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Original Article

Diet from *Mantisa religiosa*-egg case abolishes pulmonary dysfunctions triggered by sub-acute exposure to aerosolized-petroleum hydrocarbons in rat model

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SUMMARY

Daily exposure to fractionated petroleum products could be connected to respiratory disorders in industrialized countries and insectary egg of high proteins may be applied as the diet for its management. This study checked the effect of dietary supplemented *Mantisa religiosa* egg case (DSMREC) on pulmonary dysfunctions triggered by sub-acute exposure to petroleum hydrocarbons in the animal model. Animals were divided into eight groups ($n = 8$): control group; normal rats exposed to 15 ml aerosolized kerosene, petrol, and diesel, respectively for 1hr daily for 14 days; positive control rats treated with 4% DSMREC only; rats exposed to 15 ml aerosolized kerosene, petrol, and diesel, respectively and co-treated with 4% DSMREC. The results elevated lung ADA, 5¹-nucleatidase activities and MDA content following exposure to aerosols of petroleum hydrocarbons. Also, aerosols thickened the pulmonary interstitial cells, distended alveoli (emphysema), proliferated bronchial associated lymphoid tissues followed by the congestion of pulmonary blood vessels. Amazingly, 4% DSMREC prevented these alterations. Therefore, our results suggest some possible mechanisms of

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action by which 4% DSMREC prevents petroleum-induced pulmonary toxicity in rats.

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1. Introduction

The use of petroleum products from petroleum industries in developing countries particularly Africa has been identified to have certain respiratory health effects on users due to constant exposure to fractionated hydrocarbon products [1]. Exposure to high levels of air pollutants has been implicated in morbidity, cardiovascular diseases and respiratory diseases [2]. Similarly, contamination of air quality resulting from automobile exhausts has become a public misfortune because of its health hazards among urban dwellers. This incidence was associated with allergic diseases in industrialized and emerging countries [3]. Contemporary toxicology and epidemiological appraisal showed that automobile emissions consist of oxides of nitrogen (NOX), sulphur oxide (SOX), carbon monoxide (CO), particulate matters (PM), formaldehyde, polycyclic aromatic hydrocarbons (PAHS) and other uncountable noxious gases [4]. Thus, unavoidable and industrial exposures to these toxicants have been reported to enhance obstruction of lung airways responsible for the injury of the terminal bronchioles and a decrease in the pulmonary compliance [5].

Basically, acute respiratory infection (ARI) is one of the leading causes of diseases worldwide which account for the deaths of about 4–5 million in adults including children in developing countries [6]. According to the report of Gupta et al. [3], diesel exhaust particles account mostly as the motor vehicle-generated air pollutants in towns and cities. However, acute exposure to aerosols of petroleum hydrocarbons particularly diesel exhausts could cause irritation of eyes and nose, changes in lung functions, headache, fatigue, and nausea [7]. Chronic exposure is characterized by cough, increased production of sputum, and decrements of lung functions [8]. Also, chronic obstructive pulmonary diseases (COPD) such as bronchitis, emphysema and asthma, inflammation and obstruction of the airways were also implicated [8,9]. Recent findings have noted pneumonia, interstitial lung disease (a complication in breathing and lung stiffness) and lung cancer (cell growth in lung's tissues) among patients and animals suffering from pulmonary disorders and abnormal respiratory functions [10,11] on exposure to fractionated petroleum products.

The female insect known as *Mantisa religiosa* commonly recognized as mantis formula secretes foamy and liquid proteinous material to protect its eggs from winter and parasitic infestation [12]. In Asia, *Mantisa religiosa* egg case (MREC) is commonly applied traditionally to treat urinary disorders, infertility, impotence, and shingles while in West Africa, the egg case powder is used in treating chronic cough and respiratory dysfunctions without understanding its basic and mechanistic applications [12]. The conventional endpoints such as tumor necrosis factor- α (TNF- α), lavage fluid or lung tissue inflammatory cytokines responsible for the activation of inflammatory cells and pathogenesis of lung fibrosis may consequently limit the scientific claim of treatments for lung wellness if other interrelated biochemical procedures are not well elucidated. Hence, the active components from MREC may play a therapeutic roles in the treatment of pulmonary disorders, including (i) the repair of lung-induced oxidative damage via lipid peroxidation; (ii) improving the productivity of lung adenosine triphosphate (ATP) via pulmonary 5¹ nucleotidase and adenosine deaminase (ADA); and (iii) promoting the survival of bronchial associated lymphoid tissues by strengthening pulmonary interstitial cells via bioenergetic pathway mechanism. Based on these postulates, a study regarding the protection of MREC on the aforementioned mechanisms especially the use of pre-clinical therapeutic approaches in preventing pulmonary dysfunctions in the rat to juxtapose human situations was conceived. It was also projected that the outcomes would be helpful to recognize the mechanisms accounting for the lung wellness aside inflammatory cytokines. This provides the fundamental basis for the current investigation. Hence, the present study seeks to examine the effect of dietary supplemented mantis formula

egg case on some key enzymes linked with pulmonary dysfunctions triggered by sub-acute exposure of aerosolized petroleum hydrocarbons.

