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Fermented *Ofada* rice (A Nigerian recipe) attenuates renal and hepatic dysfunction induced by crude oil poisoning in Wistar Rats

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Abstract
Objectives: This work examined the antioxidative capacity of *Ofada* rice Koji (ORK) to ameliorate toxicity emanating from Bonny Light Crude Oil (BLCO) on xenobiotic targeted tissues (liver and kidney) in rats.
Methods: Twenty Wistar rats were divided into 4groups (ABCD). Short-term crude oil toxicity was conducted for seven days. Groups A (positive control) were exempted and devoid of the toxicant while B (negative control) to D were administered 4ml BLCO/kg bwt. Group C was fed with 40g of standard rat feed mixed with 60g of fermented ORK powder (60% w/w) while group D animals were given 95g of standard rat feed mixed with 5g of ascorbic acid (ASC) (5g w/w) by composition. ORK and ASC diets were given for 14 days.
Results: ORK intake reduced Malondialdehyde (MDA) formation in the kidney and liver by 46% and 61% respectively. Assessment of Catalase (CAT), Superoxide dismutase (SOD), and Glutathione (GSH), when compared with untreated control rats, were increased by 28%, 45% and 24% respectively (for kidney) and 19%, 34% and 38% respectively (for liver). ORK maintained these enzyme levels by 17%, 4% and 14% respectively (for kidney) and 7%, 14% and 17% respectively (for liver). Renal creatinine and urea were elevated by BLCO treatment but were normalized by ORK intake. The severe alteration in renal and hepatic morphology as observed with BLCO intubation was almost regularized by ORK intake.
Conclusion: Data from this experimental research expresses the ameliorative capacity of ORK in BLCO toxicity.

Keywords: Bonny Light Crude Oil (BLCO), *Ofada* rice Koji (ORK), Ascorbic acid (ASC), Antioxidant activity, Oxidative stress

Plain English Summary
This work investigated the ability of Ofada rice Koji (ORK) to limit damage to the liver and kidney of rats exposed to Bonny Light Crude Oil (BLCO). Short-term crude oil toxicity was carried out for seven days on twenty Wistar rats, which were distributed into five treatment groups. ORK intake lowered damage to kidney and liver membranes, showing a pro-toxicant protection against membrane peroxidation. In comparison to untreated control rats, the levels of Catalase (CAT), Superoxide dismutase (SOD), and glutathione (GSH) were elevated in both the kidney and liver. When ORK was consumed, the drastic changes in hepatic and renal morphology that were seen during BLCO intubation nearly normalized. The results of this experimental study demonstrate ORK's ability to reduce BLCO toxicity.

Background

Crude oil complexity in consonant with its toxicity is consequently a major occurrence in vicinities where crude oil exploration, refining and flaring are relatively high with the particular endorsement to regions defective of environmental control and assessment policies (1, 2). Activities emanating from such

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explorations are major roots of pollution to waterways, lakes, rivers, ponds, and farmlands in Delta communities of Nigeria (3). Currently, the frequency of oil spillage exposure coupled with anthropogenic activities, is predominantly and potentially the key route to aquatic and terrestrial ecosystem hazards within the crude oil-rich zones (4, 5). On entering the environment, crude oil undergoes microbial degradation, biotransformation, and photo inductions which metamorphosize into components that are more volatile, potentially labile and chemically toxic than the parent crude oil base (6). Documentation from previous literature has expounded and propounded that crude oil is endowed with myriads of toxicants and xenobiotics such as polycyclic aromatic hydrocarbons (PAH), Total petroleum hydrocarbon (TPH) and water-soluble fractions (WSF) (7). These toxicants and their metabolites when metabolized by cytochrome P_{450} in living systems are bio-transformed via phase I and phase II reactions into pro-carcinogens causing cancer and other toxicological anomalies (8, 9). The kidney, liver, lungs and skin are sensitive organs which are extremely susceptible and highly impacted by potential toxicants from crude oil (10, 11, 12, 13). Total petroleum hydrocarbons (TPHs) are a collection of hydrocarbon-based chemicals exuding from crude oil such as the aliphatic and aromatic hydrocarbons which include toluene, benzene, xylenes, flourene, naphthalene etc. Hydrocarbon proportions of crude oil (particularly TPH) are naturally hydrophobic (lipid-loving) initiating their lipophilic properties when found in bio-systems (12). Generally, these toxicities are exerted through obstructing metabolic pathways, deactivating enzymatic mechanisms and acting as uncouplers in the electron transport chain (ETC) (14, 15, 16). Furthermore, products from crude oil can form adducts or complexes with macromolecules like DNA, proteins and lipids resulting in cell mutation, amino acid modification or alteration and lipid peroxidation (14, 15). Oxidative stress via free radical production has been reported with crude oil ingestion (17). These ROS by-products when modified oxidatively lead to the peroxidation of lipid membrane that damages cellular biomolecules. Despite the defence mechanism possessed by living organisms, its protective tendency is rather insufficient in scavenging oxidants propelled from both endogenous and exogenous sources (18, 19). In this view, food and medical science are scouting for food and plant products with substantial antioxidant principles to complement or boost endogenous antioxidant systems (20, 21). It is on this premise that *Ofada* rice, a local recipe was investigated. *Ofada* rice is a frontline locally prepared species of *Oryza sativa* consumed largely in the Western province of Nigeria. Its nutritional quality has been demonstrated to surpass that of foreign parboiled rice (22). Other positive and promising qualities such as malting, have been estimated quantitatively and qualitatively high from *Ofada* rice when compared with maize and sorghum (23). Furthermore, studies have adopted fermented *Ofada* rice (*Ofada rice koji* – ORK) to ameliorate lead-induced toxicity in rats (24). This research study substantiated and evaluated the antioxidant property of microbial-fermented *Ofada* rice (*Ofada* rice *koji* – ORK) on some oxidative stress biomarkers generated in the kidney and liver of experimental rats poisoned with BLCO.

