Tumorigenic Potential of Dimethyl Sulfoxide, Anthracene in Fish and Anthracene in Dimethyl Sulfoxide in Rattus Norvegicus

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Research Article

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Posted Date: May 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-477123/v1

TUMORIGENIC POTENTIAL OF DIMETHYL SULFOXIDE, ANTHRACENE IN FISH AND ANTHRACENE IN DIMETHYL SULFOXIDE IN RATTUS NORVEGICUS

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ABSTRACT

Anthracene is a polyaromatic hydrocarbon (PAH), present in cigarette smoke and some industries. It may cause tumorigenesis of lungs and other organs. Randomized controlled trial was adopted for the study. Seven out of forty-three rats were used for determination of median lethal dose (LD_{50}), whereas thirty-six (36) rats were divided into six groups of six per group. The first group was treated daily with3ml/kg body weight ofwater; the second was treated daily withdimethyl sulfoxide (DMSO) (30mg/kg), whereas third, fourth and fifth groups were treated with anthracene in DMSO at12.5, 25

and 50 mg/kg body weight, respectively.The sixth group was treated daily withanthracene in fish (75 mg/kg)for a period of three weeks. Pre-treatment blood samples were collected on day zero (0) and thereafter on day 7, 14 and 21 respectively for haematological, cardiorespiratory, tumour kinetic, allometric parameters, as as well as oncogenic biomarkers. Findings revealed that dimethyl sufoxide and anthracene may cause adenocarcinoma of lung characterised by anaemia, neutrophilia or neutropenia, increased cancer antigen-125 (CA-125), carcinoembryonic antigen (CEA), increased heart rate and respiratory rate, superior vena cava oedema and histological changes such as reduced alveolar space, damaged alveolar sacs and infiltration of interseptal space with mononuclear cells. Micro nodules were observed in fibrosed lungs. Hence there is need to assess hazard risk of anthracene in industries and anthracene contaminated environment.

Keyword: Anthracene, Dimethyl sulfoxide, Biomarker, Lung tumour, Rat, Parameter.

INTRODUCTION

Anthracene also called paranaphthalene or green oil, is a solid polycyclic aromatic hydrocarbon (PAH) of formula $C_{14}H_{10}$, consisting of three fused benzene like rings. It is the simplest tricyclic aromatic hydrocarbon from coal tar; melts at 218°C and boils at 354°C (Lindsey *et al*., 2014;Aleshina*et al*., 2004;Haynes 2014). Although it is insoluble in water, it is soluble in most organic solvents such as carbon disulfide, alcohols, benzene, chloroform and hydronaphthalenes (Aleshina*et al*., 2004;Haynes 2014;Douben, 2003), but it is partially soluble in DMSO and Ethyl acetate.A total of 539 PAHs are present in tobacco cigarette and 16 could cause cancer including anthracene

and naphthalene (USEPA, 1986). They are cytotoxic by causing decreased organ function, damage, hyperplasia, oxidative stress, inflammation and cell proliferation (Andreoli*et al*., 2003). About 10 – 500ng have been reported for naphthalene in cigarette (Ding *et al*., 2005). Therefore, PAHs are environmental pollutants that result from incomplete combustion of organic materials present in red and white cigarette. They are 2 – 8 ring semi-volatiles (IARC, 2004). Like many other polycyclic aromatic hydrocarbons, anthracene is on the EPA's priority pollutant list. It has been identified in surface and drinking water, ambient air; exhaust emissions, smoke of cigarettes and cigars, in smoked foods, fossils and edible aquatic organisms. Exposure to humans happens mainly through tobacco smoke and ingestion of food contaminated with combustion products. One of the most common ways anthracene enters the body is through breathing contaminated air; this then gets into the lungs. Occupational exposure occurs mainly in a hazardous waste site where PAHs are disposed, working in a plant that makes coal-tar or that uses petroleum or coal or makes or uses wood preservatives.Prolonged exposure causes variety of topical and systemic adverse reactions (Faus, 1991). Human exposure to anthracene has also been associated with headache, nausea, loss of appetite, inflammation of the gastrointestinal tract, slow reactions, and weakness (Volkova, 1983).