2. Materials and methods

2.1. Chemicals

The substrates AMP and adenosine as well as trichloroacetic acid (TCA), thiobarbituric acid (TBA), bovine serum albumin, K_2HPO_4 was obtained from Sigma Chemical Co (St. Louis, MO, USA). All kits used for assays and other chemicals used in this experiment were of highest purity and of analytical grade.

2.2. Sample collection and preparation

Several cases containing eggs of *Mantisa religiosa* were purchased from Ojeé market, the ancient city of Ibadan, Nigeria. Authentication of the egg case was carried out at the Department of Botany, University of Ibadan It was thereafter pulverised into powder to enhance the quantitative characterisation of active components. The sample was designated as mantis formula egg case (MFEC).

2.3. Proximate analysis and in-vitro antioxidant activity

Proximate properties of the MREC including ash, moisture content, crude fibre, protein, fat, carbohydrate and nitrogen were analyzed. Thereafter, the total phenol of the MFEC was determined according to the method of Singleton et al. [13]. Total flavonoid content was evaluated using a method reported by Meda et al. [14]. Tannin content was quantified according to the method described by Padmaja [15]. Furthermore, the total saponin content was determined by the method described by Makkar et al. [16]. Afterward, carotenoid content was examined following the procedure of Rice-Evans [17].

2.4. Determination of trace elements

The concentrations of the eight essential trace elements namely Ca, Mg, K, Na, Mn, Fe, Cu and Zn were estimated in the *M. religiosa* egg case using atomic absorption spectrophotometer [18]. The levels of these micro nutrients were assessed because of its multiple traditional applications in Africa particularly in developing countries.

2.5. Animal handling

Male wistar rats of weight ranging within 300 g from the animal house at the University of Ibadan were used in this experiment. They were maintained on a 12 h light/dark cycle with free access to food and water and used according to the guidelines of the University of Ibadan's animal care use and research committee (UI ACUREC-2017) and also in accordance with international guidelines.

2.6. Diet formulation

The diets were freshly formulated according to a modified method of Akinyemi et al. [18] as shown in Table 1. The diets were prepared to contain 10% protein with casein, taking into consideration the crude protein content of the mantis formula egg case. The quantity of casein required to obtain 10% protein in diet was derived from the following formula:

$$\frac{100 \times 10}{y}$$

where y is the percentage crude protein in the casein.

Table 1

Diet formulation for basal and supplemented diets for control and test groups.

Treatment	CG	KSG	PSG	DSG	PMEC	KPMEC	PPMEC	DPMEC
Casein	13.9	13.9	13.9	13.9	10.0	10.0	10.0	10.0
Oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin premix	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cornstarch	72.1	72.1	72.1	72.1	72.0	72.0	72.0	72.0
Mantis formula	–	–	–	–	4.0	4.0	4.0	4.0
Total (g)	100	100	100	100	100	100	100	100

Note: Casein = 71.88% protein; 1 g of the mineral-vitamin premix contains; 3200 i.u vitamin A, 600 i.u vitamin D₃, 2.8 mg vitamin E, 0.6 mg vitamin K₃, 0.8 mg vitamin B₁, 1 mg vitamin B₂, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B₆, 0.004 mg vitamin B₁₂, 0.2 mg folic acid, 0.1 mg biotin H₂, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. The composition of *Mantis religiosa* egg case was purchased from a commercial shop in Ibadan, Nigeria. Each *Mantis religiosa* egg case weighs 0.4962 g while about thirty two (32) cases of *Mantis religiosa* egg case was used to formulate the diet.

2.7. Study design

The rats were acclimatized for two weeks and randomly divided into eight groups of eight animals each (n = 8); **CG**- Normal control group placed on a basal diet; **KSG**- Group of animals exposed to 15 ml of aerosolized kerosene for 1hr per day for 14 days (sub-acute exposure) and placed on a basal diet; **PSG**- Group of animals exposed to 15 ml of aerosolized petrol for 1hr per day for 14 days and placed on a basal diet; **DSG**- Group of animals exposed to 15 ml of aerosolized diesel for 1hr per day for 14 days and placed on a basal diet; **PMEC**- Positive control animals placed on 4% dietary supplemented Mantis formula only; **KPMEC**- Group of animals exposed to 15 ml of aerosolized kerosene for 1hr per day for 14 days and placed on 4% dietary supplemented Mantis formula; **PPMEC** – Group of animals exposed to 15 ml of aerosolized petrol for 1hr per day for 14 days and placed on 4% dietary supplemented Mantis formula; **DPMEC**- Group of animals exposed to 15 ml of diesel for 1hr per day for 14 days and placed on 4% dietary supplemented Mantis formula. 15 ml of aerosolized petroleum hydrocarbons for 1hr per day was selected following the method of Oyejisi et al. [7] and was based on the duration of average human exposure. Similarly, 4% was chosen because of the high content of crude protein in Mantis formula. Also, daily feed intake was monitored and body weight was taken before and after the experiment. These rats were sacrificed 24 h after the last treatment session.

