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Protective Role of Dichloromethane Extract of *Solanum dasyphyllumin* in Gentamicin Induced Nephrotoxicity in Mice

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ABSTRACT

This study investigated the effect of dichloromethane extract of Solanum dasyphyllum on gentamicin-induced nephrotoxicity in mice. Thirty mice were randomly divided into six groups (Groups 1-6, 5 each). Nephrotoxicity was induced using 100 mg/kg gentamicin administered intraperitoneally with concomitant administration of different doses of dichloromethane *Solanum dasyphyllum* extract (SdDMF) (100, 200, and 400mg/kg respectively) orally for 8 days. After the treatment, blood samples were obtained, allowed to clot, and centrifuged to obtain the serum which was used to determine urea, creatinine, and blood urea nitrogen (BUN). Kidney tissue malondialdehyde (MDA) and glutathione (GSH) concentrations; and catalase (CAT) activities were also determined. Result revealed a decrease in tissue MDA, serum creatinine, urea, and BUN in the extract-treated groups. Also, increased tissue CAT activities and GSH concentrations were observed in the extract-treated groups. In conclusion, this study showed that SdDMF can be reno-protective and effectively inhibit gentamicin-induced nephrotoxicity.

Keywords: Dichloromethane, *Solanum dasyphyllum*, gentamicin, nephrotoxicity, antioxidant

INTRODUCTION

Gentamicin belong to the aminoglycoside antibiotic, which has a broad spectrum activities against gram positive and gram negative bacteria, but most effective in the treatment of gram negative bacteria. Gentamicin can withstand

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heat after autoclaving[1]. Gentamicin, when administered orally shows no appreciable pharmacological effect because it is poorly absorbed from the gastrointestinal tract and hence binds to tissues and is mostly eliminated unchanged in the urine. Therefore, gentamicin is administered parenteraly via intravenous, intramuscular, intraperitoneal routes or topically. The mechanism of action involves inhibition of protein synthesis after binding to 30S subunit of the bacterial ribosome[2].

Report had shown that about 30% of patients administered with gentamicin came up with kidney toxicity but the exact mechanism of gentamicin-induced nephrotoxicity has not been ascertained. Studies had shown that there are different pathways involved in the nephrotoxic effect of gentamicin[3,4]. The involved pathways include release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), reduction of antioxidant defense mechanism and initiation of inflammatory processes. The generation of ROS leads to formation of ion–drug complex, which results in kidney damage. Serum creatinine, blood urea nitrogen, and inflammatory cytokines (Interleukins and tumor necrosis factor alpha) levels had been shown to be elevated significantly in gentamicin-induced nephrotoxicity[5,6]. Compounds with anti-oxidant and anti-inflammatory effects had been shown to have a protective role against gentamicin-induced nephrotoxicity[7]. *Solanum dasyphyllum* leaves contain various phytochemical constituents such as flavones and alkaloids which suggests its antioxidant properties. Some species of Solanum (*Solanum nigrum* and *Solanum dulmacara*) had been found to exhibit anti-inflammatory, analgesic, anti-platelet aggregation, anti-oxidative, anti-atherosclerotic, and anti-tumour properties[8,9].

From the understanding of the pathophysiology of gentamicin-induced nephrotoxicity, the two major factors promoting kidney damage are oxidative stress and inflammation. Because of the identified antioxidant and anti-inflammatory properties of *Solanum dasyphyllum*, the present study was designed to evaluate the protective role of dichloromethane extract of *Solanum dasyphyllum* against gentamicin-induced kidney damage in mice.

METHODOLOGY

Chemicals

Glutathione, Hydrogen peroxide, 5,5'-dithios-bis-2-nitrobenzoic acid and epinephrine were purchased from Sigma Chemical Co., Saint Louis, MO USA. Absolute ethanol, trichloroacetic acid and thiobarbituric acid were purchased from British Drug House Chemical Ltd., Poole, UK. All chemicals were of analytical grade and purest quality available.

Plant material and extraction procedure

Solanum dasyphyllum leaves were purchased from a local vendor in Akungba, Akoko in Ondo State, Nigeria. *Solanum dasyphyllum* leaves were air dried at room temperature, ground using domestic food processor to obtain the powder. Two hundred grams of the obtained *Solanum dasyphyllum* powder were extracted using a mixture of 80% methanol and 20% water in a soxhlet extractor for 72 hours. Fractionation of methanol extract of *Solanum dasyphyllum* (MESd) was carried out using n-hexane, dichloromethane solvent by liquid-liquid fractionation. The yield of the preparation was 6%.

