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Pretreatment with Oil Palm Leaf Extracts Confers Protection against Crude Oil Adulterated Food (COAF)

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ABSTRACT

The medicinal importance of oil palm leaf is attracting attention. The present study explored the role of various solvent extracts of the oil palm leaf in conferring protection in rats consuming crude oil adulterated food. The study comprised the use of forty (40) albino rats assigned randomly to eight (8) groups with five in each group. Rats in the control group (Group 1) served as control and were neither exposed to crude oil contaminated diet nor pre-treated with oil palm leaf extracts. Rats in group 2 were initially maintained on a normal diet. Rats in group 3-7 were pre-treated with 250 mg/kg bodyweight of various solvent extracts (aqueous, methanol, ethanol, acetone, and petroleum ether), while rats in group 8 were pre-treated with 250 mg/kg bodyweight of a blended mixture of (aqueous, methanol, ethanol, acetone, and petroleum ether) extracts of palm leaves. Each treatment was for four weeks. Thereafter, the rats in groups 2-8 were exposed to crude oil contaminated diets (4 mL per 100 g of feed) for another four weeks. The body weight of the rats and biochemical indices: hematological, liver function indices and oxidative stress indicators were determined using standard biochemical protocols. The results showed crude oil-mediated derangement of hematological parameters, liver function indices in addition to increased tissue malondialdehyde and depletion of tissue antioxidants. Pretreatment with oil palm leave extracts, however, reduced the trend in these metabolic derangements. Thus, pretreatment of animals with oil palm leaf extract confers protection against crude oil poisoning.

Keywords: Crude oil, Adulterated Food, Oil Palm Leaf, Toxicity.

Introduction

The occurrence of petroleum and its' product contamination is not limited to exploration activities but includes poor product handling and spillage.[1-5] These contaminations when not properly handled is often left to enter the food chain and have been proven to have different heights of noxiousness on both man and animals as several metabolic disease conditions have been linked to these contaminants as well.[6-8] This trend makes food chain petroleum contamination a high-risk burden in areas where ever crude oil exploration occurs as the breakdown of petroleum hydrocarbons have led to the release of free radical generating compounds and activated oxygen, nitrogen, hydrogen, and carbon metabolites into the cells leading to different types of autoimmune disease conditions often manifested as growth impairment, oxidative stress, diabetes, neurodegeneration, and cancer.[9-14]

In a bid to come to the rescue of man, researchers' all over the world have increasingly advocated the use of natural products as alternatives to synthetic adjuvants against toxic compounds as these synthetics aside being expensive may have some element of contribution to counter drug reactions occurring in biological systems.[15-17] Some of the most recent adjuvants used include *Vernonia amygdalina* extracts,[18-20] *Monodora myristica* extracts,[21-23] *Moringa oleifera* extracts,[24,25] Honey[7] and Oil palm leaf powder.[26, 27] The oil palm leaf - a major agricultural waste have been identified to generate large tones of biomass and constitutes a high level of an environmental nuisance when left after the oil palm fruit has been harvested.[28,29] In a bid to contribute to the continual craving for re-use of agricultural waste into useful products; the present study explored the role of various solvent extracts of the oil palm leaf in conferring protection in rats consuming crude oil contaminated diets.

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Materials and Methods

Materials

The crude oil (Escravos blend) was obtained from Warri Refining and Petrochemical Company. Harvest of *Elaeis guineensis* leaves was done at Ovwor Mixed Secondary School Oil palm Plantation in Ughelli, Delta State, Nigeria. This was immediately rinsed with water to remove all traces of sand particles and unwanted debris. The leaf was identified at Forestry Research Institute of Nigeria, Ibadan with a voucher number of F101173. Chemicals of high-quality analytical grade were employed for analysis.

Experimental animals

Forty (40) mature albino Wistar male rats were purchased from the animal house of the Department of Anatomy, Delta State University, Abraka, Nigeria. The experimental rats were kept in wooden cages and their weights were recorded. Acclimatization was allowed for two weeks and the rats were fed on grower's mash (Rainbow Feed Limited, Sapele, Nigeria). Approval to embark on this study involving Wistar rats was granted by the ethical committee of Faculty of Science, Delta State University, Abraka with registration number REC/FOS/19/01.