Materials and Methods

Animals

Twenty (20) healthy adult Wistar rats (100 – 200g) were used for the study. The experimental animals were deposited in improvised wooden cages. The animals were domiciled in the laboratory of Biochemistry at Delta State University, Abraka. Before the experimental treatment, the rats were allowed to acclimatize within seven (7) days of procurement. The animals were given free access to standard rat Chow twice daily as well as clean water *ad libitum*.

Fermented Ofada rice power (Ofada rice Koji – ORK) Preparation

Ofada rice was bought from "Imakun Omi" Market, in Ogun State, Nigeria. The methodology of the ferment preparation of *Ofada* rice Koji – ORK was performed as described by Yen *et al.* (25) and as modified by Aganbi *et al*. (24). 800g *Ofada* rice grains free pebble and dirt was soaked in a large beaker in distilled water between the temperatures of 80° C -85° C for an hour (1hr) at room temperature. Steaming was immediately initiated at 121° C for 15 minutes to sterilize. 10% v/w of freshly prepared *Saccharomyces cerevisiae* suspension (yeast cells) was inoculated into the sterilized ORK and cultured for 15 days at 30° C (95% relative humidity). The *Ofada* rice-inoculants constituents were subsequently stirred and mixed during the incubation period every 24 hours to accelerate the release of fermented heat. The resultant ORK ferment was heated to dryness for ten minutes in a microwave oven (L.G. Model) and adjusted to a "medium-high" Watt calibration. The dried ORK were then blended into powdery form for feed formulation.

Experimental Procedure

The Wistar rats were divided into a subset of four groups per animal (n=5). Each subset was labelled Group A to D. Animals in group A were completely excluded from bonny light crude oil (BLCO) while groups B, C, and D were administered (by oral intubation) with 4ml crude oil/kg body weight. The crude oil toxicity was performed for seven days. Standard rat feed was given to group A and B animals afterwards, which served as positive and negative controls respectively. Group C was fed with 40g of standard rat feed mixed with 60g of fermented ORK powder (60% w/w) while group D animals were given 95g of standard rat feed mixed with 5g of ascorbic acid (5g w/w) by composition. The administration of ORK and ascorbic acid with standard rat feed was given for 14 days.

Preparation of Liver and Kidney extracts

After the 14th day of feeding, the animals were sacrificed under a diethyl-ether fume-saturated chamber. While under anaesthesia, a vertical incision was made through the thoracic and abdominal region to open the internal organs. The liver and kidney were excised and washed in ice-cold 1.15% KCL solution, blotted, weighed and homogenized using Potterlvegin homogenizer in beakers containing 0.25M sucrose solution. The resulting liver and kidney homogenates were centrifuged at 10,000g for 30 minutes. The collected supernatant was kept in a fridge at about 4°C awaiting usage.