Animals

Male adult Swiss albino mice were obtained from the animal house of the Institute of Medical Research and Advanced Training, University College of Medicine, Ibadan, Nigeria. The animals were housed in well-aerated plastic cages, fed with standard mouse cubes obtained from Ladokun Feeds Nigeria Ltd and supplied with clean drinking water *ad libitum*. Handling of animals and other protocols conform to the guidelines of the US Public Health Service Guidelines[10]**.**

Study design

Thirty mice each weighing between 18 and 23 g were randomly distributed into six groups of five mice per group as follows:

Group 1: Normal control (mice received standard diet and 0.2 mls normal saline orally for 8 days)

Group 2: 100 mg/kg **Gentamicin** intraperitoneally + **0.2 mls normal saline** orally for 8 days

Group 3: 100 mg/kg **Gentamicin** intraperitoneally + **0.2 mls** 100 mg/kg SdDMF orally for 8 days

Group 4: 100 mg/kg **Gentamicin** intraperitoneally + **0.2 mls** 200 mg/kg SdDMF orally for 8 days

Group 3: 100 mg/kg **Gentamicin** intraperitoneally + **0.2 mls** 400 mg/kg SdDMF orally for 8 days

Group 6: 100 mg/kg **Gentamicin** intraperitoneally + **0.2 mls** 200 mg/kg ascorbic acid orally for 8 days

Preparation of samples

After the experimental period, blood samples were collected by cardiac puncture into plain centrifuge tubes and allowed to stand for 2 hours before centri-

fuged to obtain serum. The serum was used to determine the creatinine, urea, and blood urea nitrogen levels. Kidneys were harvested after dissection of the animals and rinsed in ice-cold 1.15% KCl, dried and weighed. The kidney samples were homogenised in 4 volumes of 50 mM phosphate buffer, pH 7.4 using a Potter Elvehjem homogeniser and centrifuged at 10000g for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF) which was used to determine catalase (CAT) activities; malondialdehyde (MDA) and glutathione (GSH) levels.

Biochemical parameters determination

Serum creatinine, urea, and blood urea nitrogen levels were determined using methods described by

Bartels and Bohmer (1972)[11]; Weatherburn (1967)[12]; and Richter and Lapainte (1959)[13] respectively. Also, kidney tissue malondialdehyde and glutathione concentrations; and Catalase activities were determined using methods described by Mihara and Uchiyama (1978)[14], Aebi (1984)[15], and Sinha *et al.* (1971)[16] respectively.

Statistical analysis

Data obtained were analysed using the graph pad prism statistical package version 6.01. Values were expressed as mean ± SEM. Differences in mean between more than two groups were compared using one-way ANOVA and Newman-Keuls post hoc test was employed for multiple comparisons of treatment groups. Level of statistical significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

In this study, a significant ($p < 0.05$) decrease in reduced glutathione concentration and catalase activities was observed in group administered with gentamicin treated with normal saline (G2) when compared with control group (G1). Also, a significant increase ($p < 0.05$) in MDA concentration was demonstrated in group administered with gentamicin (Table 1).

An increase in GSH concentration and CAT activities and a decrease in MDA concentration was observed in kidney tissue of groups treated with SdDMF (100, 200 and 400mg/kg) and ascorbic acid (G3-G6) when compared with group treated with normal saline (G2) (Figures 1-3). The observed reduction in MDA concentration was significant among MESd treated groups as compared to what is obtainable in ascorbic acid treated group. A dose-dependent significant ($p < 0.05$) increase in GSH concentrations were demonstrated in the extract treated group (100, 200 and 400mg/kg).

A significant ($p < 0.05$) increase in serum creatinine, urea and BUN levels was observed in the group treated with the Tween-80 (vehicle of extraction) when compared with the control group (Table 1). Also, a significant reduction in serum creatinine level was observed in all the extract treated groups except 100 mg/kg (Table 1). The extract and ascorbic acid treated groups showed a reduction in serum urea and BUN concentrations, with a significant ($p < 0.05$) decrease in serum urea and BUN concentrations in G5 and G6 groups.

