American Journal of Biomedical Research, 2025, Vol. 13, No. 1, 8-14
Available online at http://pubs.sciepub.com/ajbr/13/1/2
Published by Science and Education Publishing
DOI:10.12691/ajbr-13-1-2

Cardioprotective Potential of *Calotropis procera* (Giant Milkweed) Leaf Extract on Sodium Nitrite-Induced Toxicity in Wistar Rats

Oluwatosin Emmanuel Adewale, Ayorinde Victor Adediran, Olu Israel Oyewole*

Department of Biochemistry, Osun State University, Osogbo, Nigeria
*Corresponding author: oeadewal@buffalo.edu

Received January 20, 2025; Revised February 22, 2025; Accepted March 01, 2025

Abstract Sodium nitrite ($NaNO_2$) is frequently used as a preservative for curing meats like hot dogs, bacon, and ham. However, when ingested in high quantity, it gets to toxic level and induces cardiovascular toxicity and other diseases. The aim of this study is to investigate the cardioprotective and antioxidant potential of ethanolic extracts of *Calotropis procera* (*C. procera*) leave(s) on sodium nitrite-induced toxicity and oxidative stress in male Wistar rats. Thirty-two adult male rats were randomly and equally divided into four groups (n=8) and handled daily for 28 days as follows: Group 1; received distilled water only, Group 2; 1 g/L of $NaNO_2$, Group 3 1 g/L $NaNO_2$ + 250 mg/kg bw of *C. procera* leaf extract and Group 4; 250 mg/kg bw *C. procera* leaf extract. Phytochemical analysis was done. Blood samples were collected from the animals by cardiac puncture at the end of the treatment and serum samples were prepared for cardiac function assay. Heart tissues were excised for histopathological study and estimation of antioxidant capacity. Weight changes were monitored before and at the end of treatment. Results showed the presence of phytochemicals such as alkaloids, flavonoids, tannins, total phenols and saponins. Significant growth changes were observed in groups 1, 2 & 3 over the 28 days compared to the control while group 4 animals had a weight loss. Treatment with *C. procera* reduced significantly the serum induced-increase in creatinine kinase and lactate dehydrogenase. The activity of MDA was reduced by the extract while GSH, GST, CAT, GPX and CAT were in the heart homogenate were significantly increased by the extract. The extract caused no lesion to the architecture of the heart. These results showed that *C. procera* leaf extract ameliorated toxicity in rat hearts and significantly increased antioxidant capacity.

Keywords: *toxicity, cardioprotective, oxidative stress, antioxidant, sodium nitrite*

Cite This Article: Oluwatosin Emmanuel Adewale, Ayorinde Victor Adediran, and Olu Israel Oyewole, "Cardioprotective Potential of *Calotropis procera* (Giant Milkweed) Leaf Extract on Sodium Nitrite-Induced Toxicity in Wistar Rats." *American Journal of Biomedical Research*, vol. 13, no. 1 (2025): 8-14. doi: 10.12691/ajbr-13-1-2.

1. Introduction

Sodium Nitrite is one of the five major preservatives in the food industry globally. It is also used to fight harmful bacteria in processed meat, inhibiting growth of microbes, manufacture of dyes and rubber production [1]. At reduced concentrations, Sodium nitrite serve some medicinal functions including antidote to toxic chemicals, vasodilation and bronchodilation [2]. Despite these benefits, continuous intake of sodium nitrite induces numerous diseases, generates free radical and oxidative stress, induce cancer and multiple organ damage depending on level of exposure. Ansari *et al*., [1] reported induced organ damage in animals acutely exposed to a dosage of between 20-75 mg/kg body weight of sodium nitrite.

Medicinal plants have been a source of remedy to numerous ailments due to their pharmacological characteristics [3]. Currently, over 40 percent of conventional medicines are extracted from medicinal plants [4]. *Calotropis procera* is a perennial flowering plant located majorly in the arid and Semiarid region, belonging to the family of Apocynaceae [5]. It has numerous reported medicinal functions. Hence it usage in the treatment of fevers, asthma, dysentery and cold amongst others [5]. Several research has identified the medicinal activity of the extracts of *C. procera* in the management of cancer [6], diabetes [7], inflammation [8], pain management [8] as well as its antioxidant properties [7]. A major organ affected by the toxic nature of sodium nitrite is the heart. Literature is replete with information on the various medicinal tendencies of *C. procera*. However, there is paucity of information on the cardioprotective and antioxidant activity of *C. procera* on sodium nitrite induced cardio-toxicity in male Wistar rats.