2.8. Mechanistic mode of exposure to aerosols of petroleum hydrocarbons

A modified nose-inhalation exposure method as described by Oyejisi et al. [7] was used: Briefly, the cages housing the animals were positioned in respective exposure chambers. 15 ml of the petroleum-fractionated products (diesel, gasoline and kerosene) was aerosolized within the respective exposure chambers at ambient humidity and temperature. The animals were exposed for 1 h daily. Following the exposure, the animals were relocated from the exposure chambers to a petroleum-free section of the animal house to mimic natural exposure condition.

2.9. Preparation of plasma and pulmonary post mitochondrial fraction

After the treatment, the blood was collected into clean dry heparinized centrifuge tube and centrifuged at 10,000 rpm for 20 mins. The supernatant (plasma) was separated and stored at 4 °C for toxicology analysis. Likewise, the lungs were homogenized in 4 volumes of an ice cold medium, consisting of 1.15% potassium chloride and 50 mM Tris–HCl buffer with a pH 7.4 in a motor driven Teflon-glass homogenizer. The supernatant known as pulmonary post mitochondrial fraction (PPMF) was isolated at 4 °C and used for biochemistry assays.

2.10. Hepatic toxicological analysis

The activity of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [19], and alkaline phosphatase (ALP) [20] were measured using available commercially kits (Randox Laboratories Kits, St Louis, MO, USA).

2.11. Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa et al. [21] and expressed as nmoles/mg protein.

2.12. Pulmonary 5¹-nucleotidase activity assay

5¹-nucleotidase assay was carried out using the method described by Heymann et al. [22] which follows the principle that 5' — nucleotidase, a suitable marker enzyme that cleaves phosphate group from purine nucleotides AMP and GMP (Adenosine monophosphate and Guanosine monophosphate, respectively) and the inorganic phosphate (Pi) released is measured. Briefly, 0.15 ml of 10 mM AMP was incubated with 0.1 ml of 5 mM Tris KCl, followed by addition of 0.1 ml of 10 mM MgCl₂ and 0.15 ml aliquot of lung homogenate at 35 °C for 20 min. The reaction was stopped by addition of 0.5 ml 10% (w/v) trichloroacetic acid and the precipitated protein was removed by centrifugation. 0.5 ml of supernatant was added to 5.0 ml of 1.6% ammonium molybdate with 0.2 ml of sodium acetate followed by addition of 0.8 ml of 10% ferrous sulphate solution. The optical density was measured at 700 nm after shaking. The enzyme activities are reported as mmol Pi released/minute/mg of protein.

2.13. Adenosine deaminase (ADA) activity assay

ADA activity determination was performed as described by Guisti and Galanti [23] which is based on the direct measurement of the formation of ammonia, produced when adenosine deaminase acts in excess of adenosine. Concisely, 50 ml of enzyme preparation reacted with 21 mmol/l of adenosine, pH 6.5, and was incubated at 37 °C for 60 min. Results were expressed in units per mg protein (Unit/mg protein). One unit (1U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine at standard assay conditions.

2.14. Protein determination

Protein was measured by the Biuret method as described by Gornall et al. [24] using Bovine Serum Albumin (BSA) as standard.

2.15. Histopathology examination

Sequel the treatment, the lungs were separated and placed in 4% para-formaldehyde at 4 °C for 48 h. After dehydration, transparency, paraffin immersion and paraffin embedding, the cerebrum was sliced along the median anteroposterior axes at a thickness of 6 mm. The section was stained with hematoxylin and eosin for morphological observation and defining positions. Sections were read and images were captured using a light microscope.

2.16. Statistical analysis

The data in each group were expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) was used to analyze the results and the Duncan multiple test was used for the *post hoc* analysis [25]. The Statistical Package for Social Science (SPSS) 20.0 for Windows was used for the analysis and the least significance difference (LSD) was accepted at $p < 0.05$.

3. Results

3.1. Proximate properties, antioxidant capacity and essential nutrients

The results of proximate analysis, antioxidant properties and essential trace metals obtained from *M. religiosa* egg case (MREC) are presented in Tables 2–4, respectively. The protein content of MREC revealed highest (69.65%) followed by carbohydrate (12.81%), fat (12.50%) and nitrogen (12.32%),

Table 2
Proximate analysis of Mantis formula egg case.

Parameter	% composition
Dry mass	97.85
Ash	3.54
Moisture	2.15
Crude fibre	1.50
Protein	69.65
Fat	12.5
Carbohydrate	12.81
Nitrogen	12.32

Table 3
Phytochemical screening of common antioxidants in Mantis formula egg case.

Phytochemical	Concentration (mg/100 g)
Total phenol	212
Tannins	153
Flavonoids	61
Saponins	114
Carotenoids	54

Table 4
Characterization of essential trace elements in Mantis formula egg case.