Anthracene can vary in appearance from a colorless – white - pale yellow - yellow crystal-like solid with a weak aromatic odorbut exhibits a blue fluorescence under ultraviolet radiation (400–500 nm peaks) (Lindsey *et al*., 2014, Larranaga*et al*., 2016, USCG 1999].Oxidation of anthracene yields anthraquinone, the parent substance of a large class of dyes and pigments (Aleshina*et al*., 2004, Collin *et*

al., 2006); hence, used in the production of the red dye alizarin and other dyes (Lindsey *et al*., 2014), smoke screens, scintillation counter crystals and in organic semiconductor research.It is also used in wood preservatives, insecticides and coating materials. Anthracene is commonly used as a UV tracer in conformal coatings applied to printed wiring boards. The anthracene tracer allows the conformal coating to be inspected under UV light (Zeitler, 2012).

Gas chromatography/mass spectroscopy (GC-MS) could be used to detect PAHs (Vu *et al*., 2015).GC-MS could detect 4.6ng/cigarette (Zha*et al*., 2002). PAHs are present also in foodstuffs and beverages; many of them have structure - tumorigenic relationship, classified as bicyclic or tricyclic depending on their number of cyclic rings. The compounds are further subdivided into all-benzenoid and cyclopentanoid-benzenoid (Rodgman and Perfetti, 2006). Lung cancer volume can be detected and quantified in experimental rodent using contrast agent-based X-ray micro-tomography (Bidola*et al*., 2019). Lung tumour could be treated using radioactive rays (Zhang *et al*., 2016) and chemotherapy (Zha*et al*., 2018). In view of this there is need to investigate tumorigenic potential of DMSO, anthracene in fish and anthracene in DMSO with intent to assessing hazard-risk-benefit of anthracene, DMSO and fish in tumours.

MATERIALS AND METHODS

Experimental animals

A total of forty-six rats weighing 150 ± 15g were used for the study. The rats were sourced from local breeders in Makurdi, Benue state, Nigeria and kept in plastic cages. They were allowed to acclimatize for a period of two (2) weeks, before the

commencement of the experiment. They were fed with standard feeds and water was provided ad libitum. The experiments were conducted according to international guiding principles for biomedical research involving animals (C.I.O.M.S 1985), and as recommended by ethical committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi.

Acute toxicity study

The upper limit test was adopted to determine the median lethal dose (LD_{50}) of anthracene and DMSO base on OECD 425 guidelines for testing chemicals, (2000).Three rats each were administered 3000 mg/kg of DMSO and anthracene in DMSO and observed for a period of 2weeks. Median lethal dose of anthracene in 10% acetone and ethanol was determined according to Saganuwan method (2015).One each of male and female rats was administered 250 mg/kg body weight and observed for 14 days. All the two rats died within the period of two weeks. Thereafter one each of male and female rat was administered 125 mg/kg body weight and observed for two weeks. All the two rats survived for a period of over two weeks.

Sub-acute toxicity study

The rats were divided into six groups of six rats each. Group one (I) rats were administered only water,while group (II – V) were treated per Os daily with DMSO (30 mg/kg), anthracene in DMSO 12.5, 25 and 50 mg/kg body weight respectively, whereas Group (VI) rats weretreated per Os daily with 75 mg/kg of anthracene in fish, for a period of threeweeks. Pre-treatment blood samples were collected on day zero and thereafter on day 7, 14 and 21 respectively for haematological parameters which

include packed cell volume (PCV), red blood cells (RBC) count, total and differential white blood cells (WBC) count as described by CSLI (2000),Pal*et al*., (2006),CSLI (2007),Praful and DarshanGodkar (2003), Cheesbrough (1998), respectively. Serum was harvested from the whole blood for biochemical analysis of oncogenic biomarkers, CA-125 and CEA using Enzyme Linked Immunosorbent Assay (ELISA) method. The body weights of the rats were measured and recorded prior to the administration of the chemicals and thereafter weekly. The body weights were used to calculate and obtain the physiological parameters and allometric scaling. The rats were euthanized using 100mg/kg of pentobarbital. The lungs were collected for histo-pathological examination. The weight, width and breath of the lungs collected were measured for allometry.

