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Original Article

Hepatoprotective effect of *Solanum anomalum* leaf on doxorubicin-induced hepatotoxicity in male rats

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Abstract

Background and aims: *Solanum anomalum* Thonn. ex Schumach (Solanaceae) parts are used locally in ethnomedicine to treat various diseases. This work investigated the hepatoprotective potential of the *S. anomalum* to validate its ethnomedicinal uses and give scientific proof to its claimed antidotal activity in folkloric medicine.

Methods: The hepatoprotective activity of *S. anomalum* leaf extract (70-210 mg/kg) was investigated against doxorubicin (DOX)-induced liver injury in rats. Rats were divided into five groups of six rats each and treated concomitantly with DOX (2.5 mg/kg i.p) and leaf extract (70, 140, and 210 mg/kg orally) for 14 days. Vitamin C was used as a standard drug. Liver function indices, liver enzymatic and nonenzymatic antioxidants, malondialdehyde (MDA) level, and histological assessment of the liver were determined to assess the hepatoprotective potentials of the extract.

Results: DOX elevated liver function indices (AST, ALT, ALP, total and direct bilirubin) significantly ($P<0.05$). These parameters were markedly reduced by coadministration of the leaf extract (70-210 mg/kg) ($P<0.05$-0.01) when compared to the DOX-only group. Also, the extract coadministration improved total protein and albumin levels, which were reduced by DOX. Moreso, levels of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) that were decreased by DOX were significantly ($P<0.01$) elevated by the leaf extract. In contrast, the raised MDA level was reduced. The DOX-only group had severe pathological features in its histological liver section, which were comparably reduced in the extract-treated groups. The histopathological changes corroborate other biochemical parameters determined, thus indicating hepatoprotective solid activity.

Conclusion: This study's findings suggest that the leaf extract of *S. anomalum* possesses liver protective potentials, which may be due to the antioxidant activities of its phytochemical constituents. This property can be exploited in the treatment of DOX-related toxicities.

Keywords: Liver, *Solanum anomalum*, Oxidative stress, Antioxidant, Doxorubicin

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Introduction

Doxorubicin (DOX) is an anthracycline glycoside antibiotic with high efficacy against various forms of cancer, whose clinical usefulness has been limited by associated undesirable organ toxicities such as cardiomyopathy, nephrotoxicity, and hepatotoxicity despite its high therapeutic index (1). DOX toxicity has been linked to its unstable metabolite, DOX-semiquinone, which reacts with O_2, producing H_2O_2 and O_{2-} (superoxide) through the enhancement of extramitochondrial oxidative enzymes such as xanthine oxidase and NADPH oxidase and interference with mitochondrial iron export, thereby leading to inhibition of the activities of endogenous enzymatic and nonenzymatic antioxidants causing oxidative stress and damages to organs (1). Antioxidants can protect the organs by scavenging the free radicals generated by the activities of the DOX-semiquinone. The search for a potent antioxidant helpful in preventing or reducing DOX-associated organ damage has been ongoing. Plants serve as a significant reservoir of natural antioxidants utilized over the years to curb the effect of oxidative stress on the body.

Various parts of the shrub, *Solanum anomalum* Thonn. ex Schumach, are utilized as food or local medicine to treat several diseases such as diabetes, gastrointestinal disorders, infections, inflammation, and pains (2,3). The plant is cultivated domestically or in bushes growing in West and East Africa sub-regions. The fruits and leaves have been reported to possess antihyperglycemic effects in rats (3,4). Moreso, *in vivo* and *in vitro* antiplasmodial (5,6), anti-edema (7), antioxidant and antiulcer (8), anticonvulsant and depressant (9), analgesic (10), and antidiarrhoeal (11) potentials are published in the literature on the leaf extract. Secondary metabolites such

as alkaloids, flavonoids, saponins, and tannins as well as phyto compounds like diosgenin, (25(R)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been found in the leaves of the plant (4,5). In this study, we report the hepaprotective potential of the leaf extract against DOX-induced liver toxicity.