2. Methods

2.1. Plant Material

Calotropis procera (Giant milkweed) leaves were collected from Saki Town, Oyo state, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Ibadan, Ibadan, Oyo State. Leaves were air dried to a constant weight at room temperature for eight (8) weeks. The leaves were pulverized into powdery form by an electric blender and stored in an airtight plastic bowl until use.

2.2. Preparation of Extracts and Phytochemical Screening

Six hundred grams (600 g) of the powdered sample was dissolved in 3.6 litres (1:6 w/v) of absolute ethanol for 14 days to extract the phytochemicals present in the leaves after which the extract was filtered using a white muslin cloth. The mixture of ethanol and the leaf extract was poured into a rotatory evaporator at a temperature of 40^{o}C to evaporate the ethanol. The residue was collected from the round bottom flask of the rotatory evaporator into a beaker. Concentrated extract was obtained by the total removal of ethanol in a water bath. The residue (crude extract) was used to prepare the stock solution for administration. Phytochemical tests were carried out on the extract using standard procedures as described by [9,10,11,12]

2.3. Experimental Design and Animal Grouping

A total of 32 Wistar albino rats weighing between 120–150 g was obtained from the animal house, Department of Physiology, University of Ibadan, Nigeria and divided into four groups of eight rats each. The rats were housed in the Animal House, Department of Biochemistry, University of Ibadan. They were kept (eight in each group) in a ventilated wooden cage at optimum temperature and 12 hrs. light / dark cycle. Rats were given pellets and clean water ad-libitum every day. The rats were acclimatized for seven days before *C. procera* leaf extract and sodium nitrite administration commenced as described

Group I -Normal Control (not exposed to $NaNO_2$)

Group II- 1 g/L $NaNO_2$ (aq)

Group III- 250 mg/kg bw *C. procera* leaf extract only.

Group IV-1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract

The ethanolic leaf extract of *C. procera* was administered by oral gavage daily for 28 days.

2.4. Preparation of Drug

Sodium nitrite (1 g/L) was prepared by dissolving 1g of sodium nitrite salt in 1 Litre of distilled water and administered orally daily.

2.5. Preparation of Serum

At the end of the experiment, the rats were fasted for 24hours before they were sacrificed by cervical dislocation and the blood sample was collected into the plain sample bottles. The blood samples were centrifuged at 3000 rpm for 15 minutes and the serum was collected with pasture pipette and transferred unto another sample bottle and stored at 4^{o}C for the lipase and amylase activities.

2.6. Preparation of Heart Homogenates

The rats were quickly dissected and the heart removed, rinsed in 1.15% KCl, it was then weighed and homogenized in 5vol 0.1 M phosphate buffer (pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 3000 rpm for 15 mins and the supernatant was stored at -4^{o}C temperature.

2.7. Induction of Oxidative Stress

Oxidative stress was induced in the experimental rats by exposure to sodium nitrite (1 g/L) in drinking water ad libitum for 28 days.

2.8. Measurement of Cardiovascular Markers

2.8.1. Measurement of Serum Creatine Kinase Activity

Serum creatine kinase activity was measured using the modified method described by Witt and Trendelenburg [13]

2.8.2. Determination of Serum LDH Activity

Serum lactate dehydrogenase test was assayed using manufacturers protocol as spelt out in the LDH Test Kit (Himedia, CAT: CCK058-500).

2.9. Antioxidant Assay

Serum catalase (CAT) activity, superoxide dismutase (SOD) activity, glutathione s-transferase (GST) activity, glutathione peroxidase-1 (GPX) activity, glutathione (GSH) content and malondialdehyde (MDA) assay were done using the method described [14,15,16,17,18,19] respectively.

2.10. Histopathological Assay

Immediately after dissection, the heart tissues were removed and kept in 10% (v/v) neutral buffered formalin and embedded in melted paraffin wax. The tissues were partitioned with 5 µm thickness and stained with Haematoxylin- Eosin and thereafter Masson's Trichome. The specimen slice was then applied to a microscopic slide and images taken using a Zeiss Primo Star Phase contrast microscope installed with Zeiss Zen 3.3 blue edition software, scale 10 µm, magnification ×400.