Determination of haematological parameters

Determination of red blood cell (RBC) count

The RBCs were counted after making a 1:200 dilution with Hayem'sfluid. The improved Neubauer chamber was charged after 2minutes and the RBCs counted using a microscope with x40 magnification (Hayem 1889, Pal*et al*., 2006).

Determination of total white blood cell (WBC) count

The WBCs were counted after making a 1:20 dilution with Türk's solution.Erythrocytes hemolyze while leukocytes were stained for easy visualization. The improved Neubauer chamber was charged after 30 minutes and the WBCs counted using a microscope with x10 magnification(Cheesbrough, 1998).

Determination of packed cell volume (PCV)

The blood sample was drawn into a heparinized capillary tube and centrifuged at 12,000 rpm for 5minutes, and then the ratio was measured and expressed as a percentage (CLSI, 2000,Cheesbrough, 1998).

Determination of differential white blood cells count

Thin film made with a drop of blood was stained using a modified Leishman's stain technique.White blood cells were counted after air drying the slides. The slides were then observed under oil immersion objective lens of the microscope. Two hundred cells were then counted and classified. Cells counted were expressed in percentage. (Praful and DarshanGodkar, 2003; CLSI, 2007; Cheesbrough, 1998).

Determination of oncogenic biomarker

CA-125 and CEA were analysed by enzyme immunoassay and colorimetric method, using commercial Elisa kit (Accu-bind, MonbindInc.). After the reaction time, the micro wells were read using EMP microplate reader by Shenzhen *Emperor* Electronic Technology.

Calculation of physiological parameters

Physiological biomarkers were calculated using the following formulas as described bySchmidt-Nielsen (2004) and Saganuwan(2012).

Body mass ratio (Kcal/day): $3.52\ W^{0.75}$ (where W = body weight in gram)

Total o_2 consumption, V_{02} (litre O_2 h^{-1}): 0.676 x $M_b^{0.75}$ (where M_b = body weight in kilogram)

O_2 consumption per kilogram (litre h^{-1} kg^{-1}): 0.676 x $M_b^{-0.25}$

Heart rate (min^{-1}): 241x $M_b^{0.25}$

Lung ventilation rate (litre h^{-1}): 20.0 x $M_b^{0.75}$

Lung volume (litre): 0.063 x $M_b^{1.02}$

Tidal volume (litre): 0.0062 x $M_b^{1.01}$

Respiration frequency (min^{-1}): 53.5 x $M_b^{-0.26}$

Determination of tumour kinetic

Tumour size, tumour volume, tumour weight, tumour doubling time, specific growth rate, tumour cells per day, labelling index, growth factor, tumour doubling time potential and cell loss factor were determined using the method of Saganuwan (2019).

Tumour volume (mm^3) = 4/3x π x r^3(where $\pi = 3.14159$ and r = d/2).

Tumour weight (mg) = $\frac{a \times b^2}{2}$ (where *a* and *b* are the tumour length and width in millilitre)

Tumour doubling time (day) = One gram of tumour mass = 10^9 cells = 30 doubling time

Specific growth rate SGR (%/day) = $\frac{ln_2}{DT} x100$ (where DT is tumour doubling time and $ln_2 = 0.6931$)

Cell cycle per day (day) = $\frac{TW \; X \; 10^9}{1000}$(where *TW* = tumour weight and *TDT* = tumour doubling time)

Libelling index (%) = $\frac{TD}{Ts}$ (where Ts = the duration of the S phase, Tc = the duration of the cell cycle)

Growth factor (%) = $\frac{P}{Q + P}$ (where P = proliferating cells, Q = quiescent cells)

T_{pot}(where T_{pot} = tumour doubling time potential
Cell loss factor (%)= $\Phi = 1 - \frac{TD}{}$ and TD = tumour doubling time)

Tumour doubling time potential (day): $\frac{Tc}{GF}$ (where GF = growth factor, Tc = cell cycle time)

Tumour cell kill = log10 cell kill total = $\frac{T - C}{3.32} X TD$ (no treatment given in this context)

(where T = median time (days) to reach 1g (1000mg), C = median time (days) for control tumours and TD = tumour doubling time).

