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Original Article

FUMARATE ATTENUATED DOXORUBICIN-INDUCED CARDIAC INJURY THROUGH MODULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION AND TRANSFORMING GROWTH FACTOR-B1 SIGNALLING

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ABSTRACT

Doxorubicin (dox) is an anticancer agent with adverse effects, such as cardiac injury. Fumarate, a tricarboxylic acid cycle metabolite, has been shown to ameliorate cardiac injury. This study investigated the possible cardioprotective action of fumarate in dox-induced cardiac injury. Male Wistar rats were divided into four groups (n=8); control (distilled water 10 ml/kg, po), dox (10 mg/kg, ip), dox (10 mg/kg, ip) + fumarate (50 mg/kg, po) and dox (10 mg/kg, ip) + fumarate (100 mg/kg, po). The animals were treated for 10 days and euthanized. Blood was withdrawn, the heart was excised and immediately frozen for biochemical and molecular analyses. Fumarate reduced serum levels of troponin-T (57.5 %, $p<0.05$) at 50 mg/kg and (55 % $p<0.05$) at 100 mg/kg in the dox group. Malondialdehyde formation was reduced from 69.6±3.6 mmol/mg in the dox group to 52.4±3.4 nmol MDA/mg protein at 50 mg/kg and 39.4±0.9 nmol MDA/mg protein at 100 mg/kg in fumarate-treated dox rats ($p<0.001$). Similarly, catalase (CAT) (9-fold), superoxide dismutase (SOD) (3-fold), and glutathione peroxidase (GPx) (3-fold) activity, which was significantly decreased in the dox group, was reversed by fumarate at 100 mg/kg. Transforming growth factor (TGF) ß1 expression was reduced by fumarate ($p<0.001$). The expression of endothelial nitric oxide synthase (eNOS) was reduced with the administration of fumarate at 100 mg/kg (26.2±1.6, $p<0.001$) in dox-treated rats. The data have shown that fumarate ameliorated the dox-induced cardiomyopathy by increasing CAT, SOD, and GPx activities and a reduction in TGFß1 and eNOS expression.

KEYWORDS: Cardiac injury, doxorubicin, fumarate, endothelial nitric oxide synthase, Transforming growth factorß1, Troponin.

1. Introduction

The rising prevalence of malignancies worldwide has been worsened by poor treatment outcomes, primarily due to adverse events from anticancer therapy, especially chemotherapy [1]. Chemotherapeutic agents used in the management of malignancies have been associated with devastating and life-threatening adverse effects such as cardiomyopathy, anaemia, nephropathy, hepatotoxicity, gastrointestinal disturbances, emesis, and sometimes death [2,3]. In developing nations, an additional economic burden has limited the choice of chemotherapeutic agents [4]. Hence, there is a constant reliance on certain chemotherapeutic agents such as doxorubicin (dox) due to its affordability, availability, and wide spectrum of activity [5].

Dox, an anthracycline, obtained from Streptomyces peucetius var. caesius is a widely used chemotherapeutic

agent [6,7]. However, with chronic use, dox causes apoptosis-induced cardiomyopathy, which is progressive and irreversible [8]. This leads to a reduction in the pumping efficiency of the heart, potentially resulting in compensatory heart failure. For this reason, dox therapy usually involves the simultaneous administration of medications that are cardioprotective. Some of these agents, such as ß-blockers and phosphodiesterase (PDE-5) inhibitors, reduce the cardiotoxic effect of dox and slow the decline in cardiac function [5]. However, these conventional agents also have pharmacological effects that may become deleterious [5]. Thus, there is a growing search for alternative or newer therapeutic agents with limited side effects [9]. This has led to an increase in the application of natural products as alternative therapies [10,11]. For instance, resveratrol, which ameliorates dox-induced cardiotoxicity via activation of AMP-activated protein kinase (AMPK), has also been employed [5].