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Extraction of oil palm leaves

Plant extraction was carried out using five solvents namely; water, ethanol, methanol, acetone, and petroleum ether. These solvents were chosen to establish the one that will contain more active ingredient against crude oil-contaminated food toxicity. Before extraction, the collected palm leaves were dried to constant weight and ground into smooth particles with the aid of an electric waring blender (Model: 38BL54, Waring Laboratory Science, USA). The leaves (100 g each) were then weighed into 400 mL of the different solvents and allowed to dissolve for 48 h in an air-tight conical flask. The aqueous extract was soaked in water of 40^{o}C as previously described by George *et al*.[30] while the ethanol, methanol, acetone, and petroleum ether extracts were carried out in a 95% v/v solvent as previously described by Okpoghono *et al*.[21] and Achuba.[18] At the end of the 48 h period, the various samples were filtered using a clean muslin cloth and the filtrate transferred to a rotary evaporator (Model R1180, Superfit continental (P) Ltd, Mumbai, India) at 45^{o}C for concentration. The concentrated filtrates were further concentrated to dryness using a water bath and stored in a refrigerator until needed.

Preparation of extracts for pretreatment

From the crude extracts (dry samples) 2.5 g of each (water, ethanol, methanol, acetone, and petroleum ether extract) was dissolved in 25 mL of aqueous tween 80 (1% in 99 mL of distilled H_2O (v/v) which brings the concentration of each extract to 0.1 g/mL. The blended mixture was prepared by the addition of 0.5 g of each extract to give 2.5 g which was dissolved in 25 mL of aqueous tween 80.

Experimental distribution and design

The forty (40) albino rats were assigned randomly to eight (8) groups with five rats in each group after the acclimatization period. Rats in the control group (Group I) served as control and were neither exposed to crude oil contaminated diet nor pre-treated with oil palm leaf extracts. Rats in group II were initially maintained on a normal diet. Rats in group 3-7 were pre-treated with 250 mg/kg bodyweight of various solvent extracts (aqueous, methanol, ethanol, acetone, and petroleum ether) of oil palm leaf for four weeks while rats in group 8 were pre-treated with 250 mg/kg bodyweight of a blended mixture of (aqueous, methanol, ethanol, acetone, and petroleum ether) extracts of palm leaves for four weeks. This dose was chosen as a representative of equal amount of each extract administered. The extracts were administered through oral gavage for four weeks. Exposure to crude oil contaminated food (4 mL per 100 g of feed) was carried out as previously described by Achuba *et al*. (2018b) to rats in groups 2-8 for four weeks after oil palm leaf extract pre-treatment period.

Animal sacrifice, sample collection and preparation

The rats were allowed to fast overnight on the last day of the experiment. They were sacrificed by cervical decapitation and 5 mL sterile syringes with a needle were used for the collection of blood from the vena cava into properly labeled plain sample bottles and EDTA bottles for biochemical and hematological tests, respectively.

The collected blood samples were spurn using a centrifuge (TGL16E, Changsha Yingtai Instrument Company Ltd, China) at 3500 g for 15 min as previously done by Achuba[18] and the serum collected for various biochemical assays. Liver wet tissue measuring 0.5 g was homogenized in 9.0 mL of normal saline using pre-chilled mortar and pestle and the supernatant obtained was stored in the refrigerator and used for biochemical analysis following standard procedures within 48 h.

Hematological and biochemical analysis

All reagents and chemicals used for assay were of analytical grade while laboratory analysis was carried out by standard laboratory methods outlined below:

Determination of Packed cell volume (PCV); White blood cell count (WBC) and Red Blood Cell counts (RBC) were by the methods of Thrall and Weiser [32] while Hemoglobin (Hb) determination was by the protocol documented by Tietz.[33] The assay for alanine amino transferase (ALT) and aspartate amino transferase (AST) used available commercial diagnostic kit (Randox diagnostic kit) according to the manufacturers' instruction and this method was based on the method of Reitman and Frankel[34] while alkaline phosphatase (ALP) activities were carried out based on the method described by Kaplan and Righetti[35] on the principle of Alkaline phosphatase being able to catalytically break down p-nitrophenyl phosphate at pH 10.4, releasing p-nitrophenol and phosphate. Total protein (TP) was determined employing the method of Droumas[36] and Tietz[33] for albumin (ALB) levels in serum and liver of rats. Level of lipid peroxidation (MDA) was determined according to the method of Gutteridge, and Wilkins[37] while the method of Ellman[38] was used for the assay of reduced glutathione. The methods of Misra and Fridovich[39] were used for superoxide dismutase (SOD), Kaplan *et al*.[40] for Catalase.