Element	Part per million (ppm)
Calcium	10,800
Magnesium	200
Potassium	5600
Sodium	116.29
Manganese	57.98
Iron	165.48
Copper	3.89
Zinc	10.69

respectively (Table 2). Similarly, as shown in Table 3, the bioactive constituents of antioxidants depicted total phenol (212 mg/100 g), tannins (153 mg/100 g), saponins (114 mg/100 g), flavonoids (61 mg/100 mg) and carotenoids (54 mg/100 g). Lastly, characterization of essential elements from MREC revealed calcium (10,800 mg/kg), potassium (5600 mg/kg), magnesium (200 mg/kg), iron (165.48 mg/kg), sodium (116.29 mg/kg), manganese (57.98 mg/kg), zinc (10.69 mg/kg) and copper (3.89 mg/kg) (Table 4).

3.2. Effect of 4% dietary supplementation of *Mantisa religiosa* egg case (MREC) flour on psychomotor behavior and absolute body weight in animal exposed to aerosolized petroleum fractionated hydrocarbons

Following the exposure, the animals manifested dizziness, loss of appetite, decreased psychomotor movement and hair loss. The result on the absolute body weight is presented in Table 5. Group of animals exposed to aerosolized petroleum hydrocarbons (KSG, PSG and DSG) decreased by 9.56%, 6.72% and 4.29%, respectively while the control group (CG) and positive control group (PMEC) increased by 7.23% and 5.54%, respectively (Table 4A). The group of exposed animals and treated with 4% dietary supplementation of Mantis formula egg case (as shown in Table 5) was unable to reverse the reduction in absolute body weight initiated by aerosolized petroleum fractionated products. This suggests that mammalian exposure to aerosolized petroleum products may cause permanent or stable

Table 5

Effect of 4% dietary supplemented mantis formula egg case on the body weight of animals exposed to different aerosolized petroleum fractionated products.

GROUP	Absolute weight before exposure	Absolute weight after exposure	Percentage change in weight
CG	128.7 ± 9.72	138 ± 8.92	7.23**
KSG	232.2 ± 9.69	210 ± 4.62	9.56*
PSG	239.6 ± 9.15	223.5 ± 7.37	6.72*
DSG	258.9 ± 11.95	247.8 ± 10.36	4.29*
PMEC	137.1 ± 7.16	144.7 ± 8.38	5.54**
KPMEC	209 ± 5.27	198 ± 7.15	9.09*
PPMEC	268.8 ± 10.68	255 ± 14.01	5.12*
DMEC	190.1 ± 9.68	185 ± 7.6	2.63*

Key: ** % increase * % decrease.

reduction in growth pattern. As depicted in previous study (Table 6), Petrol showed highest percentage of branched chain alkanes (32%) and aromatics (30.5%). Kerosene showed 75% of branched chain alkanes while diesel revealed 75% of n-alkanes.

3.3. Effect of 4% dietary supplemented mantis formula flour (MFF) on AST and ALP activity in animals exposed to aerosolized petroleum hydrocarbons

Results obtained from this study showed that the activity of AST for KSG, PSG and DSG groups increased by 43.27%, 39.20% and 3.64%, respectively (Fig. 1) when compared to the control group (CG). Co-treatment with 4% dietary supplemented Mantis formula flour (DSMFF) however reduced the

Table 6

The average main constituents of petrol/gasoline, kerosene and diesel expressed as average percentage weight per volume (% w/v) [73].

Name	Petrol (%)	Kerosene (%)	Diesel (%)
n- Alkanes	17.3	LQ	75.0
Branched alkanes	32.0	75.0	LQ
Cycloalkanes	5.0	LQ	LQ
Olefins	1.8	5.0	LQ
Aromatics	30.5	25.0	25.0

LQ = Low quantity.

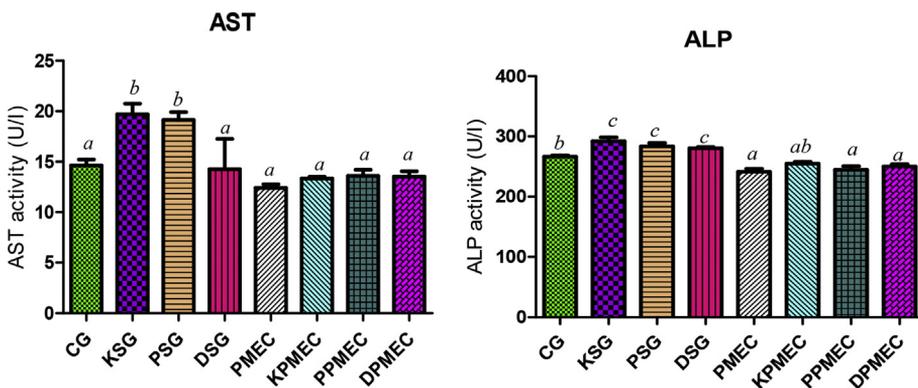


Fig. 1. Effect of dietary supplemented Mantis formula on AST and ALP activities in animals exposed to aerosolized petroleum hydrocarbons for 14 days (sub-acute exposure). Results are expressed as mean ± S.D (n = 8). Bars with different letters are significant (p < 0.05).

activity in KPMEC, PPMEC and DPMEC by 32.08%, 33.45% and 6.11%, respectively in relation to the corresponding aerosolized exposed groups (KSG, PSG and DSG) (Fig. 1). Interestingly, the positive control group (PMEC) depleted the activity of AST by 5.45% in relation to the control group (CG) (Fig. 1). Similarly, as observed in Fig. 1, there was an increase in the activity of ALP in the KSG, PSG and DSG by 9.73%, 6.50% and 5.33%, respectively when compared to the control group CG; while, co-treatment with 4% DSMFF caused a decrease by 26.26%, 21.40% and 10.41%, respectively in relation to the corresponding aerosolized exposed group. Likewise, the positive control group (PMEC) decreased the activity of ALP in relation to the CG by 9.43% (Fig. 1).

