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2, 5-Hexanedione-Induced Oxidative Damage and DNA Fragmentation: Ameliorative Role of Rutin *Ex Vivo*

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Abstract

The aim of this research was to evaluate the ameliorative effects of rutin on 2, 5-hexanedione-induced oxidative damage and DNA fragmentation in the viscera of Wistar rat *ex vivo*. Blood and tissues homogenates from normal Wistar rats weighing 150-200 g were used for the present investigation. The treatment with 2,5-hexanedione and/or rutin was for four (4), eight (8) and sixteen (16) hours incubation period at room temperature, and used non-specifically at the final concentrations of 0.0, 10.0 µM, 100.0µM, 1.0mM and 10.0mM respectively. The experiments were done in four sequential stages involving the determination of lipid peroxidation and DNA fragmentation by standard procedures. 2,5-hexanedione dose/time-dependently caused a significant ($P < 0.05$) oxidative damage in the blood and tissues via lipid peroxidation and DNA fragmentation relative to control. Rutin administration was able to significantly ($P < 0.05$) suppress the 2,5-hexanedione-induced oxidative and DNA damage. However, the effective toxicity of 2,5-hexanedione and ameliorative effects of rutin were most versus least pronounced in liver (at 10 µM) and pancreas (at 1 mM) homogenates at 8hrs incubation time, respectively. 2,5-hexanedione in the viscera of Wistar rat induces oxidative and DNA damage characterised by higher level of malondialdehyde and DNA fragmentation. However, rutin administration was able to ameliorate these effects *ex vivo.*

Keywords: 2, 5-Hexanedione, Ameliorative, DNA Fragmentation, Lipid Peroxidation, Rutin

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1. Introduction

Nature-nurture controversies pave way for environmental determinism, a system that virtually affects the balance in an ecosystem as a result of direct/indirect exposure to environmental chemicals. These chemicals include organic solvents, toxicants, pro-carcinogens and even carcinogens which are constantly in contact with humans via inhalation and accidental consumptions. An example of such chemical is n-hexane, a solvent that is constantly utilised in our day to day laboratory researches at schools, pharmaceuticals and cosmetics industrial processes (Chen *et al.*, 2015). By implication, a constant chemico-biological interaction between humans and n-hexane is relatively unavoidable. As a result of higher hydrophobicity, it has been reported that about 80-90 % of n-hexane could be absorbed and distributed to lipid-rich tissues and organs such as the brain, peripheral nerves, liver, spleen, kidneys and adrenal glands. These tissues are delicate and naturally endowed with phase I and II metabolizing enzymes which most times facilitates in the bioactivation of xenobiotics like n-hexane. Pharmacokinetics and pharmacodynamics studies have demonstrated that 2,5-hexanedione, a metabolite of n-hexane, is the causative agent associated

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with n-hexane-induced toxicities in the viscera of an organism (Chen *et al.,* 2015; Adedara *et al.,* 2014). The toxicological effects include an impairment of liver and kidney functions through an induction of oxidative stress (Adedara *et al.,* 2014). Other effects include neurotoxicity characterised by aberrant induction of apoptosis via activation of mitochondria-dependent caspase-3 pathway (Chen *et al.,* 2015). Furthermore, 2,5-hexanedione has been reported to alter the biomarkers of reproduction negatively in Wistar rats thereby causing a testicular dysfunction (Boekelheide *et al.,* 2003).

Despite an avalanche of data in relation to cellular and molecular effects of 2,5-hexanedione, there is currently paucity of information in the literature on the possible ameliorative property of natural products or rather functional foods on 2,5-hexanedione-induced oxidative damage. Rutin (quercetin-3-rhamnosyl glucoside), a natural flavone derivative, was first discovered in Buckwheat in the 19th century. It is a low molecular weight polyphenolic compound that is widely distributed in vegetables and fruits which are readily available and affordable within and around our environment for consumptions (Yang *et al.,* 2008). It is a flavonoid with antioxidant, vasodilatory, anti-inflammatory and immune-stimulating activities (Yang *et al.,* 2008; Cristina *et al.,* 2012; Kim *et al.,* 2012; Nafees *et al.* 2015). Other biological activities of rutin includes antihyperglycemic (Kamalakkannan and Prince, 2006), protection of neural damage induced by transient focal ischemia (Khan *et al.,* 2009) as well as amelioration of certain behavioral and electrophysiological alterations induced by arsenic (Sarkozi *et al.,* 2015). This study is primarily designed to evaluate the ameliorative effects of rutin on 2,5-hexanedione-induced oxidative damage and DNA fragmentation in the viscera of Wistar rat *ex vivo*.