Recently, fumarate, the tricarboxylic acid cycle (TCA) product, has been reported to be cardioprotective through upregulation of the nuclear erythroid factor-2 (Nrf2) pathway [12]. Acting through the L-arginine-nitric oxide (NO) pathway, fumarate has been reported to reduce blood pressure via renoprotective mechanisms [13]. The renoprotective actions of fumarate have been linked to modulation of hypoxia inducible factor (HIF-1α), peroxisome proliferator activated receptor (PPARα), and redox signalling [14,15]. These actions have made fumarate a ligand of interest in the cardiorenal system. NO signalling is involved in the mediation of dox-induced cardiotoxicity [13,16,17]. Therefore, fumarate may attenuate the dox-induced cardiac injury due to its effect on NO metabolism. Our study aimed to evaluate the actions of fumarate in dox-induced cardiac injury and explore the possible mechanisms involved.

2. Materials and Methods

2.1 Animals

Male Wistar rats (6-10 weeks; 120–200 g) were kept in the animal facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were placed on pelletized rat food (Top feeds Growers Mash, Super-Deluxe Animal Feed Mills Co. Ltd, Nigeria), kept in a 12-hour light/dark cycle at 26 ± 2℃; humidity, 60 ± 10%, and given ad libitum access to clean water. The protocols for this study were approved by the Institutional Ethics Committee (EC / FP / 034/07) of the Faculty of Pharmacy, University of Benin.

2.2 Experimental Procedure

Male Wistar rats (120g – 200g) were randomly assigned into four (4) groups containing eight (8) animals; group I; Control (distilled water; 10 mL/kg); group II; dox (10 mg/kg i/p); group III; dox (10 mg/kg, i/p) + fumarate (50 mg / kg po.) and group IV; dox (10 mg/kg, i/p) + fumarate (100 mg/kg po.). Dox was administered as a single dose on the first day only. The animal weights were recorded on days 0 and 10. On the last day, the animals were euthanized (ketamine + Xylazine; 100 mg/kg, ip) [18]. Blood (5 ml) was collected in plain bottles and allowed to clot. The clotted blood was centrifuged at 15000 rpm for 30 minutes and the supernatant (serum) was carefully removed for biochemical assays (troponins-T, myoglobin, and creatine kinase MB). The heart was excised and immediately frozen. The homogenization of the heart tissue tissues (for the assays of malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase assays) was as previously described [18]. The isolated heart (50 mg) was cut with a sterilised scalpel and immediately transferred to a triazole solution for transforming growth factor (TGFß1) and endothelial nitric oxide synthase (eNOS) gene expression assay.

The organ weight index was deduced using: (Heart weight (g))/(Body weight (g))

2.3 Biochemical assays

2.3.1. Troponin T, creatinine kinase MB, and myoglobin chemiluminescent assays

Troponin T was assayed with the Abbot ARCHITECT i-system® troponin T chemiluminescent microparticle immunoassay (CMIA). Briefly, the microparticle bottles were mixed by inverting 30 times followed by visual inspection to ensure resuspension of the microparticles before loading on ARCHITECT STAT TROPONIN. Caps of resuspended microparticles were carefully replaced with a septum. The contents of the kit were then placed onto the i-system. The maximum sample volume was calculated and 0.08 ml of the first ARCHITECT STAT TROPONIN test and an additional 0.03 ml of each additional ARCHITECT STAT TROPONIN test from the same sample cup were used. The samples were loaded and assayed within 3 hours. The same procedure was repeated for creatine kinase MB and myoglobin assays following the ARCHITECT i system with STAT protocol for CK MB and myoglobulin respectively [19–22]

2.3.2 Protein concentration assay

The protein content heart homogenate was determined using the Thermo Scientific® micro-BCA protein assay kit. The preparation of the BCA reagent was done by adding 25 parts MA + 24 parts MB + 0.5 parts MC. This was followed by the addition of 0.03 mL of heart homogenate to 0.5 mL of BCA working reagents and 0.497 mL of distilled water. The resulting mixture was kept at 60 ℃ and read at 562 nm using a spectrophotometer. Standard albumin concentrations (4, 8, 10, 12, 16 and 20 µg/mL) were also prepared and read at the same wavelength. A blank solution (0.03 mL of sample mixture, 0.5 mL of BCA working reagent and 0.497 of distilled water) was also prepared [23].