Materials and Methods

Plant collection

Solanum anomalum fresh leaves were collected in August 2022 from gardens in the Uruan area, Akwa Ibom State, Nigeria. A taxonomist at the University of Uyo, Uyo, Nigeria, subsequently identified and authenticated them. A specimen of the plant was deposited at the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

Extraction

Washed fresh leaves of *S. anomalum* were chopped into smaller pieces, shade-dried for two weeks, and pulverized to powder using an electric grinder. The powder (1.5 kg) was soaked for three days in 50% ethanol (7.5 L) at room temperature (28 ± 2 ˚C). Later, this was filtered, and the liquid filtrate was concentrated to dryness in *vacuo* 40˚C using a rotary evaporator (BuchiLab, Switzerland). The extract was stored in a refrigerator at -4 ˚C until used for the proposed experiments.

Animals

For these experiments, Male Wistar rats weighing 120-135 g were obtained from the Animal House of the Department of Pharmacology and Toxicology, Madonna University, Elele. The rats were properly cared for in standard cages and maintained on standard pelleted feed (Guinea feed) and water *ad libitum.*

Experimental design

This study used the repeated dose model of 14 days' duration, as described by Olorundare et al (12) earlier. Group I rats pretreated with 10 mL/kg/d of distilled water orally constituted the standard control. Group 2 rats were orally administered normal saline (10 mL/kg/d) but concomitantly treated with DOX hydrochloride (2.5 mg/kg, i.p) dissolved in normal saline intraperitoneally on alternate days for 14 days. *S. anomalum* leaf extract (dissolved in distilled water) was administered to groups 3-5 rats orally at 70, 140, and 210 mg/kg/d, respectively, 2 hours before treatment with DOX (2.5 mg/kg, i.p) on alternate days for 14 days. Vitamin C, a standard antioxidant drug, was orally administered to group 6 rats (positive control) 2 hours before treatment with DOX (2.5 mg/kg, i.p) on alternate days for 14 days. This served as a reference group.

Blood sample collection

At the expiration of the treatment period (14 days), the weights of the rats were taken, and the animals were deprived of food overnight but had free access to water. Diethyl ether anesthetized rats were sacrificed, and a cardiac puncture technique using fine 21G Needles and 5 mL Syringes was used to collect blood samples into plain sample bottles. The rats' livers were identified, harvested, and weighed. The relative weights of the livers were calculated as the liver weight divided by the body weight.

Effect of the leaf extract on liver functions indices and oxidative stress markers

Sera separated from the rats' blood samples were stored at -20 °C and further used to determine liver function indices such as conjugated and total bilirubin, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using Fortress Diagnostic Kits® according to the manufacturer's protocols (13).

The harvested liver of each rat was weighed and divided into two parts: a part of each preserved in 10% formaldehyde for histological processes, and the second part, washed with ice-cold 0.9% NaCl and homogenized in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl by using motor driven Teflon-pestle. The homogenized liver tissues were after that centrifuged at 7000 rpm for 10 min at 4˚C. Oxidative stress markers; superoxide dismutase (SOD) (14), catalase (CAT) (15), glutathione peroxidase (GPx) (16), and reduced glutathione (GSH) (17) and malondialdehyde (MDA) content (18) were determined from the supernatant. These oxidative stress markers were used to assess the antioxidative stress potentials of the extract.

Histopathological studies

The excised fixed livers of rats were used for histological processes. They were processed and stained with hematoxylin and eosin (H&E) (19) according to standard procedures at the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt. Morphological changes were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

Statistical analysis

Data collected from this study was analyzed with Instat GraphPad (GraphPad Prism software Inc. La Jolla, CA, USA) using one-way analysis of variance (ANOVA) followed by a Tukey Kramer multiple comparison post-test. Values were expressed as mean ± SEM and significance relative to the control was considered at $P<0.05$.

Results

Effect of leaf extract on liver weight of rats with DOX-induced hepatotoxicity

Rats treated with DOX (2.5 mg/kg, i.p) on alternate days for 14 days were found to have considerably increased

relative liver weights of the DOX-only treated group compared to that of the standard control group. However, this increase was not statistically significant ($P > 0.05$) compared to control. Concomitant treatment of rats with the leaf extract and DOX caused non-dose-dependent and statistically insignificant ($P > 0.05$) reductions in the relative liver weights of treated rats when compared to the DOX-only treated group (organotoxic control). A similar effect was observed with the standard drug, vitamin C (Table 1).