The findings, from this study, will further underscore the anti-oxidative and DNA protective properties of rutin against a metabolite of an environmentally-friendly chemical, 2,5-hexanedione. Furthermore, this study is first of its kind using *ex vivo* experimental modus operandi to evaluates the ameliorative effects of rutin on 2,5-hexanedione-induced oxidative damage and DNA fragmentation.

2. Materials and Methods

2.1 Chemicals and Reagents

Rutin (610.517g/mol) and 2,5-hexanedione (114.142g/mol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals/reagents used were of analytical grade except otherwise stated.

2.2 Sample Collections

Ten (10) male Wistar albino rats weighing 150–200 g were used for the present investigation. They were reared at the Animal house of the Faculty of Pharmaceutical Sciences; Ahmadu Bello University Zaria, Nigeria with the approval of Animal rights Review Committee. All animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals of the National Institutes of Health (NIH publication No. 85-23, revised 1985). The rats were humanely sacrificed, thereafter; blood and organs (brain, liver, kidney, heart, lungs and pancreas) were collected. The blood samples were immediately stored in EDTA vacutainers. For organs, excess blood were washed off and parts of the organs homogenized (1:5) in 0.01M phosphate buffer (pH 7.4), thereafter centrifuged at 10,000 rpm for 15 minutes. The supernatant (post-mitochondrial fraction) was decanted and stored at 4^0C for subsequent analysis. The supernatant was kept in ice bags each time it was taken out of the freezer for adequate preservation.

2.3 Treatments

The treatment with 2,5-hexanedione and/or rutin was for four (4), eight (8) and sixteen (16) hours incubation period at room temperature, and used non-specifically at the final concentrations of 0.0, 10.0 µM, 100.0 µM, 1.0 mM and 10.0 mM respectively. These range of concentrations were chosen based on the findings of Abolaji *et al.,* (2014) on the use of environmental toxicants for research purposes. The experiments were in four sequential stages involving the determination of lipid peroxidation and DNA fragmentation. In the first stage, rat's viscera were treated with 2,5-hexanedione and thereafter, subjected to lipid peroxidation assay. The concentrations that showed a strong induction were selected and used at the second stage for DNA fragmentation. In the third and fourth stages 2,5-hexanedione with/without rutin was used for both lipid peroxidation and DNA fragmentation assays using the selected concentrations at stage two.

2.4 Determination of Lipid Peroxidation

This was carried out according to the method described by Ohkawa and Ohishi (1979), with slight modifications. Here, 100 µL of sample was diluted with 100 µL of the

phosphate buffer (pH 7.4) followed by the addition of 100 µL of each 2,5-hexanedione and/or rutin to all tubes except the control which contained 100µL of phosphate buffer (pH 7.4) to normalize the experimental condition. They were incubated for 4/8/16 hrs. Thiobarbituric reagent (2 mL) was added to 150µL of the mixture and incubated in water bath for 1 hr at 90°C then, allowed to cool. It was later centrifuged at 3000 xg for 5 minutes and the supernatant was collected. The absorbance of the pink supernatant (TBA-malondialdehyde complex) was measured at 535 nm. Malondialdehyde formed was then calculated using the molar extinction coefficient of 1.56 x 10^5 $cm^{-1}M^{-1}$ which was the calculated slope after plotting the standard curve for malondialdehyde at different concentrations against their absorbance at 535 nm.