3.4. Effect of 4% dietary supplemented mantis formula on lipid peroxidation (index of pulmonary oxidative damage) in animals exposed to aerosols of petroleum hydrocarbons

As observed in Fig. 2, the lipid peroxidation of lung tissues showed that KSG, PSG and DSG were significantly ($p < 0.05$) elevated in Malondialdehyde (MDA) levels by 42.28%, 51.82% and 44.72%, respectively when compared to the CG. The groups co-treated with 4% DSMF were significantly ($p < 0.05$) reduced in MDA levels by 26.71%, 42.26% and 24%, respectively in relation to the corresponding aerosolized petroleum hydrocarbons as depicted in Fig. 2.

3.5. Effect of 4% dietary supplemented mantis formula on 5^1 nucleotidase activity in animals exposed to aerosolized petroleum hydrocarbons

The activity of 5^1 nucleotidase is shown in Fig. 3. It was revealed that KSG, PSG and DSG significantly ($p < 0.05$) increased the activity of 5^1 nucleotidase by 26.91%, 31.00% and 66.00%, respectively when compared to the CG. The groups co-treated with 4% DSMFF significantly ($p < 0.05$) depleted the activity of 5^1 nucleotidase by 8.48%, 17.53% and 34.28%, respectively in relation to the corresponding aerosolized petroleum hydrocarbons (Fig. 3).

3.6. Effect of 4% dietary supplemented mantis formula on adenosine deaminase (ADA) activity in animals exposed to aerosols of petroleum hydrocarbons

Following the exposure of animals to KSG, PSG and DSG, the activities of adenosine deaminase were significantly ($p < 0.05$) increased by 26.91%, 31.00% and 66.00%, respectively when compared to the control group CG. Contrariwise, the activities of ADA of the co-treated animals with 4% were

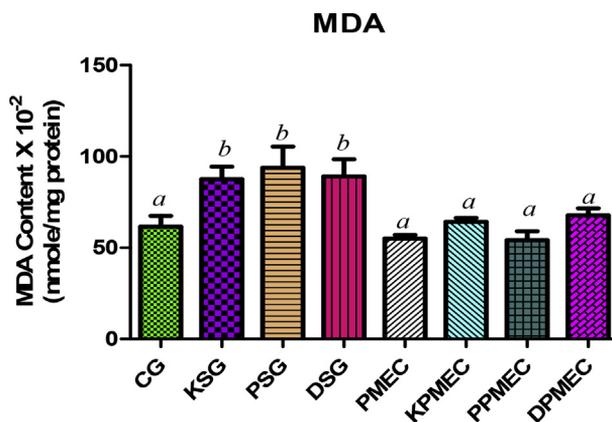


Fig. 2. Effect of dietary supplemented Mantis formula on lipid peroxidation (MDA content) in animals exposed to aerosolized petroleum hydrocarbons for 14 days (sub-acute exposure). Results are expressed as mean \pm S.D (n = 8). Bars with different letters are significant ($p < 0.05$).

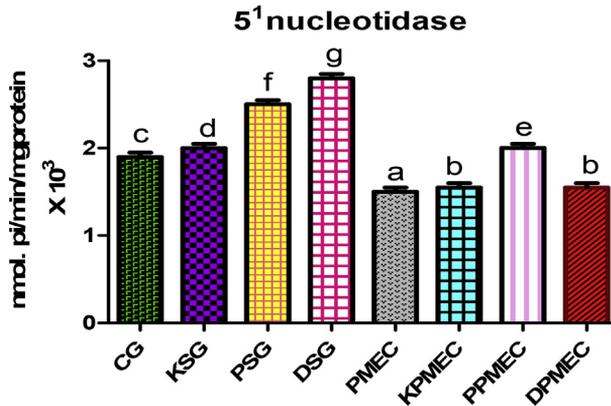


Fig. 3. Effect of dietary supplemented Mantis formula on 5¹nucleotidase activity in animals exposed to aerosolized petroleum hydrocarbons for 14 days (sub-acute exposure). Results are expressed as mean \pm S.D (n = 8). Bars with different letters are significant (p < 0.05).

remarkably (p < 0.05) depleted by 25.46%, 17.17, 44.81%, respectively when compared with the corresponding aerosolized exposed groups (Fig. 4).