Effect of Solanum anomalum leaf extract on liver function indices of rats with DOX-induced hepatotoxicity

Levels of liver function indices (AST, ALT, ALP, total and combined bilirubin) of rats administered DOX only (2.5 mg/kg, i.p) on alternate days for 14 days were observed to have been elevated significantly ($P < 0.001$) following the treatment. At the same time, significant decreases ($P < 0.001$) in total protein and albumin levels were also observed when compared to control. Significant ($P < 0.001$) non-dose-dependent decreases in these enzyme activities, and levels of total and combined bilirubin were also observed following concomitant administration of *S. anomalum* leaf extract (70-210 mg/kg) with DOX (2.5 mg/kg, i.p) for 14 days when compared with the DOX only group. However, significant ($P < 0.05$) and dose-dependent elevation of total protein and albumin levels were observed in the extract-treated groups when compared to the DOX-only treated group (Table 2).

Effect of Solanum anomalum leaf extract on liver oxidative stress markers

Administration of DOX (2.5 mg/kg, i.p) on alternate days for 14 days to rats caused significant decreases ($P < 0.05$) in enzymatic and non-enzymatic endogenous antioxidants levels (GSH, SOD, CAT, GPX, and GSH) when compared to control. An elevated level of MDA was also observed. Concomitant administration of leaf extract *S. anomalum* (70-210 mg/kg) with DOX elicited significant ($P < 0.05$-0.001) non-dose-dependent improvement in the antioxidant enzymes (SOD, CAT, GPX) levels as well as that of GSH when compared to DOX only treated group. MDA levels were similarly reduced significantly ($P < 0.05$-0.01) in the extract-treated rats relative to the DOX-only group (Table 3).

Table 1. Effect of *Solanum anomalum* leaf extract on the relative liver weight of rats with doxorubicin-induced liver toxicity

Treatment	Dose (mg/ kg)	Relative liver weight (mg)
Normal control	-	0.05 ± 0.01
Doxorubicin	2.5	0.07 ± 0.01
Vitamin C + DOX	20	0.05 ± 0.01
	70	0.06 ± 0.01
Extract + DOX	140	0.05 ± 0.01
	210	0.05 ± 0.01

Data are expressed as MEAN ± SEM (n = 6).

Effect of Solanum anomalum leaf extract on the histology of the liver of rats with DOX-induced injury

The histological section of the livers of rats treated with distilled water depicts normal central veins and normal hepatocytes. In contrast, the livers of rats treated with DOX (2.5 mg/kg) alone showed areas of necrosis of hepatocytes, with adjacent hepatocytes appearing normal. However, the livers of rats concomitantly administered with 70 and 140 mg/kg of leaf extract and DOX showed sections with normal hepatocytes and central veins. Livers of rats treated with 210 mg/kg of *S. anomalum* leaf extract and DOX showed sections of livers with normal central veins and hepatocytes containing lipid macrovesicles in their cytoplasm. Liver sections of rats concomitantly treated with vitamin C (40 mg/kg) and DOX showed sections with necrosis of hepatocytes around the portal triad. The portal triad showed mild lymphocytic infiltrate (Figures 1A-F).

Discussion

The present study investigated the effect of *Solanum anomalum* leaf extract on DOX-induced liver toxicity in rats. DOX administration was found in this study to have increased the liver weights significantly. At the same time, co-administration of the leaf extract with DOX suppressed this increase in the liver weight of rats. Injuries and toxicities to internal organs are commonly reflected in the increased weight and size of the organ (20,21), which result from inflammatory processes in the organs. DOX, an anthracycline glycoside antibiotic used against various cancer types (22), generates free radicals during its metabolism, destroying hepatic, cardiac, and kidney cells and tissues, limiting its clinical usefulness. The lowered liver weights in extract-treated groups are indicative of the hepatoprotective potentials resulting from the counteractive effect on the free radicals generated by DOX and antioxidant activities of the phytoconstituents6 such as diosgenin (23), 1-octacosanol and octacosane (24-26), squalene (27,28), β-sitosterol (29,30), and phenolic compounds in the leaf extract.