2.5 Determination of DNA Fragmentation

The quantitation of DNA fragmentation with diphenylamine (DPA) was done as described by Sellins and Cohen (1987) with slight modifications. Exactly 1:1 dilution of sample was made using phosphate buffer (pH 7.4) in well labeled tubes. Subsequently, the total of 100 µl of each 2,5-hexanedione and/or rutin was added to another set of tubes labeled B except the control tube (containing 100 µl of 0.01 M phosphate buffer) to normalize the experimental condition, it was gently mixed and incubated for 4/8/16 hrs. The cells were centrifuged at 200xg at 4°C for 10 minutes. The supernatants were carefully transferred into new tubes labeled S. A volume of 100 µl TTE solution was added to the pellets in tubes B and vortexes vigorously. To separate fragmented DNA from intact chromatin, tubes B were centrifuged at 20,000 xg for 10 minutes at 4°C. The supernatants were carefully transferred into new tubes labeled T. Then, 100 µl TTE solution was added to the small pellets in tubes B. 100 µl of 25% TCA was added to tubes T, B and S and vortexes vigorously. Precipitation was allowed to proceed overnight at 4°C. After incubation, precipitated DNA was recovered by pelleting for 10 minutes at 20,000xg at 4°C. Supernatants were discarded by aspiration. DNA was hydrolyzed by adding 160 µl of 5% TCA to each pellet and heated for 15 minutes at 90°C in a heating block. A blank was prepared with 160µl of 5% TCA alone. To each tube, 320 µl of freshly prepared DPA solution was added, vortexes and color allowed to develop for about 4 h at 37°C or overnight at room temperature. 200 µl aliquots of coloured solutions were transferred (ignoring dark particles) from each tube to a glass tube. Absorbance was read at 600 nm. Quantitation was calculated using the formula: % Fragmented DNA = T x 100 / T+B

2.6 Statistical Analysis

To address the biological variability and stability of the samples, each experiment was repeated at least three times and the results were expressed as mean ± standard deviation. Differences between the groups were analyzed by one-way Analysis of Variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 20.0. P-values < 0.05 were considered significant for differences in mean using the Least of Significance Difference (LSD).

3. Results

The induction of Lipid Peroxidation (LPO) and DNA fragmentation by 2,5-hexanedione (2,5-HD) vis-à-vis ameliorative effects of rutin in the brain, blood, heart, kidney, liver, lungs and pancreas of Wistar albino rats *ex vivo* research findings are presented in Table 1-7 stage-wise as described previously under materials and methods section.

In the brain homogenates (Table 1), there was a significant ($P < 0.05$) increase in Malondialdehyde (MDA) level at 4hrs (1 mM and 10 mM 2,5-HD), 8 and 16 hrs (100 µM, 1 mM and 10 mM 2,5-HD), respectively (stage 1). The induction of lipid peroxidation due to MDA level was more pronounced in 100 µM and 10 mM treated samples relative to control and was subsequently used in stage 2 for DNA fragmentation. At this stage, 10 mM treated samples showed higher percentage (%) fragmented DNA as compared to control. In stage 3 and 4 of the experiments, the 10 mM 2,5-HD was used with/without rutin (10 mM), where a significant ($P < 0.05$) decrease in induced MDA level (4 hrs) and DNA fragmentation (4 and 8 hrs) was observed in the presence of rutin.

From Table 2 (in the blood), 2,5-HD significantly ($P < 0.05$) increased the level of MDA at 4 hrs (1 and 10 mM) as compared to untreated samples (stage 1). In stage 2, these concentrations were used for the induction of DNA fragmentation with 1mM treated samples having greater percentage (%) of fragmented DNA when compared to control and this was selected for stage 3 and 4 experiments. Rutin (at stage 3 and 4) was able to significantly ($P < 0.05$) decrease the 2,5-HD induced MDA level and DNA fragmentation at both 4, 8 and 16 hrs incubation time.

Table 1. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the brain of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	Control	2.37±0.23	3.53±0.36	5.49±0.07
	10µM 2,5-HD	3.05±0.07	4.72±0.42	9.81±0.55
	100µM 2,5-HD	2.95±0.46	6.92±0.80*	14.83±1.31*
	1mM 2,5-HD	3.31±0.26*	5.45±0.30*	12.71±1.18*
	10mM 2,5-HD	3.38±0.13*	5.64±0.19*	11.86±1.41*
Stage 2	DNA fragmentation Assay (%)			
	Control	44.16±1.54	48.30±0.64	44.70±1.15
	100µM 2,5-HD		50.40±0.28*	55.4±1.84*
	10mM 2,5-HD	62.63±0.95*		
Stage 3	Malondialdehyde Assay (µM)			
	Control	1.25±0.30	5.80±0.33	3.48±0.04
	10mM 2,5-HD	3.63±0.21 a	6.35±0.44	4.10±0.17a
	10mM Rutin	2.93±0.15ab	5.77±0.11	3.53±0.06b
	2,5-HD + Rutin	2.25±0.13ab	5.92±0.21	4.06±0.13a
Stage 4	DNA fragmentation Assay (%)			
	Control	36.22±1.19	33.43±4.30	48.58±1.72
	10mM 2,5-HD	62.52±3.98a	51.61±2.02a	54.84±0.88a
	10mM Rutin	40.00±1.34b	42.80±1.35ab	44.81±2.24
	2,5-HD + Rutin	54.8±1.32ab	50.29±3.07a	50.51±1.58ab