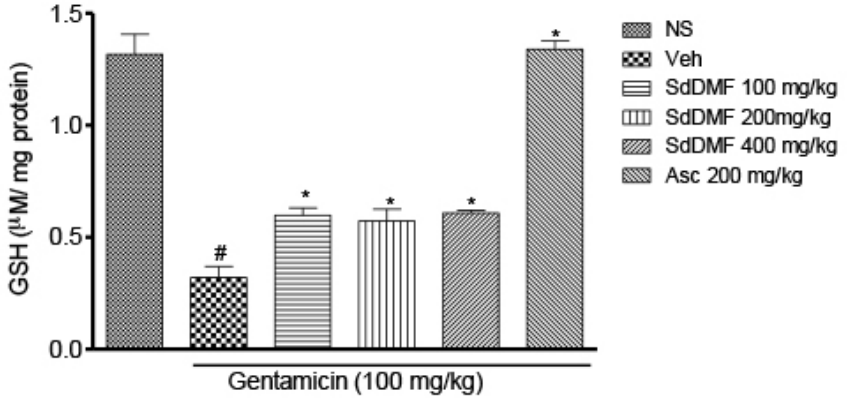

Figure 1. Effect of SdDMF on kidney reduced glutathione concentration in gentamicin-induced nephrotoxicity. Data are expressed as mean ± SEM, n=5. # $p < 0.05$ compared to normal saline (NS), * $p < 0.05$ compared to Vehicle (Gentamicin + normal saline group)

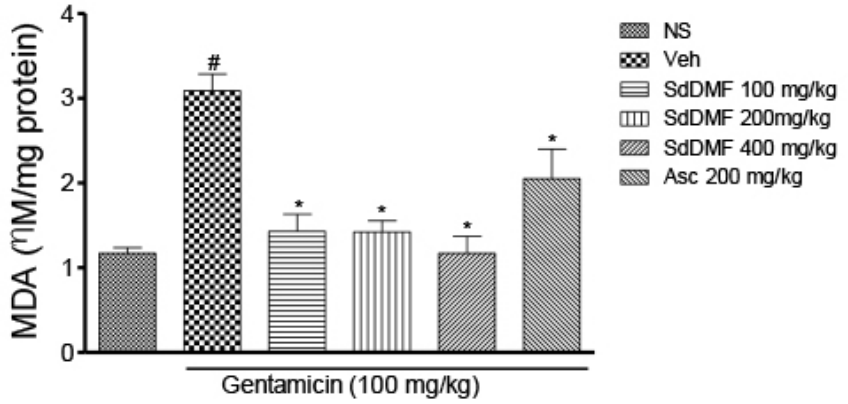

Figure 2. Effect of SdDMF on kidney lipid peroxidation in gentamicin-induced nephrotoxicity. Data are expressed as mean ± SEM, n=5. # $p < 0.05$ compared to normal saline (NS), * $p < 0.05$ compared to Vehicle (Gentamicin + normal saline group)

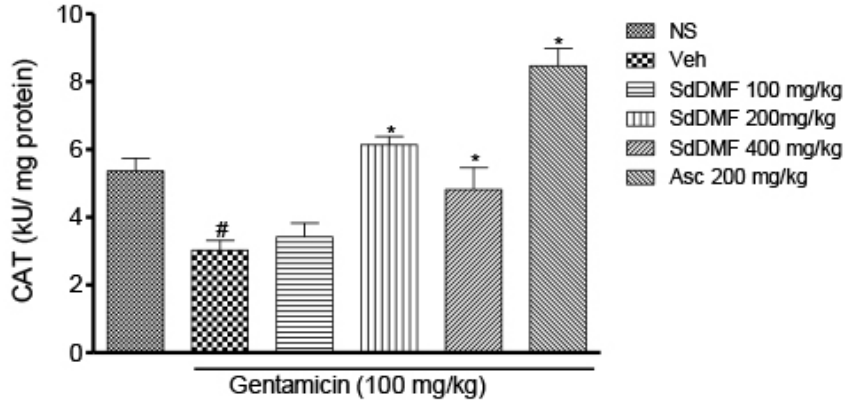

Figure 3. Effect of SdDMF on kidney catalase activities in gentamicin-induced nephrotoxicity. Data are expressed as mean ± SEM, n=5. # $p < 0.05$ compared to normal saline (NS), * $p < 0.05$ compared to Vehicle (Gentamicin + normal saline group)

Table 1. Serum nephrotoxic markers among the extract and ascorbic acid treated groups and controls