Estimation of biochemical Analytes in the Liver and Kidney

Lipid peroxidation in the tissues (liver and kidney) was estimated colourimetrically by measuring malodialdehyde (MDA) by the procedure of Niehaus and Samuelsson (49). 0.1ml of the respective homogenates were treated with 2ml of TBA –TCA-HCL reagent (TBA 0.37%: 15% TCA: 0.25N HCL - in a ratio of 1:1:1). The mixture was placed in a water bath (make) for 15 minutes, cooled and centrifuged. At 535nm the obtained supernatant was measured against a reference blank. Superoxide (SOD) activity was determined spectrophotometrically by the procedure devised by Fridovich (26) and modified by Isamah *et al.* (27). The principle for SOD determination was based on SOD inhibiting the auto-oxidation of epinephrine by $O_2^{\cdot-}$ generation. Catalase (CAT) was assayed as described by Sinha (28). Colorimetrical assay of CAT was carried out by using the dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid in the ratio of 1:3). The colour intensity was read at 620nm and the quantity of H_2O_2 was calculated for the catalase activity. Reduced glutathione was determined by the procedure of Ellman (29). Exactly 1ml of the respective homogenates (precipitated by 5% TCA) were taken and 0.5ml of Ellman's reagent (composed of 0.0198% DTNB in 1% sodium citrate) and 3ml phosphate buffer (pH 8.0) were added. The developed colour was read at 412nm. The uricase method as described by Caraway (30) was adopted in estimating kidney uric acid concentrations while kidney urea (BUN) was assayed colourimetrically by a modified Berthelot method (Urease) as described by Tietz (31). Commercially prepared reagents for urea and uric acid determination were supplied by Teco Diagnostics, 1268N, Lakeview Ave., Anaheim, USA.

Histological examination

The procedure of Ragavan and Krishnakumari (32) was adopted. In this histological procedure, the isolated kidney was placed in 10% formalin and then impregnated for microtomy sectioning using different graded solutions of alcohol from 70% to 100% to dehydrate the tissue. Afterwards, the tissues were embedded in paraffin wax and slides stained with haematoxylin and eosin (H&E). Embedded stained tissue images were captured employing a digital microscopic eyepiece (Scoptek Dcm 500, 5.0 megapixels) connected to a USB 2.0 computer.

Determination of tissue aliphatic hydrocarbons, PAHs and total hydrocarbon (TPH)

The method as described by Inyang *et al.* (7) was adopted. The aliphatic hydrocarbons and PAHs in samples administered BLCO were quantified by GC-MS. The concentrations and distribution of *n*-alkanes from (C_8 to C_{40}), isoprenoids (pristane and phytane), and a range of PAH compounds were determined in samples collected from the kidney and liver. Calibration curves were plotted, and the average response factor was generated with Agilent Chemstation chromatography software for each analyte from which the low molecular n-alkanes/high molecular n-alkanes and unresolved nalkanes/resolved n-alkanes were obtained (33).

Statistical analysis

The obtained result was expressed as Mean ± SD while the statistical significance of the treatment was analyzed by one-way analysis of variance (ANOVA). For multiple comparisons, a post-Hoc LSD was used. The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 21 Windows software.

Results

Aliphatic hydrocarbons and PAHs in rats

The mean concentrations for individual *n*-alkanes and PAHs; the sum of the *n*-alkanes and PAH

determined and the total hydrocarbon concentrations obtained are shown in Tables 1a, 1b and 1c. The quantification of total petroleum hydrocarbons for group B rats had the highest average *n*-alkane and PAH concentrations for both liver and kidney (Table 1c). Although the concentrations obtained for the hydrocarbon compounds analyzed differed slightly from the standard (ASC group), values analyzed confirmed concentrations not significantly different (P > 0.05).

Table 1a: Total Aliphatic Hydrocarbon Content(mg/L)

Component	Group A (Positive Control)		Group B (Negative Control)		Group C (ORK)		Group D (ASC)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
C8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C12	0.000	0.000	0.000	473.184	0.000	0.000	0.000	0.000
C13	0.690	0.000	6.787	0.011	6.068	0.003	2.402	0.000
C14	0.013	0.003	0.020	0.074	0.001	0.020	0.007	0.001
C15	0.049	0.021	0.015	0.033	0.035	0.073	1.414	0.007
C16	0.079	0.028	0.041	0.061	0.053	0.085	0.068	0.034
C17	0.003	0.038	0.056	0.279	0.141	0.013	0.013	0.051
Pristane	0.040	0.006	1.546	0.000	0.014	0.006	0.004	0.000
C18	0.001	0.000	0.000	0.001	0.002	0.064	0.002	0.000
Phytane	0.133	0.216	0.000	0.090	0.004	0.071	0.053	0.126
C19	0.191	0.009	0.206	0.011	0.009	0.025	0.003	0.006
C20	0.286	0.214	0.000	0.073	0.006	0.198	0.018	0.000
C21	0.120	0.382	2.303	0.049	0.122	0.596	0.136	0.000
C22	0.109	0.562	11.722	0.037	0.022	0.475	0.142	0.000
C23	0.023	0.693	0.023	0.228	0.102	2.685	0.668	0.000
C24	0.005	3.205	16.281	1.361	1.753	0.000	0.098	0.000
C25	0.000	2.247	11.527	0.119	2.570	0.000	1.083	0.000
C26	0.000	0.000	0.000	0.000	0.000	2.703	0.000	0.000
C27	0.047	0.000	107.114	0.000	17.917	0.000	0.000	0.000
C28	0.371	0.000	0.000	24.367	3.131	0.000	0.000	0.000
C29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C30	0.001	3.928	74.358	0.000	0.000	0.000	0.000	0.000
C31	0.000	0.000	0.000	0.000	0.000	15.131	0.000	0.000
C32	0.000	0.000	0.000	0.000	0.000	10.583	0.000	0.000
C33	0.001	2.538	316.639	37.328	78.583	14.776	221.19	46.291
C34	11.440	13.906	9.661	3.947	19.344	10.583	21.153	17.345
C35	6.832	6.094	5.776	0.911	46.119	6.730	13.082	14.628
C36	2.300	17.037	1.449	0.920	2.801	15.005	7.681	4.012
C37	3.197	7.420	1.452	0.078	17.518	8.770	17.923	3.934
C38	0.562	12.786	0.414	0.076	65.479	14.040	7.418	3.580
C40	0.438	2.006	0.008	0.035	68.608	17.703	30.938	4.417
Totalaliphatic (mg/l)	**26.931 ±2.29**	**73.339 ±4.35**	**567.398 ±57.34**	**543.273 ±81.13**	**330.402 ±21.41**	**120.338±5.82**	**325.495 ±38.07**	**94.432 ±8.63**