Statistical analysis

Analysis of data was carried out with the aid of the Statistical Package for Social Sciences (SPSS). Data were presented as Mean ± SD while group comparison were done using the bonferonni Posthoc test at a significant level of $p < 0.05$.

Results and Discussion

The consumption of crude oil contaminated food caused significant ($p < 0.05$) reductions in the average body weight of rats at the end of the experimental periods. However, these reductions in weight of rats were not significantly reduced in rats pretreated with oil palm leaf extracts (Table 1).

The observed petroleum-induced changes in body weight of experimental rats follow similar patterns previously reported by Ogara *et al.*[41] and Sumonu and Oloyede in rats[6, 42] and Ugwu *et al.* in *Clarias* species.[43] Crude oil-induced metabolic derangement in the environment and biological samples have been well elucidated by various studies[10,21,20,25, 41] all of which have established that foodborne petroleum cross-contamination has serious negative effects on the wellbeing and safety of all forms of life. This is buttressed by the closeness in weight of rats pretreated with the blended mixture and the rats in the control group. The efficacy of the blended mixture had been established GC-MS analysis as well as genotoxicity studies.[44,45] Findings in the present study is a further contribution to the continual search for possible protection and solution to the dangers encountered by those in oil-spill prone areas who are at all times stands a risk of being exposed to petroleum-contaminated food substances. Also, this study reported a drop in hematological indices PCV, HB, and RBC in rats while there was an observed increase in WBC parameters due to COAF consumption. The observed increase in WBC could be said to have occurred as a result of immune responses against the toxicity potentials of crude oil adulterated food (Table 2).

The white blood cell has been previously reported to be a central point of an immune boost since they contain significant amounts of leucocytes that often act as macrophages for external substances attacking the cell.[46-48] Petroleum-induced changes in hematological indices have also been reported by some authors[19, 25] which were linked to reduced hematopoiesis and increased hemolysis and destruction of the erythrocyte membrane. Moreover, pretreatment of rats with various extracts of oil palm leaf confers some protections against crude oil toxicity. This assertion is in agreement with earlier study by this same authors.[45] Also, the potency of the blended mixture relative to other extracts had been explained above. Also observed in this study is the petroleum-induced increase in liver function parameters in the liver and the serum of the experimental rats (Tables 3 and 4).

These results revealed upsurge trends in ALT, AST, and ALP activities and a concomitant reduction in protein profiles (total protein and albumin) which are consistent with petroleum contaminated diet-induced toxicity that is reported previously.[21,24,27] As earlier reported, the rise in the activities of serum aminotransferases and the alkaline phosphatase is first-line indicators of probable liver damage while their rise in the liver is often occasioned in the presence of substances that may be injurious to the hepatic cells.[49] The alkaline phosphatase

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on the other hand is a significant contributor to the detoxification process and synthesis of several energetic macromolecules needed for metabolic balance.[50] Thus when altered their leakage into the serum may be indicative of an increase in membrane permeability.[51,52] Protein profile is a significant marker of food conversion efficiency as its drop often gives an insight into possible weight loss and liver malfunction. Bearing in mind that the liver is the primary center of metabolism, the inability of the tissues to absorb enough protein from consumed food may explain the observed trend in weight loss due to COAF consumption. The observed reduction in the increasing trend of these enzyme activities in the serum and liver may not be far-fetched from the high-level mobilization of several antioxidant potentials in an oil palm leaf and may also account for the prevention of the drop in total protein and albumin in the serum and liver. All of the above are similar to the protection conferred on rats by incorporation of powdered oil palm leaf in petroleum-contaminated diets in rats by Achuba.[26; 27]

In another development, crude oil diet contamination is often occasioned with the generation of oxidative radicals which is often culminated in the depletion of several enzymatic and non-enzymatic antioxidants and increased lipid peroxidation[6,14,18,19,25-27] and is said to be no different from the one reported in this study (Table 5).