* - Statistical significant (p<0.05) when compared to control (stage 1 & 2)
a - Statistical significant (p<0.05) when compared to control (stage 3 & 4)
b- Statistical significant (p<0.05) when compared to 2,5-HD (stage 3 & 4)

Table 2. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the blood of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	control	2.49±0.54	3.76±0.09	3.56±0.05
	10µM 2,5-HD	2.88±0.09	4.00±0.23	5.06±1.27
	100µM 2,5-HD	2.07±0.32	4.19±0.32	4.39±0.05
	1mM 2,5-HD	2.68±0.27*	6.50±0.59*	5.89±0.36
	10mM 2,5-HD	3.88±0.41*	4.51±0.87	5.92±0.93
Stage 2	DNA fragmentation Assay (%)			
	control	49.13±1.94	49.0±1.41	43.25±1.86
	1mM 2,5-HD		55.15±1.34*	
	10mM 2,5-HD	50.23±0.68		50.40±1.50
Stage 3	Malondialdehyde Assay (µM)			
	control	1.77±1.33	1.84±0.53b	1.92±0.22
	1mM 2,5-HD	2.01±1.49	5.31±0.13 a	2.24±0.23
	1mM Rutin	1.62±1.61b	1.98±0.26b	1.62±0.20b
	2,5 H-D + Rutin	1.53±6.41b	1.90±0.32b	1.56±0.20b
Stage 4	DNA fragmentation Assay (%)			
	Control	33.76±1.52b	34.80±1.15b	36.40±4.10b
	1mM 2,5-HD	51.76±1.42a	57.30±1.57a	50.50±1.79a
	1mM Rutin	38.66±1.62b	43.00±1.82ab	47.70±6.36
	2,5 H-D + Rutin	46.40±0.36a	45.26±2.48ab	39.30±1.93b

* - Statistical significant (p<0.05) when compared to control (stage 1 & 2)
a - Statistical significant (p<0.05) when compared to control (stage 3 & 4)
b- Statistical significant (p<0.05) when compared to 2,5-HD (stage 3 & 4)

Table 3. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the heart of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	control	1.05±0.19	2.54±0.13	0.75±0.09
	10µM 2,5-HD	1.58±0.09*	2.93±0.23	1.09±0.06
	100µM 2,5-HD	1.84±0.09*	2.97±0.09	5.94±0.35*
	1mM 2,5-HD	1.22±0.06	2.74±0.32	0.89±0.06
	10mM 2,5-HD	1.52±0.04*	3.06±0.09	1.45±0.16 *
Stage 2	DNA fragmentation Assay (%)			
	control	48.24±1.90	29.23±1.25	26.62±0.76
	100µM 2,5-HD	50.18±0.81		53.07±1.65*
	10mM 2,5-HD		33.05±1.11	
Stage 3	Malondialdehyde Assay (µM)			
	control	4.77±0.09[b]	6.24±0.13	2.97±0.30[b]
	100µM 2,5-HD	7.45±0.19[a]	6.63±0.32	5.48±0.06[a]
	100µM Rutin	4.76±0.16[ab]	8.04±1.31	3.55±0.22[a]
	2,5-HD + Rutin	5.18±0.16[ab]	5.51±0.25	3.14±0.19[b]
Stage 4	DNA fragmentation Assay (%)			
	control	45.66±1.16	47.36±0.23	33.10±8.75
	100µM 2,5-HD	53.13±2.05[a]	52.99±1.27	50.93±1.45
	100µM Rutin	42.04±1.44	42.92±1.14	38.09±1.92
	2,5-HD + Rutin	47.35±1.71[b]	46.65±1.25	43.01±1.85

