneal administration of gentamicin (100mg/kg/day) as in group 2 along with oral administration SW10/SB (25mg/kg/day).
Group 6 (n=5): These mice received intraperitoneal administration of gentamicin (100mg/kg/day) as in group 2 along with oral administration of SW10/SB (50mg/kg/day) orally.
On the ninth day, blood samples were drawn by heart puncturing method. İn addition to this, liver was also taken from each group and stored in Neutral Buffered Formalin to study histopathological changes [35]. The hepatotoxic dose of gentamicin was selected on the basis of previous study [36].

5.4. Serum biomarkers Analysis

After completion of procedure, mice were anesthetized by chloroform. Blood samples were drawn by intracardiac puncture into the gel tube. The tubes were subjected to incubation for 15 minutes at temperature 20-25 ^{0}C after which centrifugation was done at 4000rpm for five mints. With the help of micropippette, serum was isolated from gel tube and moved to eppendrof tubes. The serum was kept at 2-8 °C and then blood biochemical tests including Alanine aminotransferase, Alkaline phosphatase, and Total bilirubin were performed with the aid of autoanalyzer & some laboratory diagnostic kits (Provided by Moon Enterprises) [37].

5.5. Histopathological examination

Liver tissues previously retained in 10% Neutral Buffered Formalin were fixed in paraffin & about four micrometer thick sections were then cut from each liver with the help of microtome and stained with hematoxylin and eosin [38].

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5.6. Statistical Analysis

The outcomes were mentioned as mean ± SEM and statistical significance between groups supplemented with drug or compound and a control group was evaluated 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 [39].

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