However, the use of plant materials by various studies have also been reported to contribute significantly to averting the free radical generating potentials of crude oil-induced oxidative damage to animals. It is also important to note that although the pretreatment with the various solvent extracts of oil palm leaf had varying levels of potencies for antioxidant enzyme depletion and increased malondialdehyde generation, the blended mixture of all solvent extracts had a better ability to confer this protection which was followed by the petroleum ether extract. The possible reason for the above trend may be linked to the extraction efficiency of the various solvent extracts used in the study thus a blended combination of the various extracts may have improved the quantity and quality of active components capable of sequestering crude oil mediated free radical generation.[45]

Table 1: Average Body Weight of Rats Pre-treated with Oil Palm Leaf Extracts before COAF consumption

Groups	Week 1	Week 4	Week 8
1	180.00 ± 44.24^{a}	190.43 ± 41.24^{a}	219.28 ± 23.98^{a}
2	170.30 ± 14.12^{b}	149.65 ± 23.22^{b}	150.53 ± 32.99^{b}
3	170.52 ± 42.12^{b}	175.70 ± 33.32^{c}	187.76 ± 21.66^{c}
4	175.60 ± 12.43^{a}	170.00 ± 23.12^{a}	181.70 ± 43.89^{c}
5	156.00 ± 18.13^{c}	177.50 ± 44.12^{a}	187.55 ± 34.98^{d}
6	180.00 ± 54.11^{a}	200.10 ± 12.43^{d}	212.63 ± 26.93^{c}
7	158.00 ± 19.14^{c}	177.80 ± 52.12^{a}	187.87 ± 42.54^{c}
8	179.15 ± 41.43^{a}	205.10 ± 32.75^{d}	208.69 ± 13.54^{d}

Values are represented in mean ± SD. n=5. Mean values with a different superscript alphabet in the same column differ significantly at $p < 0.05$.
Key: Group 1: Control, Group 2: COAF only. Group 3: 250 mg/kg water extract of *E. guineensis leaves* + COAF, Group 4: 250 mg/kg methanol extract of *E. guineensis* + COAF, Group 5: 250 mg/kg ethanol extract of *E. guineensis* leaves + COAF, Group 6: 250 mg/kg acetone extract of *E. guineensis* leaves +COAF, Group 7: 250 mg/kg petroleum ether extract of *E. guineensis* leaves + COAF, Group 8: 250 mg/kg blended mixture of *E. guineensis* leaves extracts + COAF.

Table 2: Effect of Oil Palm Leaf Extracts on Selected Hematological Indices of Rats consuming COAF

Groups	PCV (%)	Hb (mg/dL)	WBC ($x10^{9}$/L)	RBC ($x10^{12}$/L)
1	48.00 ± 4.74^{a}	42.14 ± 7.18^{a}	7.82 ± 5.71^{a}	36.33 ± 4.57^{a}
2	23.00 ± 5.09^{b}	11.31 ± 3.67^{b}	25.24 ± 12.45^{b}	10.45 ± 3.46^{b}
3	32.60 ± 8.08^{c}	26.24 ± 5.68^{c}	18.50 ± 6.16^{c}	18.30 ± 4.03^{c}
4	38.00 ± 3.53^{c}	36.45 ± 5.76^{d}	12.52 ± 3.67^{ac}	24.28 ± 3.09^{d}
5	36.00 ± 4.74^{c}	38.42 ± 3.97^{d}	15.39 ± 5.16^{c}	20.26 ± 7.89^{c}
6	39.00 ± 3.16^{c}	33.53 ± 2.65^{c}	13.42 ± 4.58^{c}	22.14 ± 5.18^{cd}
7	41.00 ± 3.60^{ac}	35.44 ± 3.27^{cd}	11.51 ± 5.56^{c}	27.40 ± 5.57^{d}
8	44.10 ± 4.72^{ac}	41.10 ± 5.18^{ad}	8.61 ± 5.43^{ac}	34.32 ± 4.55^{a}