* - Statistical significant ($p<0.05$) when compared to control (stage 1 & 2)
a - Statistical significant ($p<0.05$) when compared to control (stage 3 & 4)
b- Statistical significant ($p<0.05$) when compared to 2,5-HD (stage 3 & 4)

Table 4. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the kidney of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	Control	2.30±0.29	2.48±0.45	1.13±0.35
	10µM 2,5-HD	2.09±0.72	3.65±0.06*	2.20±0.48*
	100µM 2,5-HD	3.50±0.81	3.61±0.09*	0.90±0.11
	1mM 2,5-HD	2.50±0.42	6.05±0.25 *	2.01±0.04
	10mM 2,5-HD	2.50±0.63	3.95±0.29 *	1.65±0.46
Stage 2	DNA fragmentation Assay (%)			
	Control	42.16±1.03	50.45±1.03	45.52±0.83
	10µM 2,5-HD			55.71±1.95*
	100µM 2,5-HD	53.06±1.90*		
	1mM 2,5-HD		62.04±1.74*	
Stage 3	Malondialdehyde Assay (µM)			
	Control	3.38±0.04	6.26±0.04	3.44±0.03
	1mM 2,5-HD	3.99±0.44[a]	6.88±0.27[a]	3.74±0.04[a]
	1mM Rutin	3.31±0.04[b]	6.18±0.04[b]	3.48±0.21[b]
	2,5-HD + Rutin	3.55±0.09	4.30±0.03[ab]	3.61±0.04
Stage 4	DNA fragmentation Assay (%)			
	Control	31.34±1.24[b]	28.38±1.25 [b]	25.91±1.40 [b]
	1mM 2,5-HD	52.52±2.29 [a]	61.92±2.47 [a]	57.29±0.92 [a]
	1mM Rutin	44.49±1.79[ab]	41.76±1.37[ab]	44.96±0.75[ab]
	2,5-HD + Rutin	43.69±1.79[ab]	39.87±1.21[ab]	45.23±1.05[ab]

* - Statistical significant ($p<0.05$) when compared to control (stage 1 & 2)
a - Statistical significant ($p<0.05$) when compared to control (stage 3 & 4)
b- Statistical significant ($p<0.05$) when compared to 2,5-HD (stage 3 & 4)

Table 5. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD on the liver of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	Control	3.21±0.67	1.127±0.41	3.67±0.20
	10µM 2,5-HD	3.428±0.49	2.43±0.23*	3.27±0.23
	100µM 2,5-HD	3.50±1.07	2.09±0.09*	3.33±0.59
	1mM 2,5-HD	7.72±0.14*	2.37±0.41*	4.27±0.45
	10mM 2,5-HD	2.98±0.31	2.25±0.07*	2.74±0.35
Stage 2	DNA fragmentation Assay (%)			
	Control	45.7±0.05	37.9±3.62	32.2±1.98
	10µM 2,5-HD		82.3±2.33 *	
	1mM 2,5-HD	51.4±0.06*		55.1±0.21 *
Stage 3	Malondialdehyde Assay (µM)			
	Control	4.35±0.00	5.73±0.06	5.28±0.08
	10µM 2,5-HD	4.78±0.04	8.0±0.00[a]	7.09±0.43[a]
	10µM Rutin	4.42±0.19	5.8±0.00[b]	5.52±0.93[b]
	2,5 HD + Rutin	4.64±0.09	5.87±0.06[b]	5.19±0.05[b]
Stage 4	DNA fragmentation Assay (%)			
	Control	42.4±1.10[b]	37.4±1.45[b]	37.6±0.49[b]
	10µM 2,5-HD	53.8±1.93[a]	85.4±1.23[a]	65.4±1.56[a]
	10µM Rutin	52.6±1.46[ab]	45.0±0.56[b]	57.9±0.87[ab]
	2,5 HD + Rutin	59.0±1.94[ab]	58.2±0.52[ab]	67.0±1.09[ab]

* - Statistical significant ($p<0.05$) when compared to control (stage 1 & 2)
a - Statistical significant ($p<0.05$) when compared to control (stage 3 & 4)
b- Statistical significant ($p<0.05$) when compared to 2,5-HD (stage 3 & 4)