2.11. Statistical Analysis

All data were expressed as Mean ± Standard deviation (i.e. mean ± S D). One-way Analysis of Variance ANOVA was used to analyze the data and significant difference was determined at $P<0.05$.

3. Results

3.1. Phytochemical Screening

The phytochemical screening of *Calotropis procera* revealed that the ethanolic extract contains terpenoids, tannins, Saponins, flavonoids, steroids, cardiac glycosides, alkaloids and phenolics while anthraquinones is absent. Table 1.

Table 1. Qualitative Analysis of Phytochemicals in ethanolic extract of *C. procera* leaves

Phytochemicals	Observation
Alkaloids	++
Flavonoid	+
Tannin	++
Phenolics	+
Anthraquinones	Not detected
Cardiac Glycosides	+
Steroids	++
Saponin	+
Terpenoids	+

++ indicates strongly present, + indicates present

3.2. Quantitative Estimation of Secondary Metabolites

The quantitative estimation of the secondary metabolites present in *Calotropis procera* revealed that flavonoids, alkaloids, saponin, tannins and total phenols were most abundant (Table 2).

Table 2. Quantitative phytochemical analysis of *C. procera* leaves

Phytochemicals	Composition
Alkaloids (%)	1.37±0.05
Flavonoid (mg QE/g)	0.17±0.00
Tannin (mg QE/g)	3.60±0.00
Total Phenol (mg/g)	9.65±0.45
Saponin (%)	1.89±0.00

3.3. Effect of Ethanolic Extract of *C. procera* Leaves on the Weight of the Animals

Table 3 reveals weight changes in rats over the period of administration of the ethanolic extract of *C. procera* leaves.

Table 3. Effect of ethanolic extracts of *C. procera* on the weight of rats exposed to sodium nitrite

Group	Mean Weight (g) Day 0	Mean Weight (g) Day 28	Mean Weight Gain (g)
Group I	138.00±2.41	159.00±3.17	21.00
Group II	135.00±3.41	189.00±7.45	54.00
Group III	141.00±1.41	130.00±1.47	-11.00
Group IV	133.00±2.41	166.00±5.36	33.00

Group I: Control, Group II: 1 g/L NaNO2, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L NaNO2 (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Difference in mean weight was expressed.

The results revealed weight gain in the control group, group exposed to only sodium nitrite and the group with sodium nitrite and *C. procera* extract after 28 days. However, a reduction in weight was observed in the group exposed to only the extract of *C. procera*.

3.4. Effects of *C. procera* on Cardiovascular Markers of Rats Exposed to Sodium Nitrite

The results of the Serum creatine kinase-myocardial band (CK-MB) activity and Lactate dehydrogenase (LDH) activity are presented in Figure 1 & Figure 2 respectively. Sodium nitrite resulted in significant increase in creatinine kinase and lactate dehydrogenase activities when compared to the control (Figure 1 & Figure 2). However, animals exposed to sodium nitrite and treated with *C. procera* and the group exposed to only *C. procera* had significantly reduced creatinine kinase and lactate dehydrogenase activities (Figure 1 & Figure 2).

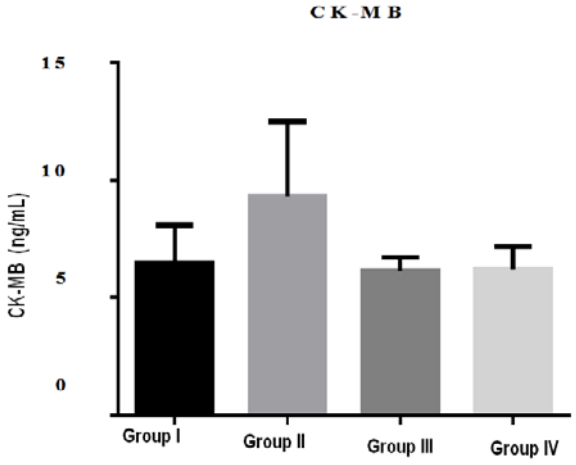

Figure 1. Effect of *C. procera* on creatinine kinase activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