2.3.3. Catalase assay

Catalase (CAT) assay was carried out spectrophotometrically using the method of Koroliuk et al [24]. Briefly, 0.01 mL of the sample was incubated with 100 µmol/mL of hydrogen peroxide (H_2O_2) in 0.05 mmol/L of Tris-HCl buffer pH = 7 for 10 minutes. This was followed by the addition of 0.05 mL of 4% ammonium molybdate to form a yellow complex of ammonium molybdate and H_2O_2 which was measured at 410 nm. One unit of catalase activity was defined as the amount of enzyme required to degrade 1 µmol of H_2O_2 per minute.[24]

2.3.4. Superoxide dismutase assay.

The principle behind this assay was based on the generation of superoxide radicals produced by xanthine oxidase. Xanthine oxidase reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a formazon complex (red dye). Briefly, 0.3 mL of mixed

substrate was added to 0.2 mL of diluted sample. The samples were mixed, and 0.75 mL xanthine oxidase was added to the mixture. The absorbance of the resulting mixture was read at 505 nm using a spectrophotometer. Superoxide dismutase (SOD) activity was deduced according to the instruction in the assay kits (Ransod®-Randox Lab, Antrim, UK) and expressed as U/mL [25].

2.3.5. Glutathione peroxidase assay.

Briefly, a reaction mixture contained 1 mM of glutathione, 0.2 mM of nicotinamide adenine dinucleotide phosphate (NADPH), and 1.4 IU of glutathione reductase in 0.05 M of potassium phosphate buffer, pH 7.0 was prepared. The reaction was initiated by the simultaneous addition of sample (0.3–0.8 mg) and 0.25 mM of H2O2. The change in absorbance at 340 nm was observed for 45 minutes and 1 U of glutathione peroxidase (GPx) activity was defined as the amount required to oxidize 1 mM NADPH/min [26].

2.4 Reactive substances (TBARS) assay

Briefly, 0.25 ml of the homogenised heart was mixed with 0.5 mL working solution (15 % trichloroacetic acid, 0.375 % thiobarbituric acid, and 0.25 N hydrochloric acid). The reaction mixture was placed in boiling water for 10 minutes. The cooled samples were centrifuged at 3000 rpm for 10 minutes. 0.2 ml of each supernatant was placed in microplates, and the absorbance was measured at 535 nm. Quantification of reactive substances with thiobarbituric acid was determined by interpolation from a standard curve of malonaldehyde (MDA) [27].

2.5 Gene Expression Assays

2.5.1 Separation of total ribonucleic acid (RNA)

Isolation of the RNA from the excised heart was done with the Quick-RNA Miniprep Kit®. The RNA was subjected to DNase I (NEB, Cat: M0303S) treatment to remove DNA contaminants. Quantification of the RNA was done spectrophotometrically at 260 nm.

2.5.2. Deoxyribonucleic acid (cDNA) conversion

One (1 µg) of purified RNA was converted to cDNA using the ProtoScript II cDNA synthesis kit®. The reaction was a 3-step reaction with varied temperatures and durations: I., 65 °C for 5 minutes, II., 42 ° C for 1 h, and III., 80 ° C for 5 minutes [28].

2.5.3. Amplification and agarose gel electrophoresis

Amplification involved the use of OneTaqR2X Mix® with the primers (Inqaba Biotec, South Africa); endothelial nitric oxide synthase (eNOS), forward primer: TGGAGCGAGTTGTGGATTG reverse primer: CTACTGGGTCAAAGACAAGAGG. glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward primer CTGGCAGCTCTTCTCAAAGC, reverse primer CCAGGTCATAGAGAGGCTCAA. GAPDH, forward prime CTCCCTGGAGAAGAGCTATGA, reverse primer AGGAAGGAAGGCTGGAAGA. Briefly, 0.025 ml of a mixture containing cDNA forward and reverse primer and Ready Mix Taq PCR mix® was denatured at 95 C for 5 minutes, followed by 30 cycles of further denaturation at 95 ◦C for 30 s, hardening for 30 s, and extension at 72 ◦C for 60 s) and a final extension at 72 ◦C for 10 minutes. The amplified genes were subjected to 1.0 % agarose gel electrophoresis. The resultant bands were analysed with the image J® software.[29]. The same protocol was used for the expression of transforming growth factor (TGFß1) using the primers: forward: CGAGGTGACCTGGGCACCATCCATGAC and reverse: CTGCTCCACCTTGGGCTTGCGACCCAC.