In this study, 14 days of administration of DOX (2.5 mg/kg, i.p) on alternate days to rats elevated significantly ($P < 0.001$) AST, ALT, ALP, total and combined bilirubin levels and lowered complete protein and albumin levels relative to organotoxic control. Thus portraying severe damage to the liver. Concomitant administration of *Solanum anomalum* leaf extract (70-210 mg/kg) with DOX (2.5 mg/kg, i.p) for 14 days lowered the levels of these enzymes and that of total and combined bilirubin in the groups treated with the extract relative to the organotoxic control group. Assessment of liver function can be made by determining the liver function indices (serum ALT, AST, ALP, bilirubin (total and direct), total cholesterol, total protein, and albumin) (31). In cases of liver injuries, blood levels of these enzymes and molecules will increase due to leakages of the cytoplasmic content into the bloodstream, which serves as an indicator of liver

Table 2. Effect of *Solanum anomalum* leaf extract on the liver function parameters of rats with doxorubicin-induced liver toxicity

Treatment	Dose (mg/ kg)	Total Protein (g/ dL)	Albumin (g/dL)	Total Bilirubin (µmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	Combined Bilirubin (µmol/L)
Normal control	-	64.04 ± 2.80	42.24 ± 1.22	5.43 ± 1.06	12.04 ± 1.49	41.28 ± 2.18	32.26 ± 0.96	4.05 ± 0.13
DOX	2.5	44.0 ± 2.80	22.0 ± 1.52	9.86 ± 0.12	26.0 ± 2.05	64.00 ± 2.33	47.33 ± 1.45	6.56 ± 0.23
Vitamin C + DOX	20	59.33 ± 1.76^{b}	33.0 ± 0.57^{a}	6.53 ± 0.48^{c}	11.16 ± 0.55^{c}	45.00 ± 2.46^{a}	31.66 ± 2.72^{b}	4.10 ± 0.37^{b}
	70	64.66 ± 0.66^{c}	35.66 ± 1.20^{b}	6.76 ± 0.31^{c}	9.96 ± 1.01^{c}	49.33 ± 4.25	33.33 ± 1.45^{a}	4.76 ± 0.17^{a}
Extract + DOX	140	80.66 ± 2.33^{c}	46.33 ± 2.33^{c}	6.23 ± 0.14^{c}	13.40 ± 0.83^{c}	43.33 ± 3.84^{a}	34.0 ± 2.51^{a}	4.50 ± 0.36^{b}
	210	61.33 ± 1.85^{c}	35.66 ± 2.96^{b}	6.83 ± 0.54^{b}	16.46 ± 1.00^{b}	48.33 ± 4.91	35.0 ± 2.88^{a}	4.86 ± 0.42^{a}

Data are expressed as mean ± SEM, Significant at $P < 0.001$ when compared to normal control; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, relative to control (n = 6).

Table 3. Effect of *Solanum anomalum* leaf extract on liver oxidative stress markers of rats with doxorubicin-induced toxicity