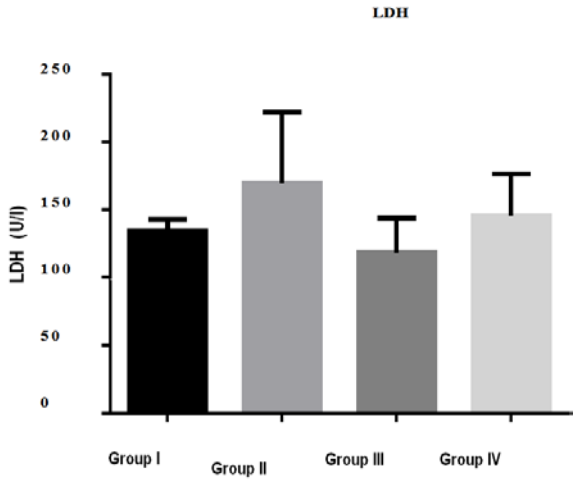

Figure 2. Effect of *C. procera* on lactate dehydrogenase activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

3.5. Effects of *C. procera* on MDA, GSH, SOD, CAT, GSK AND GPX in Rats Exposed to Sodium Nitrite

The results of the antioxidant markers (MDA, GSH, SOD, CAT, GST and GPX) are presented in Figures (3 to 8). 1 g/L of sodium nitrite resulted in a significant increase ($p<0.05$) in the MDA level, GSH level and GPX activity when compared to the control group. However, animals exposed to sodium nitrite and treated with *C. procera* and the group exposed to only *C. procera* had significantly reduced MDA activity, increased GSH and GPX respectively when compared with group I and II (Figure 3, Figure 4 & Figure 8). Furthermore, sodium nitrite reduced significantly ($p<0.05$) the activities of SOD, CAT & GST when compared to the control (Figure 5, Figure 6, & Figure 7). Administration of *C. procera* treatment to the group exposed to sodium nitrite and the group treated with only *C. procera* had significantly increased ($p<0.05$) SOD and CAT when compared to group II. (Figure 5 & Figure 6). However, GST activity was significantly increased after administration of *C. procera* only (Figure 7). The group that had *C. procera* treatment after induction with sodium nitrite had no significant difference when compared to the group that had only sodium nitrite.

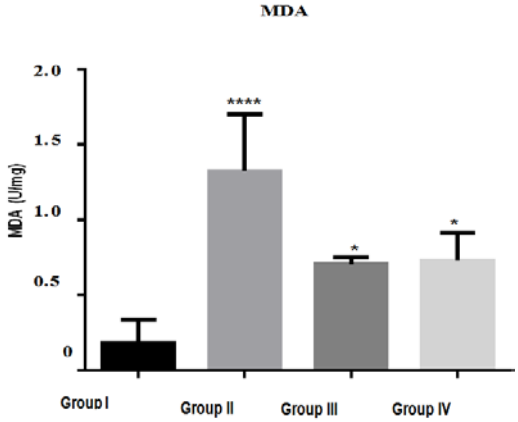

Figure 3. Effect of *C. procera* on MDA activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

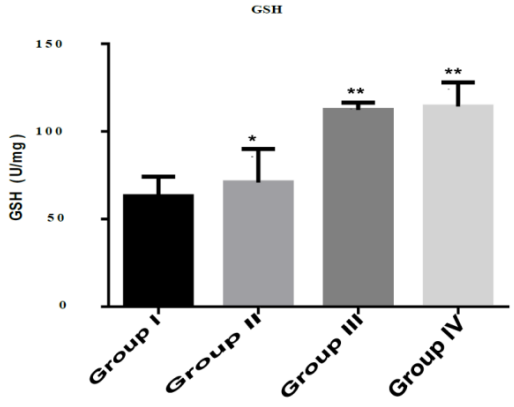

Figure 4. Effect of *C. procera* on GSH of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

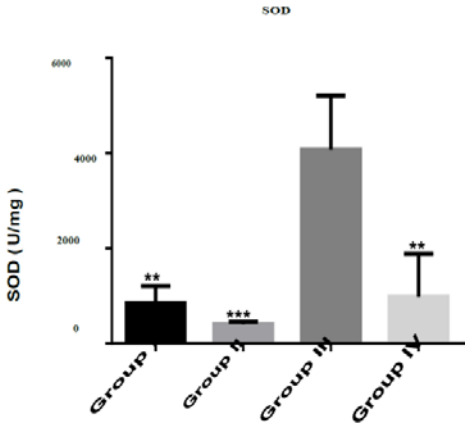

Figure 5. Effect of *C. procera* on SOD activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