3.7. Histopathological investigation

Histopathological examination of the lung tissues (Fig. 5) were examined after sub-acute exposure to aerosols of petroleum hydrocarbons. Group of animals aerosolized with petroleum hydrocarbons (KSG, PSG and DSG) depicted proliferative thickening of pulmonary interstitial cells (PTPIC), distended alveoli (emphysema) marked proliferation of bronchial associated lymphoid tissues (MBALT) followed by the congestion of pulmonary blood vessels (CPBV). Although, the groups of animals (KPMEC, PPMEC and DPMEC) co-supplemented with Mantis formula showed slight proliferative thickening of pulmonary interstitial cells (PTPIC) with clear airways but no chronic damage (Fig. 5). Also, mild hyperplasia of the bronchial epithelial cells (MHBEC) and trivial congestion of pulmonary blood vessels (TCPBV) were observed. Additionally, the animals treated with PMEC only indicated normal pulmonary interstitial cells (NPIC) and normal blood vessels (NBC), showing clear airways (bronchioles and alveoli)

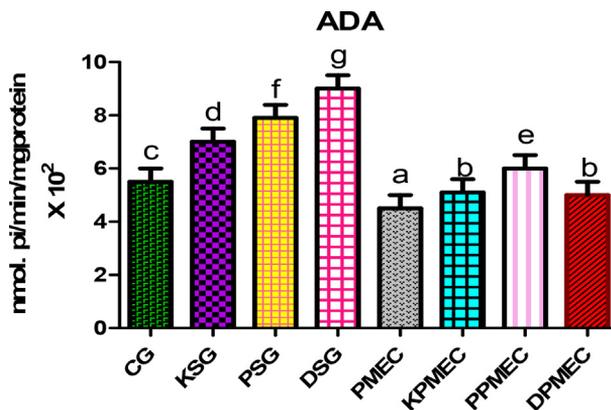


Fig. 4. Effect of dietary supplemented Mantis formula on adenosine deaminase (ADA) activity in animals exposed to aerosolized petroleum hydrocarbons for 14 days (sub-acute exposure). Results are expressed as mean \pm S.D (n = 8). Bars with different letters are significant (p < 0.05).

i.e. absence of pulmonary emphysema. Also, control animals showed clear airways (CA) (bronchi and alveoli) with normal proliferation of the bronchial associated lymphoid tissues (NBALT), although it showed minor congestion of pulmonary blood vessels but no remarkable lesions to the lungs (NVLL).

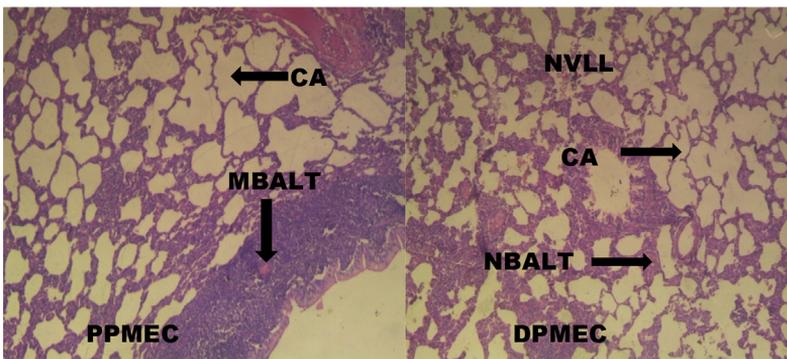
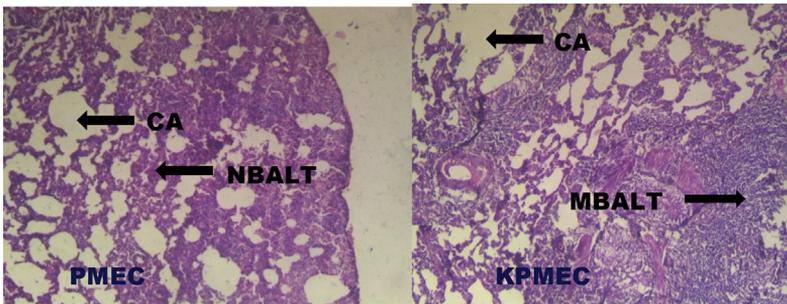
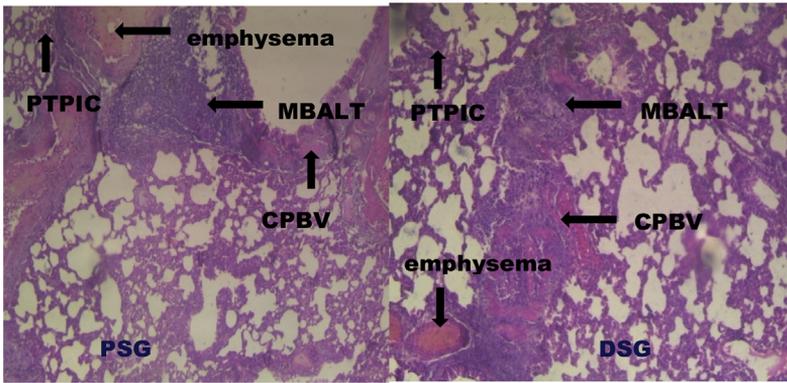
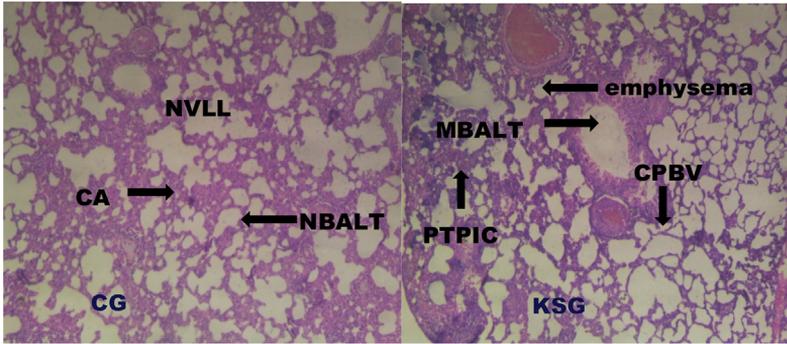
4. Discussion