Table 1b: Poly Aromatic Hydrocarbon Content (mg/L)

Component	Group A (Positive Control)		Group B (Negative Control)		Group C (ORK)		Group D (ASC)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Acenaphthene	0.000	18.827	0.216	18.827	0.217	0.000	0.027	0.000
Acenaphthylene	0.138	3.406	0.249	3.406	0.661	0.013	0.160	0.022
Anthracene	0.895	0.029	0.293	0.029	0.345	0.029	0.015	0.044
1,2-Benzanthracene	0.763	14.741	0.005	14.741	1.586	0.893	0.000	0.015
Benzo(a)pyrene	0.000	10.416	1.059	10.416	0.000	0.964	0.087	0.067
Benzo(b)fluoranthene	0.000	1.034	0.018	1.034	0.000	0.031	0.764	0.022
Benzo(g,h,i)perylene	0.000	3.825	2.074	3.825	9.087	1.146	3.791	0.024
Benzo(k)fluoranthrene	9.119	5.572	51.957	5.572	4.822	2.970	14.401	12.824

Chrysene	3.528	2.985	3.708	2.985	3.766	0.364	0.000	1.226
Dibenz(a,h)anthracene	0.766	2.067	0.938	2.067	6.108	0.376	0.000	0.362
Fluoranthene	0.000	17.432	3.181	17.432	16.401	9.872	0.010	16.493
Fluorene	0.000	27.881	0.036	27.881	32.212	19.905	0.000	8.246
Indeno(1,2,3-cd) pyrene	0.000	0.016	15.741	0.016	0.000	0.000	0.000	0.000
Naphthalene	0.000	0.000	47.852	0.000	0.000	0.000	0.000	0.000
Phenanthrene	0.000	0.000	0.000	0.000	11.410	0.000	0.000	0.000
TotalPAH (mg/L)	**15.209 ±2.42**	**9.830 ±8.67**	**127.327 ±17.29**	**108.23 1±8.67**	**86.615 ±8.85**	**36.563 ±5.46**	**19.255 ±3.76**	**39.345 ±5.36**

Table 1c: Total Petroleum Hydrocarbon Content (mg/L)

Component	Group A (Positive Control)		Group B (Negative Control)		Group C (ORK)		Group D (ASC)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Aliphatics	**26.931±2.29**	**73.339 ±4.35**	**567.398± 57.34**	**543.273 ±81.13**	**330.402 ±21.41**	**120.338 ±5.82**	**325.495 ±38.07**	**94.432 ±8.63**
PAH	**15.209 ±2.42**	**9.830±8.67**	**127.327 ±17.29**	**108.231 ±8.67**	**86.615 ±8.85**	**36.563 ±5.46**	**19.255 ±3.76**	**39.345 ±5.36**
TPH (mg/L)	**42.140 ±5.29**	**83.169 ±14.91**	**694.725 ±75.18**	**651.504 ±89.62**	**417.017 ±29.38**	**156.901 ±11.24**	**344.750 ±46.54**	**133.777 ±13.95**