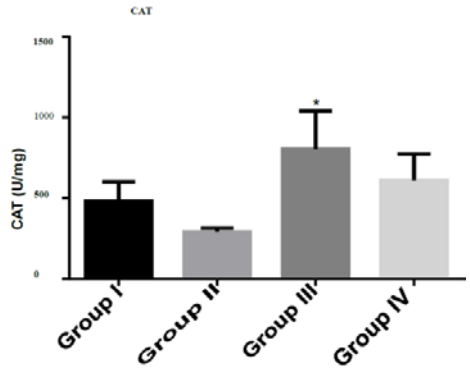

Figure 6. Effect of *C. procera* on CAT activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

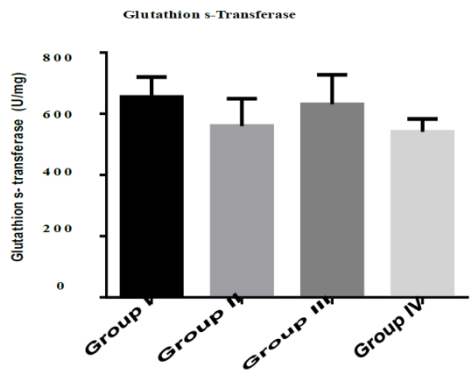

Figure 7. Effect of *C. procera* on GST activity of rats exposed to sodium nitrite

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

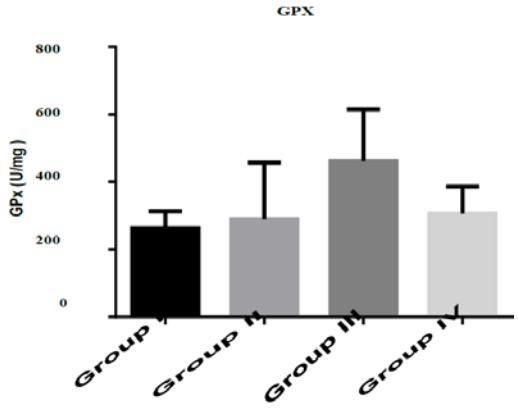

Figure 8. Effect of *C. procera* on GPX activity of rats exposed to sodium nitrite.

Group I: control, Group II: 1 g/L $NaNO_2$, Group III: 250 mg/kg bw *C. procera* leaf extract only, Group IV: 1 g/L $NaNO_2$ (aq) +250 mg/kg bw of *C. procera* leaf extract. Values are expressed as mean ± SD. Significantly different from the control group, $p<0.05$

3.6. Histopathological Examination of the Heart Section

The photomicrographs of the heart sections of rats exposed to sodium nitrite and treated with *C. procera* are presented in Figure 9. There were no visible lesions in all the groups.

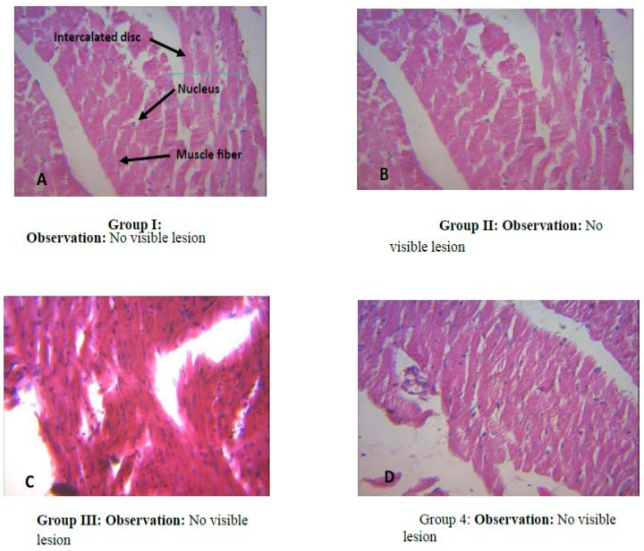

Figure 9. Photo micrograph of a section of rat hearts stained with hematoxylin and eosin (x400)