2.6 Statistical analysis

Data are presented as mean±SEM. Data from all assays were subjected to a one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for differences between treatment groups. Data analyses were performed using the Graph pad Prism® 6 software and $p<0.05$ was considered significant.

3. Results

3.1 Fumarate reversed the weight loss and cardiomegaly in doxorubicin-induced cardiac injury.

Table 1 shows that body weights increased by 21 % ±1.3 g, $p<0.01$ in the control group after 10 days. Treatment with a single dose of dox caused a drop in body weight from 153.6±1.5 to 121.8±7.4 g, $p<0.001$. Fumarate did not cause any significant change in the body weight of rats treated with dox. Heart weight was increased in rats treated with dox (0.0052±0.0), compared to the control (0.042±0.0003, $p<0.001$) (Table 2). Similarly, fumarate (50 mg/kg) caused no significant change in the heart size of dox-treated animals (0.0051±0.0 vs 0.0052±0.0). However, fumarate administration at 100 mg/kg, reversed the increase in heart size in the dox group (0.0052±0.0 vs 0.0042±0.0).

Table 1. Body weights of Wistar rats treated with dox and placed on fumarate for 10 days.

Groups	Dose	Weights (g)	
		Day 0	Day 10
Control	10 ml/kg	124.9±3.2	151.2±1.3**
Doxorubicin	10 mg/kg	153.6±1.5	121.8±7.4###
Fumarate	50 mg/kg	121.3±3.6	120.0±5.3
Fumarate	100 mg/kg	122.5±0.8	128.4±2.02

Control (distilled water). **p<0.01 compared to control. ###p<0.001 compared to doxorubicin.

Table 2: Organ weights in Wistar rats with dox-induced cardiac injury treated with fumarate for 10 days.

Groups	Dose	Heart
Control	10 ml/kg	0.0042±0.0000
Doxorubicin	10 mg/kg	0.0052±0.0003**
Fumarate	50 mg/kg	0.0051±0.0003*
Fumarate	100 mg/kg	0.0042±0.0002##

Control (distilled water). **p<0.01, *p<0.05, compared to the control. ##p< 0.01, compared to the doxorubicin group.

3.2 Elevated serum levels of troponin T, creatine kinase MB, and myoglobin in dox-treated animals were significantly reduced by fumarate.

As illustrated in Fig. 1a, dox treatment increased serum levels of troponin-T above control, 0.0±0.0 to 0.43±0.1 µg/ml, $p<0.001$. Fumarate exerted a 41.8 %, $p<0.05$ reduction in troponin-T levels in animals treated with dox. There was a similar 5-fold increase in creatine kinase-MB (Ck-MB) level in animals that only received dox ($p<0.001$), and fumarate caused a 2-fold, $p<0.01$ reduction in Ck-MB levels in these animals (Fig. 1b). Myoglobin levels increased from 0.6±0.0 ng/ml in the control group to 1.8±0.2 ng/ml, $p<0.001$ in the dox group. Fumarate significantly reduced

myoglobin levels in the dox group, from 1.8±0.2 ng/ml to 0.9±0.3 ng/ml at 50 mg/kg and 0.5±0.0 at 100 mg/kg. (Fig.1 1c).