Acute respiratory infections (ARI) initiated by exposure to complex mixtures of petroleum hydrocarbons are the leading cause of diseases worldwide and had been accounted for the deaths of several millions of people in developing countries [26]. Approximately, 36 million Americans suffer chronic lung disease induced by a number of etiologies [27,28]. It was also recently reported that exposure to mixed fuels or kerosene fractions in Asian countries caused abnormal pulmonary functions characterized by cough, cold, congestion, and phlegm [29]. However, in this study, the pulmonary protective capacity of the *Mantisa religiosa* egg case (MREC) flour against exposure to fractionated petroleum hydrocarbons was investigated. The proximate analysis confirmed that MREC contains a very high level of proteins as previous studies suggested that dietary protein particularly essential amino acids could protect against pulmonary fibrosis and lung damage [30–32]. This is in line with the study that highly protein-rich foods could improve the pulmonary immune system by combating lung diseases and infections [33]. Similarly, this study depicted the presence of macronutrients namely calcium, potassium, magnesium, sodium, manganese, iron, copper and zinc. The predominantly occurrence of calcium and potassium suggest normal cell and tissue growth as well as development and maintenance of functional alveoli [34]. The intake of dietary calcium in the body was linked to cardiovascular and tracheobronchial wellness capacitated by thoracic diaphragms [35]. Additionally, manganese, copper, and zinc exert synergistic actions in scavenging lung's free radicals [36,37] while phenols, tannins, flavonoids, saponins, and carotenoids are of enormous significance to the lungs because they offer protection against lung's intrinsic inflammation, pulmonary epithelial apoptosis and chronic obstructive pulmonary disease (COPD) [38,39].

The high activities of plasma AST and ALP in animals exposed to petroleum hydrocarbons is an indication of hepatotoxicity, hemotoxicity, mutagenicity, genotoxicity, carcinogenicity, and immunotoxicity [40,41]. The increase was linked to the interaction of petroleum constituents with the hepatic biomolecules to initiate alteration of the hepatocellular competence cum pulmonary function [42]. A recent study showed that hepatic biomarkers were evidenced among industrial workers with hepatocellular carcinoma or liver necrosis [43]. Positively, depleted activities of AST and ALP by co-treatment with 4% dietary supplemented mantis formula in animals exposed to aerosolized fractionated petroleum products suggest reversal of liver failure associated with pulmonary dysfunctions [44–46]. This is because diets fortified with macronutrients and phenolic compounds with proteins could delay lung inflammation, hepatic cirrhosis and ROS prophylactics in liver tissues [47–49] in experimented mammals.

Furthermore, the remarkable elevation of malondialdehyde (MDA) content in animals exposed to fumes of petroleum hydrocarbon products indicates the production of free radicals [7] in the lung. A study had reported that exposure to petrol fumes in an enclosed chamber increased the MDA content of the lung [50]. However, the hiked MDA level was abridged by treating with 4% DSMF suggested the protective effect of MREC against pulmonary toxicity triggered by exposure to petroleum products animal model. In addition, the reduction of MDA level in lung rat treated with MREC flour clearly demonstrated the mopping of free radicals by its phenolic compounds and macronutrients [7].

Specifically, ecto-51-nucleotidase converts extracellular AMP to adenosine when extracellular ATP is dephosphorylated into ADP, AMP, and adenosine at the exterior of bronchial epithelial cells [51,52]. The increased activity of 51-nucleotidase following the exposure to aerosols of fractionated petroleum products validates the hydrolysis of 51-AMP catalyzed by 51-nucleotidase as the central cause of adenosine production. This implies that locally released nucleotides specifically for pulmonary wellness are depleted by ecto-enzymes confined on the lung cell surface [53]. This supports the studies that extracellular nucleotides and nucleosides are essential signaling molecules in the lung for its stability and constancy [54,55]. Additionally, a composite linkage of surface ectonucleotidases regulates nucleotide and nucleoside levels in alveolar lining fluid [56] whereas adenosine mediates the lung



workability via the production of energy [57]. Conversely, the reduction of AMP hydrolysis following treatments with 4% DSMFF suggests high conservation of ATP in the lung [58]. Similarly, the reduction of 51-nucleotidase activity in the lungs via gene knockout or 51-nucleotidase depletion in vitro recovered pulmonary endothelial cell function and restore diaphragm potency [59] in animal models. While up-regulation of 51-nucleotidase has been associated with asthma, emphysema and tracheal disorders [60–63] and is reflected as one of the chief biological mechanisms that damage pulmonary endothelial cells.