4. Discussion

C. procera is widely used in traditional medicine across various locations globally such as North Africa, Middle East Asia, and South-East Asia [20]. Currently, various researches are on-going, exploring the pharmacological strength of the plant. At present, it is being extensively explored for its potential pharmacological applications [20]. This study investigated the antioxidant and cardioprotective potentials of the ethanolic extracts of *C. procera* leaves on sodium nitrite induced toxicity in male Wistar rats. The results showed that the ethanolic extracts of *C. procera* leaf was rich in alkaloids, flavonoids, tannins, total phenols and saponins. This composition from this study corresponded to the report of Mainasara *et al.* [21] that identified phytochemicals such as alkaloids, tannins, flavonoids, saponins and cardiac glycosides [21]. However, Ahmad Nejhad *et al.* [22] reported the aqueous extract of *C. procera* to be rich in phenols and flavonoids.

Administration of sodium nitrite increased serum level of CK-MB and LDH. However, the treatment with *C. procera* and the administration of only *C. procera* attenuates the sodium nitrite induced increase of both CK-MB and LDH. These findings are similar to the report of Prananda *et al.* [23] who reported ISO- induced increase in serum CK-MB and LDH which was subsequently reversed following the treatment of *Vernonia amygdalina* ethanol extract. The report from this study further demonstrates the cardioprotective ability of *C. procera* by its ability to reduce the CK-MB and LDH activities in the serum of the animals. Cao *et al.* [24] identified from his review of numerous mouse model study that cardioprotective function can be achieved by manipulating myocardial creatinine level and concluded that the creatinine kinase is a viable therapeutic target to protect against myocardial ischemic reperfusion injury. Increase in serum CK-MB and LDH is an indication of damaged membrane of the cardiac muscle cells into circulation due to leakages. This is a sign of cardiotoxicity. Elevations in LDH and CK-MB levels represent their leakage from the damaged membranes of cardiomyocytes into circulation and were previously shown to be indicators of cardiotoxicity [25,26].

Furthermore, results from this study on the antioxidant potential of *C. procera* revealed a reversal of sodium nitrite toxicity induced increase in serum MDA, GSH and GPX following treatment using the ethanolic extracts of *C. procera* leaves post induction. Gaweł *et al.* [27] reported that an increase in the production of malondialdehyde could be as a result of an increase in free radicals. Malondialdehyde is a known biomarker of oxidative stress that is used in monitoring disease level [28]. Garcia, *et al.* [29] identified increased MDA level as a sign of lipid peroxidation due to oxidative stress and a decreased MDA level to be indicative of enhanced antioxidant capacity. Hence, the reduction in MDA level reported in this study is an indication of the antioxidant capacity of the ethanolic extract of *C. procera* leaf. Glutathione on the other hand is known to be one of the strongest agents against oxidative stress due to generation of free radical and functions in slowing down disease progression [30]. The reduction of GSH concentration increases the tendency of oxidative damage [31]. Hence, the increase in GSH level reported in this study further strengthens the antioxidant capacity of *C. procera*. Glutathione peroxidase (GPX) is an enzyme that is used in cellular reduction of hydroperoxide thus protecting the membrane lipids from oxidation of peroxides [32]. A decrease in the activity of GPX could

trigger oxidative stress whereas an increase reveals its antioxidant capacity. The result from this study revealed an increase in GPX activity after treatment with *C. procera* leaf extract further strengthening the antioxidant potential of the plant. Other results from the study revealed a sodium nitrite induced decrease in SOD, CAT and GST. This was attenuated after treatment with *C. procera*. The SOD is vital in managing reactive oxygen species at the cellular level. Therefore, a decrease in the activity exposes the cell to oxidative stress, leading to early death of the cell [33]. This further strengthens the antioxidant capacity of *C. procera* considering its ability to increase the activity of SOD. Catalase (CAT) on the other hand is a vital antioxidant enzyme that moderates oxidative stress to a large extent by degrading hydrogen peroxide to water and oxygen within the cell [34]. An increase in catalase activity is essential in maintaining the antioxidant capacity of a cell by limiting the effectiveness of hydrogen peroxide. The results of the antioxidant capacity from this study corresponds with the report of Adewale *et al*. [35] who identified a decrease in SOD, CAT and GST after the administration of sodium nitrite and this decrease was attenuated by curcumin. Furthermore, the antioxidant potential of *C. procera* was reported by [22]. Hence the antioxidant potential of *C. procera* must be due to the reported evidence of increased level in GSH, SOD, CAT, GPX & GST coupled with a decrease in MDA of the treated rats.