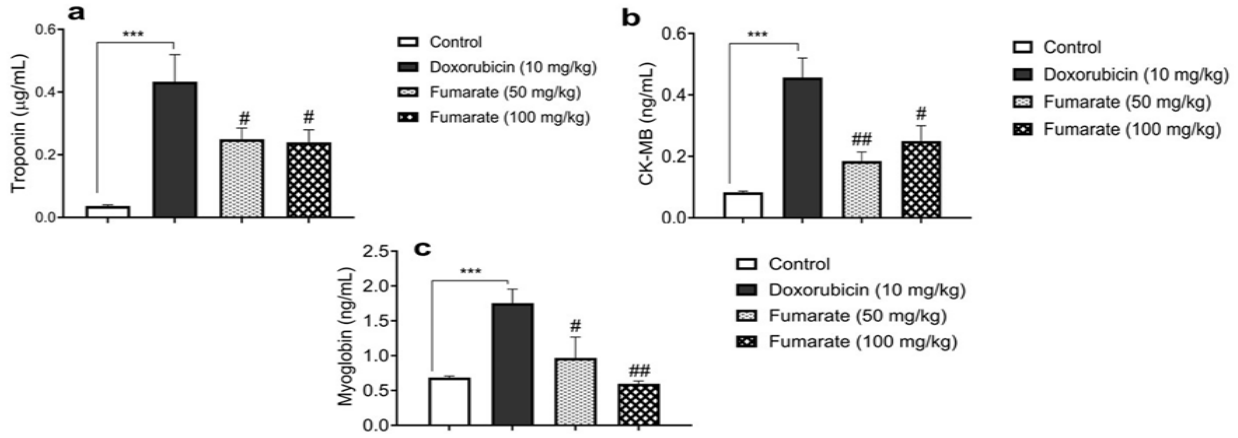

Fig. 1: Effect of fumarate on serum levels of troponin-T, creatine kinase MB, and myoglobin in doxorubicin-treated animals. ***p<0.001 vs control, #p<0.05, ##, p<0.01 vs doxorubicin group

3.3 Fumarate reduced cardiac malonaldehyde levels in dox-induced cardiac injury.

Figure 2 indicates that TBARS increased from control levels (25.3±1.8 nmol MDA/mg protein) to (69.6±3.6 nmol MDA/mg protein, p<0.001) in the dox group. Fumarate reduced TBARS by 24.8 % at 50 mg/kg and 43.5 % at 100 mg/kg in rats treated with dox.

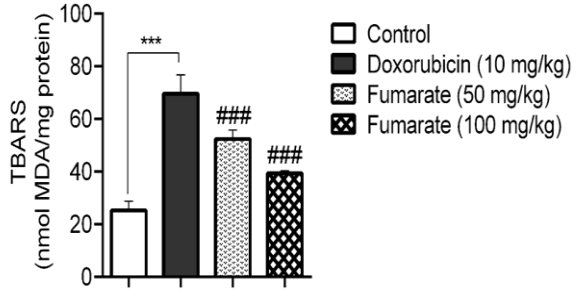

Fig. 2. Thiobarbituric acid reactive substances (TBARS) expressed as malondialdehyde levels in doxorubicin rats treated with graded doses of fumarate. ***p<0.001 vs control, ###p<0.001 vs doxorubicin group.

3.4 Fumarate upregulated the cardiac activities of catalase, superoxide dismutase, and glutathione peroxidase in animals treated with dox.

As shown in Figure 3a, dox caused an 82.4 % decrease in CAT activity, compared to control (p< 0.001). Fumarate exerted a 6-fold and 9-fold increase in CAT activity at 50 and 100 mg / kg, respectively (p<0.001). Consistent with this, SOD activity was increased from 12.8±1.4 units/mg in the dox group to 34.2±2.3 and 41.8±3.4 units/mg after treatment with fumarate at 50 and 100 mg/kg, respectively (p<0.001) (fig 3b). GPx activity was also increased in a dose-dependent manner, from 1.1±0.0 nmol/mg in animals that received dox only, to 2.1±0.0 nmol/mg at 50 mg/kg and 4.1 ±0.1 nmol/mg at 100 mg/kg of fumarate (p<0.001) (fig 3c).

oxide synthase.