Parameters/ Treatment	Dose (mg/kg)	SOD (U/mL)	CAT (U/g of protein)	GPx (µg/mL)	GSH (µg/mL)	MDA (µmol/mL)	Relative liver weight (mg)
Normal control	-	0.52 ± 0.01	2.54 ± 0.01	0.090 ± 0.001	2.20 ± 0.01	0.27 ± 0.02	0.05 ± 0.01
DOX	2.5	0.14 ± 0.001^{d}	0.21 ± 0.01^{d}	0.024 ± 0.001^{d}	0.60 ± 0.01^{d}	0.73 ± 0.01^{d}	0.07 ± 0.01
Vitamin C + DOX	20	0.37 ± 0.07^{a}	2.05 ± 0.19^{c}	0.066 ± 0.05^{b}	1.47 ± 0.12^{c}	0.44 ± 0.06^{b}	0.05 ± 0.01
	70	0.38 ± 0.02^{a}	1.31 ± 0.03^{c}	0.047 ± 0.001^{a}	1.07 ± 0.01^{b}	0.42 ± 0.03^{c}	0.06 ± 0.01
Extract + DOX	140	0.35 ± 0.03^{a}	1.03 ± 0.05^{b}	0.051 ± 0.02^{a}	1.14 ± 0.05^{c}	0.49 ± 0.01^{b}	0.05 ± 0.01
	210	0.29 ± 0.02	1.67 ± 0.09^{c}	0.747 ± 0.01^{c}	1.80 ± 0.01^{c}	0.53 ± 0.03^{c}	0.05 ± 0.01

Data are presented as mean ± SEM. Significant at $P < 0.001$ compared to normal control; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, relative to organotoxic control (n = 6).