5. Conclusion

This study reports the cardioprotective and antioxidant capacity of ethanolic extracts of *C. procera* leaf against sodium nitrite induced toxicity in male Wistar rats. The sodium nitrite- induced anomaly in creatinine kinase and LDH, as well as the antioxidants markers monitored were attenuated by treatment with *C. procera*. The ethanolic extracts of *C. procera* in an effective manner ameliorated sodium nitrite-induced cardiac toxicity and oxidative stress by improving creatinine kinase, LDH and inhibiting lipid peroxidation which was supported by the histopathology of the heart showing no observed lesion.

References

[1] Ansari, F. A, Ali, S. N, Arif, H, Khan, A. A, and Mahmood, R. Acute oral dose of sodium nitrite induces redox imbalance, DNA damage, metabolic and histological changes in rat intestine, PLoS One 12 (4) e0175196, 2017.

[2] McNally, B, Griffin, J. L and Roberts, L.D. Dietary inorganic nitrate: from villain to hero in metabolic disease? Mol. Nutr. Food Res. 60 (1) 67–78, 2016.

[3] Bou Malhab, L. J, Bajbouj, K, Shehab, N. G, Elayoty, S. M, Sinoj, J, Adra, S, Taneera, J, Saleh, M. A, Abdel-Rahman, W. M, Semreen, M. H, Alzoubi, K. H, Bustanji, Y, El-Huneidi, W, and Abu-Gharbieh, E. Potential anticancer properties of calotropis procera: An investigation on breast and colon cancer cells. Heliyon. May 26; 9(6): e16706.

[4] Sen, T. and Samanta, S. K. Medicinal plants, human health and biodiversity: a broad review Adv. Biochem. Eng./Biotechnol., 147, pp. 59-110, 2015.

[5] Al-Rowaily, S. L, Abd-ElGawad, A. M, Assaeed, A.M, Elgamal, A. M, Gendy,A, Mohamed, T. A, Dar, B. A, Mohamed, T. K, and Elshamy, A. I. Essential oil of *Calotropis procera*: comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds Molecules, 25 2020.

[6] Al-Qahtani, M. M, Farah, M, Abou-Tarboush, F, Al-Anazi, K, Al-Harbi, N, Ali, M, and Hailan, W. Q. Anticancer effects of *Calotropis procera* latex extract in mcf-7 breast cancer cells Phcog. Mag., 16, pp. 550-556, 2020.

[7] Nadeem, M, Mumtaz,M. W, Danish, M, Rashid, U, Mukhtar, H, Anwar, F and Raza, S. A *Calotropis procera*: UHPLC-QTOF-MS/MS based profiling of bioactives, antioxidant and anti-diabetic potential of leaf extracts and an insight into molecular docking J. Food Meas. Char., 13, pp. 3206-3220, 2019.

[8] Basu, A, and Chaudhuri, A. K. N. Preliminary studies on the anti-inflammatory and analgesic activities of *Calotropis procera* root extract J. Ethnopharmacol., 31, pp. 319-324, 1991.

[9] Sofowora, A. Medicinal Plants and Traditional Medicine in Africa, Spectrum BooksLtd., Ibadan. 289, 1993.

[10] Evans, W.C., Evans, D., and Trease, G. E. Trease and Evans Pharmacognosy. Edinburgh Elsevier. 4(24): pp 35-56, 2009.

[11] Harborne, J. B. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London, Pp. 279, 1973.

[12] Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., and Khan, I. A. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. *Evidence-based Complementary and Alternative Medicine*. 25: 3 pp 8-75, 2014.

[13] Witt, I., and Trendelenburg, C. Gemeinsame Studie zur Erstellung von Richtwerten für klinische-chemische Kenngrössen im Kindesalter [Joint study to establish reference values for clinical chemical parameters in childhood (author's translation)]. Journal of *Clinical Chemistry and Clinical Biochemistry*. 20(4): pp 235–242, 1982.

[14] Claiborne, A. Catalase activity. In: Greenwald, R.A., Ed., *CRC Handbook of Methods for Oxygen Radical Research*, CRC Press, Boca Raton, pp 283-284, 1985.

[15] Misra, H., and Fridowich, I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biology and Chemistry*. 247: pp 3170-3185, 1972.