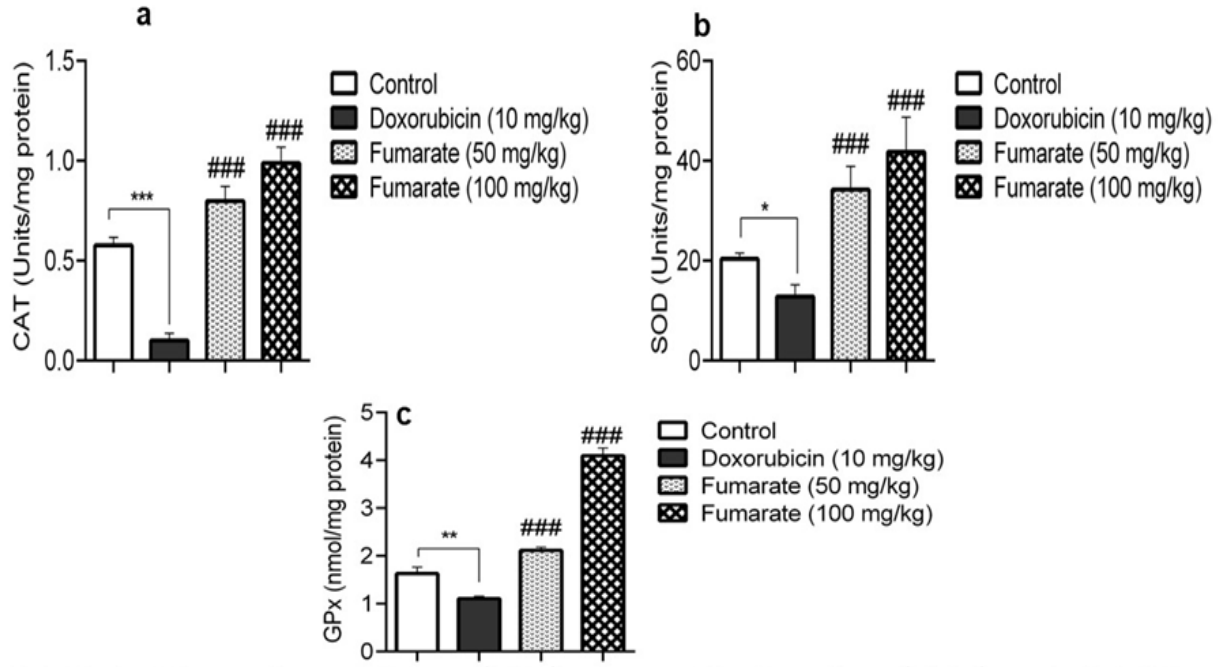

Fig. 3: Effect of fumarate on catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities in doxorubicin-induced cardiac injury. *p<0.05, **p<0.01, ***p<0.001 vs control, ###p<0.001 vs doxorubicin group

3.5 Effect of fumarate on TGFß1 expression in dox-induced cardiac injury.

TGFß1 expression was increased from 103.3±1.3 in the control group to 165.3±2.9 in rats that received dox. Fumarate significantly reduced TGFß1 expression at 50 mg/kg in dox-treated rats (165.3±2.8 vs 137.3±1.7) and 100 mg/kg (165.3±2.8 vs 151.8±1.3) (Fig. 4).

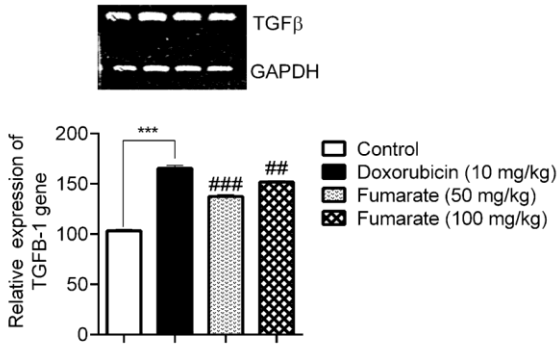

Fig 4: Transforming growth factor (TGFβ1) expression in rats with doxorubicin-induced cardiac injury treated with graded doses of fumarate. ***p<0.001, compared to control. ###p<0.001, ##p<0.01 vs doxorubicin group. Glucose-6-phosphate dehydrogenase (GAPDH).

3.6 Fumarate reduced the expression of endothelial nitric

Fig 5 shows that endothelial nitric oxide synthase (eNOS) expression increased significantly from 33.2±1.9 in the control group to 88.6±1.9 in animals treated with dox. Treatment with fumarate caused a reduction in eNOS expression to 37.2±1.4 at 50 mg/kg and 26.2±1.6 at 100 mg/kg.