Groups/ Parameter	G1 n=5	G2 n=5	G3 n=5	G4 n=5	G5 n=5	G6 n=5	F	P value
Creatinine (mg/dL)	1.00±1.3	4.10±0.1#	2.20±1.5	2.14±0.1*	2.00±1.8*	1.00±1.2*	715.4	0.000
Urea (mg/dL)	1.00±1.3	4.10±0.1#	2.20±1.5	2.14±0.1*	2.00±1.8*	1.00±1.2*	191.9	0.000
BUN (mg/dL)	4.41±0.3	19.14±1.2#	11.00±0.4	9.78±0.6	9.00±1.4*	8.14±1.5*	641.7	0.000

Data are expressed as mean ± SEM, n=5. # $p < 0.05$ compared to normal saline, * $p < 0.05$ compared to Vehicle (Gentamicin + normal saline group). BUN = Blood Urea Nitrogen, G1= Control (normal saline), G2 = 100mg/kg Gentamicin + normal saline, G3 = 100mg/kg Gentamicin + 100 mg/kg SdDMF, G4 = 100mg/kg Gentamicin + 200 mg/kg SdDMF, G5 = 100mg/kg Gentamicin + 400mg/kg SdDMF, and G6 = 100mg/kg Gentamicin + 200mg/kg ascorbic acid.

The administration of gentamicin has been a major cause of nephrotoxicity and this is responsible for its limitation in clinical use. Patients administered with gentamicin are usually being monitored for nephrotoxic side effect expressed as tubular damage[6,17]. The mechanisms involved in tubular damage include activation of oxidative stress resulting from generation of excess reactive oxygen species (ROS) and eventual renal impairment[18,19]. Studies have shown that gentamicin reduces the antioxidant effect of SOD and CAT by stimulating the release of free radicals which damage lipid bilayer of the kidney and eventually causes multiple complications linked to lipid peroxidation[20,21]. The kidney is a major site for drugs and chemicals metabolism because of its ability to extract and concentrate xenobiotics, and also because of its large blood flow which is about 20% of cardiac output[18].

Results obtained from this study revealed a significant ($p < 0.05$) increase in serum creatinine, urea and BUN levels among gentamicin treated group (G2) when compared with the control group (G1). Creatinine, urea and BUN are markers of renal damage and increase serum concentrations of these markers indicate alterations in renal functions caused by gentamicin. Gentamicin had been reported to be nephrotoxic thereby impairing renal functions[22,23,24,25]. The damage is secondary to high ROS released. Previous works had also demonstrated an increase in serum concentrations of urea, creatinine and albumin/creatinine ratio in gentamicin nephrotoxicity[18, 26]. This was said to occur as a result of reduction in glomerular filtration rate[27, 28].

Meanwhile, results from this study also showed a significant reduction ($p < 0.05$) in serum creatinine, urea and BUN concentrations in extract and ascorbic acid treated groups (G3-G6) when compared with gentamicin treated group (G2). These findings corroborate that reported by Amin *et al.* (2018)[29]. Tissue CAT activities; and GSH and MDA concentrations are measures of existing oxidative stress at tissue level. While GSH and CAT are endogenous antioxidants that act to scavenge free radicals, MDA is an indirect measure of the degree of lipid peroxidation and predictive of level of cellular damage in living system. The observed reduction in serum creatinine, urea and BUN in extract and ascorbic acid treated groups (G3-G6) is an indication of protective roles played by phytochemicals with antioxidant properties present in the extract and also that of ascorbic acid. Antioxidant compounds such as vitamin C and garlic extract have been demonstrated to prevent kidney toxicity caused by gentamicin administration and reduction of symptoms associated with kidney disorder[3,30].

Generally, the nephroprotective effect of a compound relies on its ability to prevent the generation and release of free radicals and its anti-inflammatory potentials[31]**.** Thus, the SdDMF protect the kidney from gentamicin-induced toxicity through improvement in oxidant status and a possible antioxidant activity.

Consequently, Our study demonstrated that administration of gentamicin with SdDMF scavenges free radicals, increases the concentration of antioxidant markers, and these eventually causes the reduction of the lipid peroxidation marker (MDA) and gentamicin toxicity in a dose-dependent manner. Further studies needed to be carried out to confirm the possible mechanism of action of SdDMF in ameliorating the nephrotoxicity of gentamicin.

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