Values are represented in mean ± SD. n=5. Mean values with a different superscript alphabet in the same column differ significantly at $p < 0.05$.
Key: Group 1: Control, Group 2: COAF only. Group 3: 250 mg/kg water extract of *E. guineensis leaves* + COAF, Group 4: 250 mg/kg methanol extract of *E. guineensis* + COAF, Group 5: 250 mg/kg ethanol extract of *E. guineensis* leaves + COAF, Group 6: 250 mg/kg acetone extract of *E. guineensis* leaves +COAF, Group 7: 250 mg/kg petroleum ether extract of *E. guineensis* leaves + COAF, Group 8: 250 mg/kg blended mixture of *E. guineensis* leaves extracts + COAF.

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Table 3: Effect of Oil Palm Leaf Extracts on Serum Selected Liver Functions Parameters of Rats Consuming COAF

Groups	ALT (Units/mL)	AST (Units/mL)	ALP (Units/mL)	TP	ALB
1	50.10 ± 6.18 [a]	30.10 ± 8.44[a]	180.43 ± 5.70[a]	56.38 ± 4.76[a]	38.26 ± 3.54[a]
2	121.30 ± 16.53[b]	110.10 ± 7.82[b]	328.22 ± 71.25[b]	18.38 ± 5.4[b]	7.80 ± 5.49 b
3	100.10 ± 7.55[c]	80.10 ± 7.79[c]	298.47 ± 2.76 [c]	34.46 ± 2.93[c]	17.36 ± 1.66 [c]
4	65.10 ± 4.95[d]	68.10 ± 4.97[d]	228.41 ± 6.03 [d]	44.32 ± 3.02[d]	28.96 ± 3.00[a]
5	80.30 ± 6.12[e]	84.10 ± 5.20[e]	283.59 ± 9.70[e]	36.22 ± 4.78 [c]	19.28 ± 4.35 [c]
6	68.30 ± 9.66[d]	77.20 ± 4.82[f]	261.47 ± 9.77[f]	39.42 ± 3.22 [d]	24.34 ± 2.43 [d]
7	61.10 ± 6.38[d]	58.30 ± 6.15[d]	200.15 ± 7.79[g]	39.36 ± 19.81[ad]	31.43 ± 8.00[a]
8	51.20 ± 5.15[a]	49.20 ± 6.44[a]	183.46 ± 8.92[a]	52.07 ± 2.75[ae]	39.05 ± 3.62 [a]

Values are represented in mean ± SD. n=5. Mean values with a different superscript alphabet in the same column differ significantly at $p < 0.05$.
Key: Group 1: Control, Group 2: COAF only. Group 3: 250 mg/kg water extract of *E. guineensis leaves* + COAF, Group 4: 250 mg/kg methanol extract of *E. guineensis* + COAF, Group 5: 250 mg/kg ethanol extract of *E. guineensis* leaves + COAF, Group 6: 250 mg/kg acetone extract of *E. guineensis* leaves +COAF, Group 7: 250 mg/kg petroleum ether extract of *E. guineensis* leaves + COAF, Group 8: 250 mg/kg blended mixture of *E. guineensis* leaves extracts + COAF.

Table 4: Effect of Oil Palm Leaf Extracts on Selected Tissue Liver Functions Parameters of Rats Consuming COAF

Groups	ALT (Units/mL)	AST (Units/mL)	ALP (Units/mL)	TP	ALB
1	60.10 ± 6.89[a]	35.10±5.85[a]	190.21 ± 10.98[a]	58.36 ± 3.99[a]	40.38 ± 3.83[a]
2	140.20 ± 3.58[b]	111.00±8.49[b]	370.14 ± 36.08[b]	23.61 ± 4.94[b]	15.52 ± 7.06[b]
3	111.30 ± 12.60[c]	90.10 ± 18.9[c]	300.54 ± 19.16[c]	31.40 ± 2.23[c]	22.69 ± 1.44[c]
4	78.10 ± 12.70[d]	54.20 ± 5.46[d]	227.36 ± 15.06[d]	49.36 ± 3.39[d]	31.40 ± 2.20[d]
5	100.20 ± 7.71[c]	76.70 ± 8.95[e]	260.30 ± 12.79[e]	37.30 ± 2.47[e]	22.85 ± 2.87[c]
6	90.30 ± 11.39[d]	60.10 ± 14.69[d]	240.30 ± 15.50[f]	42.28 ± 3.82[d]	26.38 ± 3.75[e]
7	71.11 ± 7.56[e]	44.20 ± 11.15[a]	217.20 ± 8.38[g]	54.29 ± 2.57[a]	35.49 ± 3.15[ad]
8	62.47 ± 8.20[a]	34.20 ± 6.19[a]	198.10 ± 19.21[a]	56.66 ± 4.73[d]	39.24 ± 3.36[a]