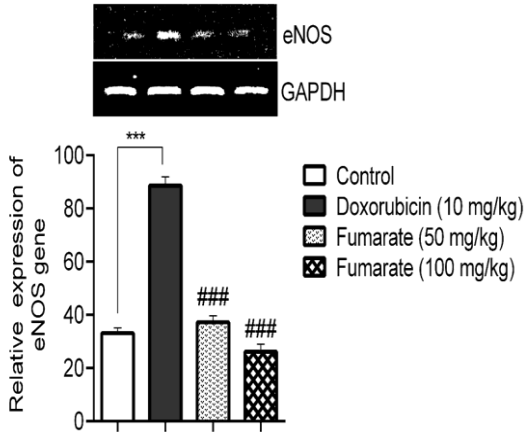

Fig. 5: Effect of fumarate on the expression of endothelial nitric oxide synthase (eNOS) in doxorubicin-induced cardiac injury. ***p<0.001 vs control, ###p<0.001 vs doxorubicin group. Glucose-6-phosphate dehydrogenase (GAPDH).

4. Discussion

This study addresses the novel actions of fumarate in dox-induced cardiac injury. Cachexia, a gradual loss of body weight, is a common pathological feature of malignancy and is usually exacerbated by chemotherapeutic agents such as dox [30]. In this study, body weight significantly decreased in the dox group, and fumarate did not cause any significant changes in the

weights of animals treated with dox. Similarly, organ hypertrophy or atrophy could indicate an underlying pathology and thus serve as an index of toxicity [31]. Cardiomegaly in animals is a structural malformation that could lead to cardiac failure. Cardiomegaly has been reported to occur due to oxidative stress and apoptosis, which interrupt calcium homeostasis needed for cardiac contractility [32,33]. Interestingly, at the highest dose, fumarate reversed cardiomegaly in dox-treated rats. This reversal is indicative of a potential cardioprotective effect, corroborating observations from earlier studies [16].

Recent mechanistic studies have shown that dox-induced cardiac injury is mediated by alterations in mitochondrial redox homeostasis, dysregulated calcium metabolism, and reduced AMPK signalling [34]. Due to its chronic application in the management of malignancies, these signalling mechanisms become exacerbated [35]. For example, altered redox homeostasis favours the peroxidation of cardiac cell membrane structures that causes the formation of aldehydes such as malondialdehyde (MDA). [8] MDA formation is usually regulated by the endogenous antioxidant enzyme system, namely CAT, SOD, and GPx. [36] These enzymes usually curb the activity of reactive radicals and reverse the damage induced by these radicals. Consequently, the activities of these enzymes are significantly depleted in excessive radical production (oxidative stress). [37] This study presents a classical observation in dox-treated animals: a dox-induced increase in the levels of MDA, which depleted the activities of CAT, SOD, and GPx. Fumarate, however, reversed these reductions by eliciting a dose-dependent increase in the activities of these enzymes. This observation is consistent with previous reports where fumarate improved the redox state and acted as an antioxidant through upregulation of antioxidant genes [12].

Troponin T, creatine kinase (CK-MB), and myoglobin are highly expressed in cardiac injury, and these biomolecules serve as established markers of cardiac injury [38]. Troponins are regulatory biomolecules involved in cardiac muscle contraction and are only released into circulation when there is an injury to the cardiac myocytes. Thus, troponins have high specificity for cardiac injury [39]. Troponin T, CK-MB, and myoglobin levels were significantly elevated in dox-treated animals, confirming the cardiotoxic effect of dox. Fumarate reduced the levels of these cardiac injury markers, indicating a possible protective effect on dox-induced injury.