Table 6. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the lungs of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (µM)			
	Control	1.31±0.16	3.90±0.38	1.44±0.09
	10µM 2,5-HD	1.35±0.19	4.47±0.37	1.86±0.06
	100µM 2,5-HD	1.56±0.32	4.94±0.05*	2.14±0.15*
	1mM 2,5-HD	1.45±0.03	4.42±0.05	1.54±0.00
	10mM 2,5-HD	1.76±0.16	5.28±0.11*	3.27±0.07*
Stage 2	DNA fragmentation Assay (%)			
	Control	36.6±0.08	38.5±1.67	40.8±0.79
	10mM 2,5-HD	51.2±0.20*	58.9±2.17*	56.4±2.47*
Stage 3	Malondialdehyde Assay (µM)			
	Control	1.07±0.04[b]	1.80±0.19[b]	2.33±0.21[b]
	10mM 2,5-HD	3.80±0.07[a]	5.28 ±0.09[a]	5.75±0.04[a]
	10mM Rutin	0.66±0.09[ab]	0.81±0.13[ab]	0.94±0.09[ab]
	2,5-HD + Rutin	1.28±0.06[b]	2.44±0.17[b]	2.59±0.13[b]
Stage 4	DNA fragmentation Assay (%)			
	Control	31.2±1.20[b]	34.2±0.94[b]	26.7±1.99[b]
	10mM 2,5-HD	50.4±0.67[a]	59.0±0.84[a]	56.0±0.97[a]
	10mM Rutin	21.4±1.39[ab]	21.7±1.12[ab]	25.0±0.00[a]
	2,5-HD + Rutin	28.3±3.02[b]	26.7±1.99[ab]	30.8±1.00[b]

* - Statistical significant ($p<0.05$) when compared to control (stage 1 & 2)
a - Statistical significant ($p<0.05$) when compared to control (stage 3 & 4)
b- Statistical significant ($p<0.05$) when compared to 2,5-HD (stage 3 & 4)

Table 7. Induction of lipid peroxidation and DNA fragmentation by 2,5-HD in the pancreas of wistar rat vis-à-vis ameliorative effect of rutin

	Experiment/Treatment	4hrs	8hrs	16hrs
Stage 1	Malondialdehyde Assay (μM)			
	Control	1.35±0.00	5.64±1.90	3.56±0.21
	10μM 2,5-HD	1.43±0.16	8.04±1.31*	6.84±0.89*
	100μM 2,5-HD	2.24±0.32*	6.90±1.48	1.97±0.36
	1mM 2,5-HD	1.51±0.04	10.44±0.63*	4.87±0.63
	10mM 2,5-HD	4.68±0.73*	4.23±0.91	11.82±1.31*
Stage 2	DNA fragmentation Assay (%)			
	Control	29.69±2.12	30.94±0.69	32.2±0.03
	1mM 2,5-HD		51.51±2.64*	
	10mM 2,5-HD	42.36±0.63*		52.3±1.96*
Stage 3	Malondialdehyde Assay (μM)			
	Control	5.71±0.32	5.96±0.23[b]	6.09±0.23[b]
	1mM 2,5-HD	6.13±0.41	8.69±0.83[a]	9.76±0.77[a]
	1mM Rutin	5.26±0.63	5.74±0.16	5.88±0.29
	2,5-HD + Rutin	4.42±0.13[a]	5.57±0.17[b]	5.58±0.32[b]
Stage 4	DNA fragmentation Assay (%)			
	Control	32.86±1.59[b]	40.83±7.68[b]	42.15±0.99[b]
	1mM 2,5-HD	44.28±1.50[a]	52.89±2.6[a]	56.29±1.04[a]
	1mM Rutin	30.51±1.18[b]	37.15±1.19[b]	39.77±5.19[b]
	2,5-HD + Rutin	25.78±1.32[ab]	34.72±1.53[ab]	37.8±1.43[ab]

* - Statistical significant ($p<0.05$) when compared to control (stage 1 & 2)
a - Statistical significant ($p<0.05$) when compared to control (stage 3 & 4)
b- Statistical significant ($p<0.05$) when compared to 2,5-HD (stage 3 & 4)

As depicted in Table 3 (in the heart homogenates), 2,5-HD treated samples showed a significant ($P < 0.05$) increase in MDA level relative to control at 4 and 16 hrs incubation period (stage 1). In stage 2, 100 μM and 10 mM were used for their possible DNA fragmentation induction where it was observed that only 100 μM treated samples (16hrs) presented with a significant ($P < 0.05$) % fragmented DNA as compared to control. The same concentration (100 μM) of 2,5-HD and rutin was used in stage 3 and 4 experiments, and it was revealed that administration of rutin significantly ($P < 0.05$) led to reduction in 2,5-HD induced MDA level and DNA fragmentation.