Biochemical indices for endogenous Antioxidant Enzyme Status

Upon BLCO exposure, a significant ($p < 0.05$) reduction in the activities SOD and CAT were observed both in the kidney and liver of all crude oil-treated groups (Tables 2 and 3). The renal CAT activity dropped by 28%, 17%, and 10%, while its hepatic activity was lower by 19%, 7% and 13% respectively when juxtaposed with the positive control. Consequently, kidney SOD activity decreased by 45%, 4%, and 12%, whereas 34%, 14% and 16% reduction were revealed in the liver correspondingly when compared with the positive control animals. Moreover, observation in CAT and SOD activity upon ORK treatment followed a similar trend in both tissues with a % increase of 13% and 43% (Table 2) for kidney tissues and 13% and 30% (Table 3) respectively for liver cells when juxtaposed with the negative control group. In addition, upon BLCO administration, the index for lipid peroxidation (MDA) increased significantly by 56%, 19%, and 8% for renal tissue and 75%, 31% and 13% for hepatocytes when juxtaposed with the positive control. MDA for both tissues upon ORK was lower by a percentage change of 46% (kidney) and 33% (liver) when compared with the negative control. ASC administration depicted a similar pattern as the ORK administration. Further assessment for both ORK and ASC administration, elevated kidney and liver GSH levels by 8% and 10% respectively and 18% and 9% respectively. Kidney creatinine and urea upon ORK intake significantly ($P<0.05$) dropped by 73% and 49% respectively while liver total protein (TP) had an increased value of 7.06±0.65 when compared with the negative control group of 4.75±0.84.

Table 2: Effect of Fermented *Ofada* rice and Vitamin C on BLCO induced toxicity in Kidney

Treatment groups	kidney (unit/GSH)	Kidney MDA (moles MDA/g wet tissue)	Kidney CAT (unit/mg protein wet tissue)	Kidney SOD (unit/mg protein wet tissue)	Creatinine (mg/dl)	Urea (mg/dl)
Positive control A	31.39±0.89	0.25±0.02	22.21±1.58	26.14±1.21	0.97±0.07	22.06±0.72
Negative control B	25.35±0.66 (24%)*	0.39±0.01 (56%)*	16.11±1.52 (28%)*	14.31±0.56 (45%)*	1.59±0.24 (64%)*	45.69±1.57 (107%)*
ORK C	27.45±0.31 (14%)* (8%)**	0.21±0.03 (19%)* (46%)**	18.40±1.43 (17%)* (13%)**	24.99±2.09 (4%)* (43%)**	0.92 ±0.07 (5%)* (73%)**	30.76±1.43 (39%)* (49%)**
ASC D	27.75±0.35 (13%)* (10%)**	0.23±0.02 (8%)* (41%)**	19.98±1.60 (10%)* (19%)**	23.00±0.79 (12%)* (38%)**	1.21±0.12 (25%)* (31%)**	36.26±0.72 (64%)* (26%)**

*% Change with respect to TPH free (Negative control group), A**%Change with respect to TPH toxicant treatment group (Positive control group), BData are expressed as Mean ± SD of 5 experimental cases. Significantly difference was set at (p<0.05)

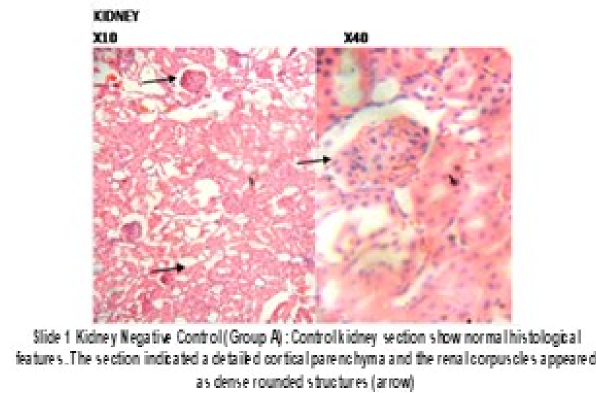
Table 3: Effect of Fermented *Ofada* rice and Vitamin C on BLCO induced toxicity in Liver

Treatment groups	Liver GSH (mg/ml)	Liver GST (µmol/mg protein wet tissue)	Liver MDA (moles MDA/g wet tissue)	Liver CAT (unit/mg protein wet tissue)	Liver SOD (unit/mg protein wet tissue)	Total protein (mg/dl)
Positive Control A	32.18±0.46	0.11±0.03	0.16±0.02	23.00±0.05	31.67±0.12	7.98±0.64
Negative control B	23.37±0.66 (38%)*	0.051±0.06 (116%)*	0.28±0.01 (75%)*	18.65±0.34 (19%)*	20.83±0.32 (34%)*	4.75±0.84*
ORK C	27.57±0.47 (17%)* (18%)**	0.13±0.07 (18%)* (61%)**	0.21±.04 (31%)* (33%)**	21.33±0.85 (7%)* (13%)**	27.15±1.99 (14%)* (30%)**	7.06±0.65
ASC D	25.40±0.30 (27%)* (9%)**	0.07±0.06 (36%)* (37%)**	0.18±0.02 (13%)* (56%)**	19.93±0.02 (13%)* (6%)**	26.67±1.1 (16%)* (28%)**	6.55±0.62