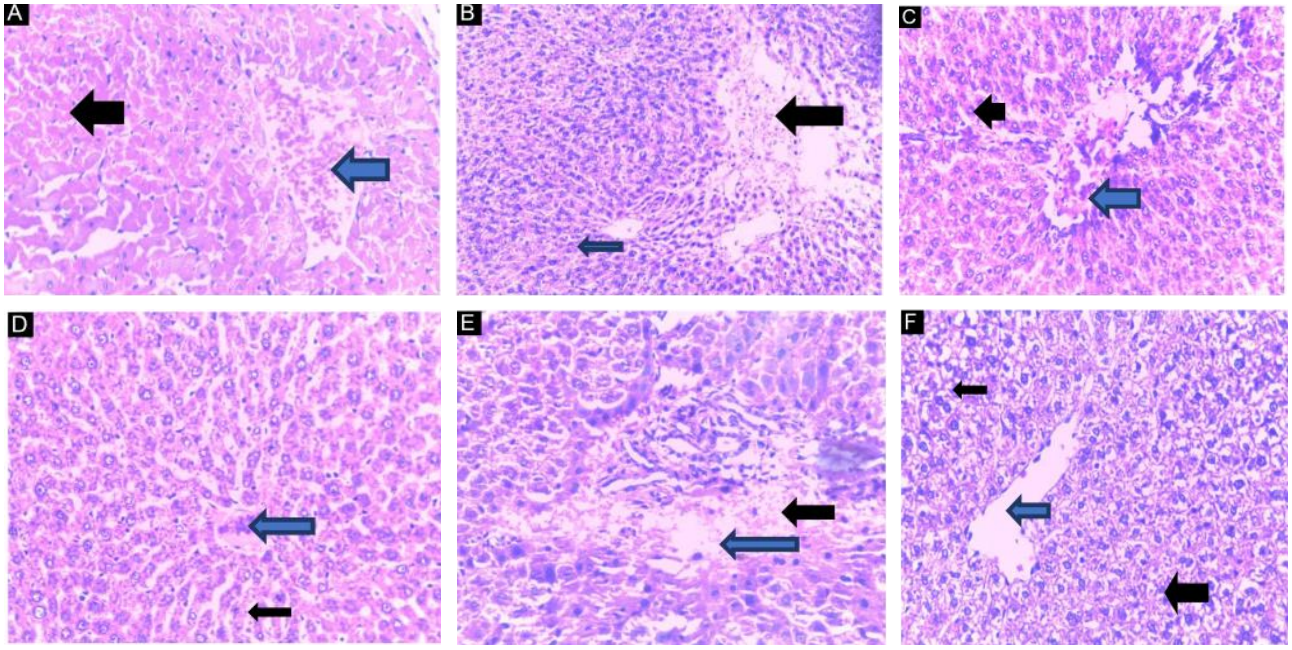

Figure 1. Section of rat livers (A) treated with distilled water showing normal central vein (blue arrow) and normal hepatocytes, (B) treated with doxorubicin (2.5 mg/kg) alone showing section of the liver with areas of necrosis of hepatocytes (black arrow) and normal adjacent hepatocytes (blue arrow), (C) treated with 70 mg/kg of *S. anomalum* leaf extract and doxorubicin showing section of liver with normal hepatocytes (black arrow) and normal central veins (blue arrow), (D) treated with 140 mg/kg of *S. anomalum* leaf extract and doxorubicin showing section of liver with normal central veins (blue arrow) and normal hepatocytes (black arrow), (E) treated with 210 mg/kg of *S. anomalum* leaf extract and doxorubicin section of liver with normal central vein (blue arrow) and hepatocytes containing lipid macrovesicles in their cytoplasm (black arrow) and (F) treated with vitamin C (40 mg/kg) and doxorubicin showing section of liver with necrosis of hepatocytes (blue arrow) around portal triad showing mild lymphocytic infiltrate (black arrow).

damage (32). Observed lowered levels of these enzymes and molecules by the leaf extract in this study are a result of the counteractive activities of phytoconstituents of the leaf extract against free radical scavenging activities, thereby protecting the liver against oxidative stress by free radicals generated by DOX. The effect may partly be due to the antioxidant activities of its phytoconstituents. This result agrees with an earlier study by Offor et al (33) in which significant protection of the liver against Lead-induced liver injury by fruit extract of this plant was reported. Also, Gupta et al (34) said the hepatoprotective effect of *Solanum xanthocarpum*. Thus, the liver protective

activity of *Solanum anomalum* was confirmed.

The administration of DOX (2.5 mg/kg, i.p) in this study to rats caused significant lowering ($P<0.05$) of oxidative stress markers levels such as GSH, SOD, CAT, GPX, and GSH but elevated MDA when compared to control. High MDA level often indicates increased lipid peroxidation and, therefore, oxidative stress activity, which has been reported to be associated with DOX administration (35-37). This trend was observed in this study. Concomitant administration of leaf extract *S. anomalum* (70-210 mg/kg) with DOX improved significantly ($P<0.05$-0.001) though non-dose-dependently the levels of the antioxidant enzymes (SOD, CAT, GPX) and GSH relative to control. The extract treatment lowered MDA levels of the extract-treated rats significantly ($P<0.05$-0.01). It has been documented that DOX suppresses the activities of endogenous antioxidants, as was the case in this study. The leaf extract's potential to reduce the level of MDA shows a reduction in lipid peroxidation and generation of free radicals, which might have been scavenged by the phytoconstituents present in this extract, hence the protective effect on the liver.

DOX was found in this study to cause necrosis of liver cells, leading to damage and obstruction of liver functions. The leaf extract, however, counteracted this effect. DOX-mediated hepatotoxicity is seen as focal damage in hepatocytes, vascular damage, and steatosis (38). Free radicals generated by DOX metabolite DOX semiquinone have been implicated as a significant factor in DOX hepatotoxic action (39). The antioxidant potentials of the phytoconstituents in the leaf extract may have been the mechanisms of hepatoprotective activity of the leaf extract recorded in this study.

Conclusion

The findings of this study show that the leaf extract of *Solanum anomalum* has the potential to counteract the injurious effect of DOX on the liver. This activity can be attributed to its phytochemical constituents' antioxidant and antioxidative stress activities. Thus, the leaf can be used to alleviate and prevent DOX-induced hepatotoxicity.

Authors' Contribution

Conceptualization: Jude E. Okokon, Donald Williams Whyks, Oyepata Simeon Joseph.
Data Curation: Jude E. Okokon, Donald Williams Whyks, Oyepata Simeon Joseph.
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Visualization: Jude E. Okokon, Donald Williams Whyks, Oyepata Simeon Joseph.
Writing–original draft: Jude E. Okokon, Donald Williams Whyks, Oyepata Simeon Joseph.
Writing–review & editing: Jude E. Okokon, Donald Williams Whyks, Oyepata Simeon Joseph.

Competing Interests

The authors declare that there is no conflict of interest.

Ethical Approval

The University Animal Ethics Committee (UAEC) approved the animal study protocol for animal research. Madonna University Nigeria, Elele campus, Rivers State, Nigeria (MU/FP/AE/22/ 23).

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