Values are represented in mean ± SD. n=5. Mean values with a different superscript alphabet in the same column differ significantly at $p < 0.05$.
Key: Group 1: Control, Group 2: COAF only. Group 3: 250 mg/kg water extract of *E. guineensis leaves* + COAF, Group 4: 250 mg/kg methanol extract of *E. guineensis* + COAF, Group 5: 250 mg/kg ethanol extract of *E. guineensis* leaves + COAF, Group 6: 250 mg/kg acetone extract of *E. guineensis* leaves +COAF, Group 7: 250 mg/kg petroleum ether extract of *E. guineensis* leaves + COAF, Group 8: 250 mg/kg blended mixture of *E. guineensis* leaves extracts + COAF.

Table 5: Effect of Oil Palm Leaf Extracts on Liver Lipid Peroxidation and Antioxidants of Rats Consuming COAF

Groups	MDA ($nmolg^{-1}$tissue)	CAT ($nmolg^{-1}$tissue)	SOD ($unitsg^{-1}$tissue)	GSH
1	3.62 ± 0.13	89.21 ± 7.95 [a]	58.31± 7.71 [a]	11.19 ± 1.54 [a]
2	15.43 ± 0.23	35.53 ± 8.02 [b]	16.30 ± 4.52 [b]	0.68 ± 0.36 [b]
3	9.89 ± 1.23	49.41 ± 3.48 [b]	35.38 ± 11.31 [c]	3.30 ± 0.58 [c]
4	6.34 ± 1.26	68.20 ± 8.96 [c]	50.42 ± 7.90 [a,d]	7.16 ± 2.44 [a]
5	8.62 ± 2.45	51.26 ± 7.17 [d]	40.43 ± 12.05 [e]	4.81 ± 1.57 [c]
6	6.17 ± 0.93	59.27 ± 9.51 [d]	45.26 ± 8.12 [e]	6.28± 4.12 [a,c]
7	5.4 ± 0.32	69.25 ±15.35[c]	48.47 ± 6.79 [e]	9.24 ± 2.95 [a]
8	3.4 ± 0.99	84.36 ± 7.49 [a]	54.52 ± 13.20 [a]	10.45 ± 1.08 [a]

Values are represented in mean ± SD. N = 5. Mean values with a different superscript alphabet in the same column differ significantly at $p < 0.05$.
Key: Group 1: Control, Group 2: COAF only. Group 3: 250 mg/kg water extract of *E. guineensis leaves* + COAF, Group 4: 250 mg/kg methanol extract of *E. guineensis* + COAF, Group 5: 250 mg/kg ethanol extract of *E. guineensis* leaves + COAF, Group 6: 250 mg/kg acetone extract of *E. guineensis* leaves +COAF, Group 7: 250 mg/kg petroleum ether extract of *E. guineensis* leaves + COAF, Group 8: 250 mg/kg blended mixture of *E. guineensis* leaves extracts + COAF.

Conclusion

The search for a more effective way of averting the negative effects of crude petroleum cross-contamination is a continuum. This study however has established that the increased fortification of biology systems by way of increased consumption of plant materials could be a major turning point in this search. However, a major takeaway from the present study is that the high efficiency of the blended mixture of different solvent extracts of the oil palm leaf gives an insight into possible effectiveness of a blend and combination of different antioxidant metabolites in the management of crude oil-stimulated metabolic derangement and the increased exploration of agricultural wastes into useful components for the management of crude petroleum toxicity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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