TGFß1 is a key driver of fibrosis [40] TGFß1, acting through Smad-signalling modulates cell proliferation and vascular injury. Fumarate reduced TGFß1 signalling. This reduction in TGFß1 expression may indicate an antifibrotic effect, which possibly improves dox-induced cardiac injury. Recent reports have shown that selectively targeting TGFß1 ameliorates fibrosis [41], and the actions of fumarate have lent credence to these reports. eNOS is an integral enzyme in the cardiovascular system. It regulates vascular homeostasis via the actions of the potent vasodilator, nitric oxide (NO) [42]. However, in the presence of reactive radicals, eNOS undergoes uncoupling, leading to enzyme dysfunction and an increased production of toxic radicals such as peroxynitrite and hydrogen peroxide. Therefore, the overexpression of eNOS is central to the etiology of cardiovascular myopathy [42]. Our study presents a similar observation: an increase in the expression of eNOS in dox-treated animals. Previous studies have shown that dox binds to eNOS (causing eNOS uncoupling) and modifies its action, leading to an increase in the formation of reactive radicals [43]. Similarly, an overexpression of eNOS directly increases the production of NO and NO can react with superoxide anion to form reactive radicals such as peroxynitrite. Peroxynitrite can attack structural proteins and trigger apoptosis in a direct feed-forward cascade involving the activation of caspases, causing cardiotoxic effects (Radi, 2013). Fumarate exerted a dose-dependent reduction in the expression of eNOS in dox-treated animals. This reduction in the expression of eNOS may seem beneficial considering its pathological role in dox-induced cardiac injury.

One of the unique attributes of fumarate is its downstream regulation of nitric oxide signalling [46]. It has been reported that fumarate, acting through the novel aspartate-oxaloacetate pathway, increases NO and reduces blood pressure. However, in our present study, fumarate selectively reduced the expression of eNOS, which was necessary to prevent further production of reactive radicals responsible for dox-induced cardiac injury. Subsequently, the reduction in the expression of eNOS in fumarate-treated dox rats indicates that fumarate modulated the activity of eNOS to exert its potential cardioprotective action.

The observation in this study is contrary to the reported pro-NO production property of fumarate. [13] It suggests that fumarate may augment or reduce NO production depending on existing pathological or physiological influences. [46] Once again, fumarate is reported to exert actions outside the TCA cycle. This is instructive because it supports the newly reported duality of the metabolites of the TCA cycle [47]. Aside from being an antihypertensive with cardio-renoprotective actions, fumarate may modulate genes to protect the cardiac system from exogenous agents such as dox. The actions of fumarate on TGFß1 and eNOS signalling in the etiology of dox-induced cardiac injury may represent a novel mechanistic approach [48] to the management of dox-induced cardiac injury. This could mean that the TCA cycle and its metabolites may also act as endogenous detoxifiers, especially against chemotherapeutic agents. These novel findings may reveal new therapeutic possibilities in the management of dox-induced cardiomyopathies.

Although the findings in this study seem promising, there is the need for further studies to adequately validate our expositions. Limitations involving the lack of quantification of pathological markers such as nuclear erythroid factor (nrf2) and nuclear factor kb (nfκb) would provide clarity on the pleiotropic actions of fumarate in this study. Similarly, the use of a male only rats and the single-dose, acute induction of dox which does not mirror the established cumulative cardiotoxic effect of dox needs to be highlighted. It is also necessary to state that data from the cardiac biomarkers assay were obtained using human immunoassay kits. The adaptation of these assays to our current study was based on previous studies [21, 49] and although they may serve as comparative indicators, the observations have been very valid to the expositions in this study.

4. Conclusions

Fumarate possibly attenuated dox-induced cardiac injury by downregulating TGFß1, eNOS signaling. There was an additional increase in the activities of CAT, SOD and GPX. This may have led to a reduction in dox-induced oxidative damage. These findings suggest that fumarate could act as a potential therapeutic ligand for mitigating dox-induced cardiac injury which is a contributing factor to mortality in cancer[3].

Author Contributions: Conceptualization, OE, AJE and II; methodology, OE, EBO, NE, SOA, EAE, and ER; validation, VE, OE, AJE. and II.; investigation, OE, EBO, NE, SOA, EAE, ER and AJE.; resources, OE and AJE.; data curation, OE, VE and AJE.; writing—original draft preparation, OE, VE, AJE and II.; writing—review and editing, OE and AJE.; visualization, OE and AJE.; supervision, OE, AJE, VE and II.; project administration, OE.; funding acquisition, OE, EBO, NE, SOA, EAE, AJE and ER. All authors have read and agreed to the published version of the manuscript.

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