Elevated lung ADA activity is a novel index for detecting broncho-pulmonary disorder [63] and pulmonary dysfunctions [64,65]. Thus, increased pulmonary ADA activity on exposure to aerosolized petroleum hydrocarbons is a consequential effect of the removal of lung's extracellular adenosine [63]. The depleted adenosine impaired adenosinergic signal transduction of the bronchi and alveoli by accumulating alveoli monocytes and macrophages during exposure to aerosols of fractionated petroleum hydrocarbons [66]. Some studies had reported that patients suffering from Chronic Obstructive Pulmonary Disease (COPD), squamous carcinoma, contagious pleuroparenchymal tuberculosis, and bronchopneumonia had uncontrollable production of ADA activity [67–69]. The reduction of ADA activity in the rats' pulmonary tissues was certainly associated with the action of macronutrients and proteins as essential minerals and amino acids particularly glutamate, could modulate the activity of ADA enzymes [70,71]. In addition, the remarkable down-regulation of ADA activity following co-treatment with 4% DSMFF is consistent with the other report on the modulatory effect of ADA activity by diets prepared from plant seeds [72].

Cytological analysis of the lung tissues of the exposed experimental animals depicted proliferative thickening of pulmonary interstitial cells, distended alveoli (emphysema) marked proliferation of bronchial associated lymphoid tissues followed by the congestion of pulmonary blood vessels. This outcome was linked to the synergistic toxicity effect of petroleum fractions including n-alkanes, branched alkanes, cycloalkanes, and olefins [73]. Studies have also reported that exposure to petroleum hydrocarbons impairs type II pneumocytes causing alveolar collapsed [6,8], hemorrhagic alveolitis, interstitial inflammation, intra-alveolar hemorrhage, edema, hyperemia, bronchial necrosis, and vascular necrosis [74,75]. However, co-treatment with 4% dietary supplemented *Mantisa religiosa* egg case mitigated and prevented the accumulation of fibroblasts or connective-tissue cells that could lead to the excessive production of collagen, thereby making pulmonary tissues more flexible to depict potent lung's respiratory capacity [76]. This study, therefore, suggests that high expression of Sirt6 protein responsible for pulmonary fibrosis and lung damage via activating TGF- β 1/Smad3 molecules [76] had been inhibited by the intake of MREC flour. Nevertheless, further mechanistic studies are required to juxtapose this observation.

5. Conclusion

Sub-chronic exposure to fumes of petroleum products for 14 days significantly increased the activity of pulmonary ADA, 5¹-nucleotidase and MDA content cum liver damage by initiating proliferate thickening of pulmonary interstitial cells, distended alveoli (emphysema), marked proliferation of bronchial associated lymphoid tissues followed by the congestion of pulmonary blood vessels. Diet fortified with 4% *Mantisa religiosa* egg case inhibited the activity of pulmonary ADA, 5¹-nucleotidase

Fig. 5. Pulmonary histopathology changes on rat treated with dietary supplementation of Mantis formula in animals exposed to aerosolized petroleum hydrocarbons for 14 days (sub-acute exposure) (original magnification \times 400). **CG** showed clear airways (CA) (bronchi and alveoli) with normal proliferation of the bronchial associated lymphoid tissues (NBALT) (thick arrow), although showing minor congestion of pulmonary blood vessels indicating no visible lesions to the lungs (NVLL). Group of animals (**KSG**, **PSG** and **DSG**) aerosolized with petroleum hydrocarbons depicted proliferative thickening of pulmonary interstitial cells (PTPIC), distended alveoli (emphysema) marked proliferation of bronchial associated lymphoid tissues (MBALT) following by the congestion of pulmonary blood vessels (CPBV). Although, the groups of animals (**KPMEC**, **PPMEC** and **DPMEC**) co-supplemented with Mantis formula showed slight proliferative thickening of pulmonary interstitial cells (PTPIC) with clear airways. Also, mild hyperplasia of the bronchial epithelial cells (MHBECC) and trivial congestion of pulmonary blood vessels (TCPBV) were observed. Lastly, the animals treated with **PMEC** only indicated normal pulmonary interstitial cells (NPIC) and normal blood vessels (NBC), showing clear airways (bronchioles and alveoli) i.e. absence of pulmonary emphysema.

and MDA content. Therefore, we can suggest that MREC administration may have immense therapeutic potential for subjects exposed to fumes of petroleum products. However, further studies with elucidation of molecular mechanism of action of MREC should be elucidated before starting the clinical test.

Conflict of interest

All authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yclnex.2019.04.001>.

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