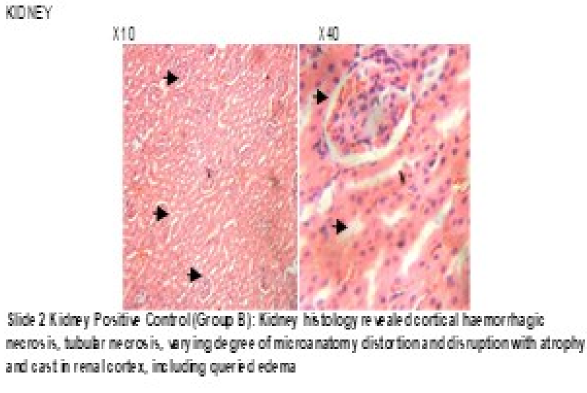
*% Change with respect to TPH free (Negative control group), A**%Change with respect to TPH toxicant treatment group (Positive control group), BData are expressed as Mean ± SD of 5 experimental cases. Significantly difference was set at (p<0.05)

Histological Analysis

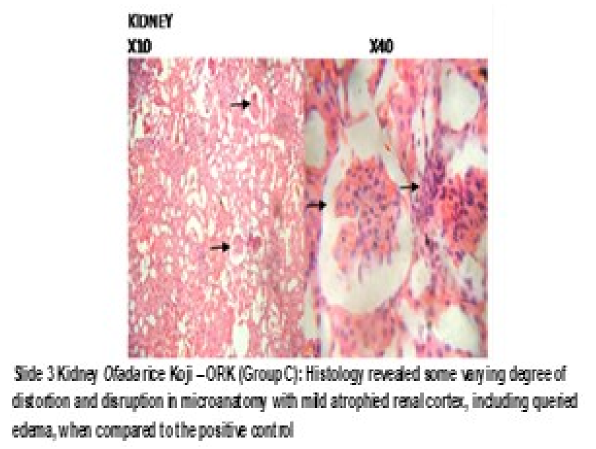
Histological evaluation (Slides 1-8) demonstrated that BLCO had a deleterious effect on both liver and kidney biopsies. In the kidney, groups treated with BLCO demonstrated renal degeneration, distortion and disruption in the microanatomy with atrophied renal cortex, including queried oedema. Liver sections displayed distortion of the lobular pattern of the liver with several foci of oedema and congestion. Observation depicts some areas with periportal inflammatory cells with extensive fibrosis and marked or conspicuous congestion of the intervening veins. Liver tissue features were accompanied by marked periportal hepatolysis and cast within the parenchyma.



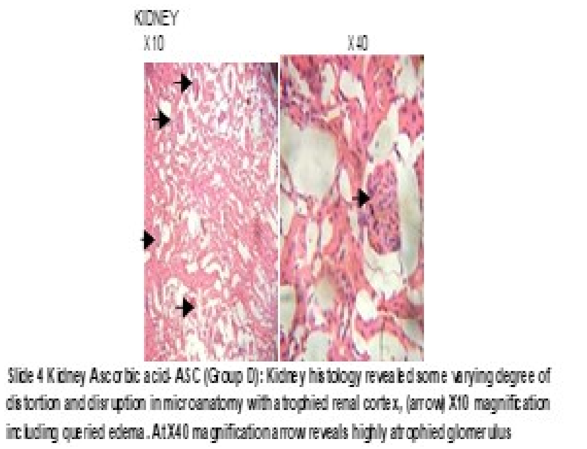
Slide 1 Kidney Negative Control (Group A): Control kidney section show normal histological features. The section indicated a detailed cortical parenchyma and the renal corpuscles appeared as dense rounded structures (arrow)



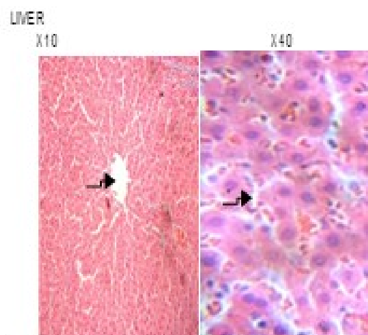
Slide 2 Kidney Positive Control (Group B): Kidney histology revealed cortical haemorrhagic necrosis, tubular necrosis, varying degree of microanatomy distortion and disruption with atrophy and cast in renal cortex, including queried edema



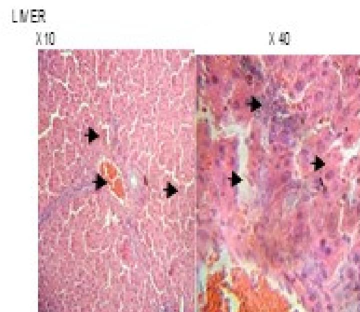
Slide 3 Kidney *Ofada* rice Koji –ORK (Group C): Histology revealed some varying degree of distortion and disruption in microanatomy with mild atrophied renal cortex, including queried edema, when compared to the positive control