[16] Flohé, F. and Günzler, W. A. Assays of glutathione peroxidase. Methods in Enzymology 105, pp 114-120, 1984.

[17] Hafeman, D. G., Sunde, R. A., and Hoekstra, W. G. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *The Journal of nutrition*, *104*(5), pp 580–587, 1974.

[18] Moron, M. S., Depierre, J. W., and Mannervik, B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta*, *582*(1), pp 67–78, 1979.

[19] Ohkawa, H., Ohishi, N., and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 95(2): pp 351–358, 1979.

[20] Kaur, A., Batish, D. R., Kaur, S., and Chauhan, B. S. An Overview of the Characteristics and Potential of *Calotropis procera* From Botanical, Ecological, and Economic Perspectives. Front. Plant Sci. 12:690-806, 2021.

[21] Mainasara, M. M., Aliero, B. L. Aliero, A. A. and Yakubu, M. Phytochemical and Antibacterial Properties of Root and Leaf Extracts of *Calotropis procera*. Nigerian Journal of Basic and Applied Science (March, 2012), 20(1): pp 1-6, 2012.

[22] Ahmad Nejhad, A., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., and Mehrnia, M. A. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of *Calotropis procera* leaf aqueous extract. Scientific reports, 13(1), 14716, 2023.

[23] Prananda, A. T., Dalimunthe, A., Harahap, U., Syahputra, R. A., Nugraha, S. E., Situmorang, P. C., Fah, Y. T., Siahaan, J. M., Velaro, A. J., Bilakaya, B., and Harahap, M. A.Y. Phytochemical profiling and cardioprotective activity of *Vernonia amygdalina* ethanol extract (VAEE) against ISO-induced cardiotoxicity in rats. Pharmacia 70(3): pp 758-796, 2023.

[24] Cao, F., Zervou, S., and Lygate, C. A. The creatine kinase system as a therapeutic target for myocardial ischaemia-reperfusion injury. *Biochemical Society transactions*, *46*(5), pp 1119–1127, 2018.

[25] Yen, H.C., Oberley, T.D., Vichitbandha S., Ho Y.S., and St Clair D.K. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. J. Clin. Investig; 98: pp 1253–1260, 1996.

[26] Hadi N., Yousif N.G., Al-amran F.G., Huntei N.K., Mohammad B.I., Ali S.J. Vitamin E and telmisartan attenuates doxorubicin induced cardiac injury in rat through down regulation of inflammatory response. BMC Cardiovasc. Disord; 12: 63, 2012.

[27] Gaweł, S., Wardas, M., Niedworok, E., and Wardas, P. Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiadomosci lekarskie (Warsaw, Poland: 1960)*, *57*(9-10), pp 453–455, 2004.

[28] Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S., and Minciullo, P.L. Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules*, *28*, 5979, 2023.

[29] Garcia, D., Lima, D., da Silva, D. G. H., and de Almeida, E. A. Decreased malondialdehyde levels in fish (Astyanax altiparanae) exposed to diesel: Evidence of metabolism by aldehyde dehydrogenase in the liver and excretion in water. *Ecotoxicology and environmental safety*, *190*, 110107, 2020.

[30] Raj Rai, S., Bhattacharyya, C., Sarkar, A., Chakraborty, S., Sircar, E., Dutta, S., and Sengupta, R. Glutathione: Role in Oxidative/Nitrosative Stress, Antioxidant Defense, and Treatments. ChemistrySelect, 6, pp 4566–4590, 2021.

[31] Giustarini, D., Milzani, A., Dalle-Donne, I., and Rossi, R. How to Increase Cellular Glutathione. *Antioxidants (Basel, Switzerland)*, *12*(5), 1094, 2023.

[32] Sarıkaya, E., and Doğan, S. Glutathione Peroxidase in Health and Diseases. IntechOpen.

[33] Landis GN, Tower J. Superoxide dismutase evolution and life span regulation. Mech Ageing Dev. 2005; 126: 365–79.

[34] Nandi, A., Yan, L. J., Jana, C. K., and Das, N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxidative medicine and cellular longevity*, *2019*, 9613090, 2019.

[35] Adewale, O. O., Samuel, E. S., Manubolu, M., and Pathakoti, K. Curcumin protects sodium nitrite-induced hepatotoxicity in Wistar rats. *Toxicology reports*, *6*, pp 1006–1011, 2019.