As seen in Table 4, the renal homogenates treated with 2,5-HD showed a significant ($P < 0.05$) increase in MDA level relative to control at 8 and 16 hrs with 10 μM, 100 μM and 1 mM having greater effects (stage 1). These concentrations were used for stage 2 experiment, and both of them significantly ($P < 0.05$) induces DNA fragmentation at 4, 8 and 16 hrs, respectively, as compared to control with1mM treated samples having more pronounced effects. At stage 3 and 4, rutin was able to ameliorate the 2,5-HD induced LPO and DNA fragmentation significantly ($P < 0.05$).

Experiment conducted with hepatic homogenates (Table 5) shows the ability of 2,5-HD to significantly ($P < 0.05$) increase the level of MDA at 4hrs (1mM) and 8hrs (all the concentrations) as compared to control samples (stage 1). In stage 2, 100 μM and 1 mM were used for DNA fragmentation assay where a significant ($P < 0.05$) induction was observed relative to control. However, 10 μM treated samples at 8 hrs seem to have the highest percentage (%) fragmented DNA which was selected for stage 3 and 4 experiments. At these stages, rutin significantly ($P < 0.05$) decrease the 2,5-HD induced MDA level (8 and 16 hrs) and DNA fragmentation (4, 8 and 16hrs), respectively.

As depicted in Table 6 (in the lungs homogenates), 2,5-HD induced a significant ($P < 0.05$) increase in MDA level as compared to the untreated samples at 8 and 16 hrs, with 10 mM treated samples having the highest MDA level (stage 1) and thus, selected for subsequent experiments. At stage 2, 2,5-HD significantly ($P < 0.05$) increased the % fragmented DNA relative to control. In the presence of rutin at same concentration, a drastic significant ($P < 0.05$) reduction of 2,5-HD induced MDA level and DNA fragmentation were recorded.

From Table 7 (pancreatic homogenates), 2,5-HD

showed a significant ($P < 0.05$) increase of MDA level having more pronounced effects in 1 and 10 mM treated samples relative to control in stage 1. At stage 2, both concentration significantly ($P < 0.05$) increased the percentage (%) fragmented DNA as compared to control. Administration of rutin at same concentration, significantly ($P < 0.05$) led to reduction of elevated MDA level and DNA fragmentation caused by 2,5-HD (stage 3 and 4).

Overall, going through the research findings from Table 1-7, the effective toxicity of 2,5-HD *ex vivo* seems to be most and least pronounced in liver (at 10 µM) and pancreas (at 1 mM) tissues at 8 hrs incubation time, respectively. However, the ameliorative effects of rutin also followed the same pattern being most and least effective in liver (at 10 µM) and pancreas (at 1 mM) tissues at 8hrs incubation time, respectively.

4. Discussion

A toxicological study entails evaluating the metabolic effects of environmentally friendly toxic chemicals at certain concentrations, a situation normally encountered by the population in industrialized countries. Example of such chemical is 2,5-hexanedione which is the main toxic metabolite of n-hexane. Basically after exposure, n-hexane is absorbed into circulation and transported to the liver, the major site of metabolism. In the liver, n-hexane is metabolized to various metabolites that are then distributed in the blood to various organs and tissues, including the liver, kidney, and brain (Marnett, 1999). It is initially hydroxylated by the action of mixed function oxidases to form either 1- or 3-hexanol in a detoxification pathway or 2-hexanol in a bioactivation pathway. Through the bioactivation pathway, 2-hexanol is converted to 2-hexanone and 2,5-hexanediol. Both of these metabolites are then further metabolized to 5-hydroxy-2-hexanone, 2,5-hexanedione, and 4,5-dihydroxy-2-hexanone. 2,5-Hexanedione is believed to be the major toxic metabolite produced in humans (Perbellini *et al.,* 1990; Mayan *et al.,* 2002; Song *et al.,* 2012; Cardona *et al.,* 1996). The blood, brain, liver, kidney, heart, lungs and pancreas participate in different active biochemical processes involving oxidative metabolism and transport functions, respectively (Adedara *et al.,* 2014), hence the basis of their usage in the current study.