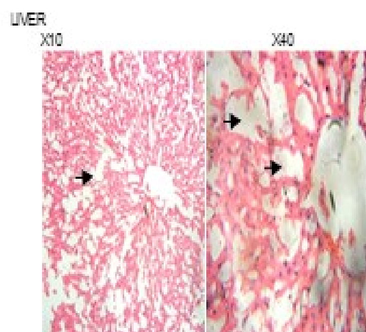
Slide 4 Kidney Ascorbic acid- ASC (Group D): Kidney histology revealed some varying degree of distortion and disruption in microanatomy with atrophied renal cortex, (arrow) X10 magnification including queried edema. At X40 magnification arrow reveals highly atrophied glomerulus



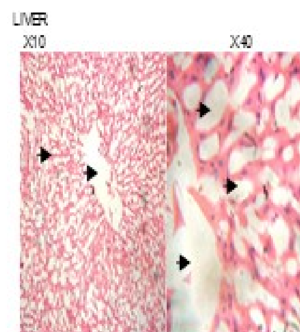
Slide 5 Liver Negative Control (Group A): The hepatocytes appear distinct with well differentiated nucleus at both low and high power. Liver histology appears normal (arrow)



Slide 6 Liver Positive Control (Group B): Visible histological changes at low power and high power with dilation of sinusoidal space (long arrow) besides multifocal hepatocytic coagulative portal biliary hyperplasia (arrow) and inflammatory changes at X40 magnification



Slide 7 Liver Ofada rice Koji – ORK (Group C): Visible histological degeneration at low power and high power with dilation of sinusoidal space (long arrow) besides multifocal hepatocytic coagulative portal biliary hyperplasia (arrow) and ballooning with hydropic degeneration



Slide 8 Liver Ascorbic acid- ASC (Group D): Visible histological degeneration at low power and high power with dilation of sinusoidal space (long arrow) besides ballooning with hydropic degeneration

Discussion

Anthropogenic activities coupled with environmental pollutants have consequences on the reduction and extinction of species in biodiversity with natural population diminution (34, 35, 36). This study examined the short-term consequences of BLCO on the renal and hepatic tissues of rats and the antioxidant potential of *Ofada* rice koji (ORK) on BLCO toxicity. Total petroleum hydrocarbon (TPH) compositionally encompasses an enormous family of chemical compounds that originally emanate from crude oil as analyzed and represented in Table 1a to 1c. They are found in the range of C_6 to C_{35} as a mixture containing hundreds to thousands of hydrocarbons including aliphatic, alicyclic and benzene aromatic compounds (7, 37). Since BLCO aggregates into aliphatic hydrocarbons and PAH components, its toxicity may antithetically affect the enzymatic and non-enzymatic antioxidant system of biological entities in both the kidney and liver (which are sites of biotransformation).

Studies have connected SOD with its atoxic and detoxifying propensity against superoxide anion or radical ($O_2{}^{\cdot-}$) (26). This atoxic effect protects against cellular disintegration from $O_2{}^{-}$ (38). As observed from this experiment (Tables 2 and 3), the reduction in tissue SOD and CAT activities upon BLCO exposure substantiates the response of cells to increase production of ROS by superoxide radicals. SOD enhances the dismutation of $O_2{}^{\cdot-}$ to H_2O_2 which is further metabolized by catalase into H_2O (39). The generation of H_2O_2 from the SOD reaction can either convert SOD into a pro-oxidant or inactivates the SOD mechanism (40). The inactivation of SOD occurs because H_2O_2 accelerates cysteine oxidation in CAT enzyme thereby decreasing SOD activity (41). Consequentially, the decreased SOD and CAT activity creates a metabolic imbalance between the ROS generation and antioxidant induction in cells thereby facilitating further release of ROS culminating into oxidative stress. The pharmacokinetics and pharmacodynamics of BLCO may have contributed immensely to the significant decline ($p<0.05$) identified in the SOD and CAT activity in both tissues, sites of xenobiotic biotransformation (42). Following ORK intake, it was obvious that ORK impeded nephrotoxicity and hepatotoxicity initiated by BLCO which was evident by the elevated level of SOD and CAT activities. Thus, this study postulates that ORK can provide cellular protection against the pro-toxicant of BLCO via its antioxidant potential. Traditionally, processed *Ofada*” rice encompasses a three-stage moisture treatment carried out by first parboiling, then soaking, followed by steaming and drying. Its nutritional and health-based benefits have been linked to the fermentative microbial activities

adopted during soaking (43). Reports have elucidated that soaking contributes to organoleptic, nutritional and physical changes in rice (44).

As expressed in this study, the reduced activities of antioxidant enzymes (SOD and CAT) in BLCO-exposed rats may have been ensured by H_2O_2 production and lipid peroxidation (45). Lipid peroxidation by free radical chain reaction is a universally accepted mechanism by which cell membranes are disrupted leading to oxidative stress and consequently apoptosis (46). The elevated MDA level suggests increased oxidative stress which can culminate in grave derangements in cellular metabolism and alteration of intracellular Ca^{2+}resulting in cell destruction (47). Previous studies elucidate that BLCO contains toxicants or xenobiotics such as nickel, vanadium, asphaltenes, nitrogen, and low molecular weight sulphur which complex into compounds that may contribute to the escalating BLCO toxicity experienced by the animals (47). Nephrotoxicity studies of Bonny light crude oil (BLCO) orally exposed to albino rats revealed destroyed renal capability, caused elevation of creatinine concentration to a significant level and significantly decreased urea concentration (14). Radical scavengers or antioxidants may react directly or indirectly with radical anions to terminate peroxidation chain reactions thereby enhancing the cell stability, quality and viability (48, 49). Pretreatment with ORK as observed from this study decreased MDA formation, indicative of its ability to avert oxidative tension in cells and as well decelerate lipid peroxidation in the kidney and liver probably via radical quenching, radical addition, electron transfer or radical recombination (50).

Reduced glutathione (GSH) a major constituent of the glutathione redox cycle, is a non-enzymatic antioxidant biomolecule, crucial in protecting against chemically stimulated cytotoxicity (42). By conjugation, GSH participates as an anti-oxidative agent in reactive intermediate and H_2O_2 elimination. The detoxification of toxic metabolites is essentially the role of GSH and studies have indicated that tissue necrosis is an index of GSH disintegration (51, 52). Administration with BLCO as demonstrated in this study revealed decreased GSH in both tissues and it corroborates previous studies (45). The drop in GSH expedites ROS release climaxing into oxidative stress with a cascade of events distorting cell functionality and integrity (45). However, ORK pretreatment significantly prevented BLCO-induced tissue GSH reduction. This may probably be achieved as a result of reduced bio-activation of BLCO radical species by ORK. The increased GSH level by the ORK diet protects cellular DNA, proteins and lipid bilayer against oxidation via the glutathione-redox cycle and this was reflected in the histological examination (Slide 3 and 7). This study presently revealed that BLCO-depleted GSH in the rat tissues were blocked by this phenomenon. GSH is enormously translated via GST activity and it forms abduct with toxicants of BLCO. Tables 2 and 3 showed that ORK induced the activity of renal and hepatic GST by preventing BLCO-BLCO-cytotoxicity. Myriads of natural chemo-preventive biomolecules have been prescribed to lower the bioavailability of potentially active neutrophils and electrophiles capable of disrupting lipid bilayer, DNA and protein structures by converting them into excretable metabolites via GST induction (2). From this research, it can be stated that nephrotoxicity and hepatotoxicity associated with BLCO administration were ameliorated by ORK consumption which was operational by participating in the quenching or mopping of reactive metabolites via elevated GSH and GST activities. Supportive research exists that corroborates the ameliorative principle of ORK when administered to rats induced with lead toxicity (24).

Conclusion

In conclusion, this study has shown that fermented *Ofada* rice can ameliorate renal and hepatic dysfunction induced by crude oil poisoning in Wistar Rats. The amelioration was mediated via the quenching or mopping of reactive metabolites as indicated by elevated GSH and GST activities, which showcased the antioxidant prowess of fermented *Ofada* rice.

List of Abbreviations

ASC: Ascorbic acid
BLCO: Bonny Light Crude Oil
CAT: Catalase
GSH: Glutathione
MDA: Malondialdehyde
ORK: *Ofada* rice Koji
SOD: Super oxide dismutase

Declarations

Ethics considerations and consent to participate

All experimental activities were carried out in compliance with guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NRC, 2010). Animal maintenance and care were subjected to strict adherence and guidelines as approved by the Delta State University Ethical Committee (DSUEC), Abraka Nigeria (DSU/BMS/03/022021).

Consent for publication
All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license. We otherwise convey all copyright ownership, including all rights incidental thereto, exclusively to the journal when published.

Availability of data and materials
The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Competing interests
The authors have no conflicts of interest to declare.

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Author Contributions
The study's inception and design involved input from all authors. AE, MJC, and OOB gave the conceptual framework for this research work. AE and OET handled the material preparation, and data collecting, and OOC and OOB carried out the data analysis. MJC and OOC wrote the manuscript's initial draft and AE, OOB, and OET proofread it with feedback. The last draft was reviewed by all authors and got their approval.

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