Some environmental chemicals exert their toxicity through generation of Reactive Oxygen Species (ROS) which are well known to oxidize the biological systems resulting in the pathogenesis of several diseases (Adedara *et al.,* 2014; Boekelheide *et al.,* 2003) like cancer, hypertension, diabetes, etc. Equally, from the same habitat, so many antioxidant-rich foods containing flavonoids like rutin are readily available and affordable, and as such, their moderate consumption might go a long way in preventing and treating the aforementioned diseases. In this study, we present for the first time the ameliorative effects of rutin on 2,5-hexanedione-induced oxidative damage and DNA fragmentation in the viscera of Wistar rat *ex vivo*.

Our findings indicate that 2,5-hexanedione induced damaging effects in the blood, brain, liver, kidney, heart, lungs and pancreas via lipid peroxidation and DNA fragmentation, having more pronounced effects in liver homogenates. The administration of 2,5-hexanedione has been implicated in oxidative stress due to generation of H_2O_2. The deleterious chemical effects of H_2O_2 molecules can be divided into the categories of direct activity, originating from their oxidizing properties, and indirect activity in which they serve as a source for more deleterious species, such as hydroxyl radicals and hypochlorous acid (Kohen and Nyska, 2002). Reactive oxygen species attack cellular components containing polyunsaturated fatty acid residues to produce peroxyl radicals which undergo a cyclization reaction to form endoperoxides and eventually trans-4-hydroxy-2-nonenal and MDA (Marnett, 1999). The dose/time-dependent increase in the MDA levels observed in this study clearly indicate a state of stress in the tissues possibly induced by 2,5-hexanedione or its metabolites. On the other hand, the ability of 2,5-hexanedione to induce DNA damage has also been reported in mesenchymal stem cells from rat bone marrow and testes (Chen *et al.,* 2015; Boekelheide *et al.,* 2003). However, pharmacokinetics and pharmacodynamics studies have demonstrated that 2,5-hexanedione, a metabolite of n-hexane, is the causative agent associated n-hexane-induced toxicities in the viscera of an organism (Chen *et al.,* 2015; Adedara *et al.,* 2014), supporting the observed higher rate of DNA fragmentations in the current study possibly due to induction of oxidative stress/or intercalation of genetic materials by 2,5-hexanedione or its metabolites. The oxidative and DNA damaging effects of 2,5-hexanedione on delicate organs and tissues have been reported (Song *et al.*, 2012; Ladefoged and Perbellini, 1986; Myall *et al.,* 1990; DeCaprio *et al.,* 1982; Carney *et al.,* 2002), which invariably further corroborated our findings.

Our findings also revealed that rutin administration *ex vivo*, drastically ameliorates the toxicities earlier induced by 2,5-hexanedione through lipid peroxidation and DNA damage characterised by higher level of MDA and fragmented DNA. Rutin is a kind of flavonoid glycoside known as Vitamin P with antioxidant, vasodilatory, anti-inflammatory and immune-stimulating activities (Yang *et al.,* 2008; Marcarini *et al.,* 2011; Kim *et al.,* 2011; Nafees *et al.,* 2015). Other biological activities of rutin includes antihyperglycemic (Kamalakkannan and Prince, 2006) and protection of neural damage induced by transient focal ischemia (Khan *et al.,* 2009). Many cellular protective effects have also been attributed to rutin, which are the results of its high radical scavenging activity and antioxidant capacity (Guo *et al.,* 2007). These properties are potentially beneficial in preventing diseases associated with oxidative stress and protecting the stability of the genome as currently observed from our research findings.

5. Conclusion

The findings from this research revealed deleterious effects of 2,5-hexanedione in the viscera of Wistar rats via an induction of oxidative and DNA damage characterised by higher level of malondialdehyde and DNA fragmentation. However, rutin being a strong antioxidant was able to ameliorate these effects *ex vivo*. Further work should be conducted *in vivo* in this regard.

6. Conflicts of Interest

Authors declare that there is no conflict of interest with regards to the publication of this paper.

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