Histopathology

The tissue samples of the lung obtained from the sacrificed rats were fixed in 10% formalin. The tissue were dehydrated through graded concentration of ethanol (70%, 95% and 100%), then cleared in xylene and embedded in paraffin wax. The embedded tissues were stained with Hematoxylene (1^0 dye) and Eosin (2^0 dye) (H & E) and observed under a light microscope with x100 magnification. The lesions observed were photographed using Vanox T Olympus photographing microscope (Drury *et al*., 1976).

Statistical analysis

All data generated during the study were expressed as mean ± standard error of mean (S.E.M) and analysed statistically by one way analysis of variance (ANOVA). Sifnificant difference was considered at $p \leq 0.05$ using Post Hoc Turkey test. IBM SPSS version 24 computer statistical software was used for the analysis.

RESULTS

The limit dose test of 3000 mg/kg each of anthracene in DMSO and anthracene in DMSO was survived by the rats, thereby projecting LD_{50} to be above 3000mg/kg. Also the $LD_{50}$of anthracene in 10% acetone and ethanol was estimated to 180.7 ± 36.1 mg/kg body weight (Table 1). The abnormal signs observed in the male rats administered anthracene in 10% acetone and ethanol are squeaking, lying in ventral recumbency, nodding of head, partial paralysis, whereas the female rats showed calmness, dullness, high respiration, mild squeaking and partial paralysis before death. Post mortem lesions observed in both male and female rats were congested lungs and heart, empty stomach with normal consistency and areas of necrosis in the liver except for shrunken testis in the male.The signs observed in the rats for the acute toxicity study using DMSO where not as characterized as those for anthracene in 10% acetone and ethanol but include dullness, death in few minutes , high respiration and weight loss. Post mortem lesions include hydrothorax, focal areas of necrosis on the lungs, congested and micro granulations on the liver, full stomach and empty intestine.Effects of DMSO, anthracene in DMSO and anthracene in fish on body weight, total blood volume, packed cell volume, erythrocyte volume and erythrocyte counts are presented in Table 2. There was significant decrease ($p<0.05$) in all the parameters at dose level of 50mg/kg of anthracene in DMSO on day 7, 14 and 21 except for plasma volume on day 21. Anthracene in DMSO at a dose level of 12.5mg/kg and 25 mg/kg, DMSO (30 mg/kg) and anthracene in fish (75mg/kg) showed significant increase ($p<0.05$) in the haematological parameter, except on day 14 and 21 for packed cell volume.Total white blood cells counts and granulocytes increased significantly ($p<0.05$) on day 21 in

50mg/kg ofanthracene in DMSO treated rats, whereas monocytes and lymphocytes decreased significantly ($p<0.05$) in the same group. Similar significant increase or decrease was observed in other treated groups (Table 3) except the group administered water. Cancer antigen 125 (CA-125) and carcinoembryonic antigen (CEA) were significantly increased in all the treated groups (Table 4). Body calorie, total oxygen consumption, lung ventilation rate, lung volume and tidal volume were significantly increased in 50mg/kg of anthracene in DMSO treated group, whereas respiration frequency and heart rate were significantly decreased ($p<0.05$) in the same treated group. However, reverse was the case in DMSO and anthracene in DMSO (12.5 and 25mg/kg) treated groups. Anthracene in fish (75mg/kg) treated group was not affected significantly (Table 5).The tumorigenic potential of anthracene on haematological parameters shows significant increase in the group administered water, DMSO and anthracene in DMSO 12.5 and 25 mg/kg except the 50 mg/kg treated group. However the group treated with 75 mg/kg body weight had the parameters restored to normal, but RBCs decreased in all the groups treated with DMSO, anthracene in DMSO and anthracene in fish ($p<0.05$) (Table 6). There was significant difference in leucocytes counts in all the treated groups ($p<0.05$) (Table).The euthanized rats were observed for post mortem lesions. Before euthanasia, the rats administered 50 mg/kg had alopecia from 14day after administration, Increased respiration and lethargy.After euthanasia, stomach and intestine were empty. Lungs were paleand enlarged weighing an average of 2.1g, measuring 3.6 x 1.4cm with macro nodular lesions measuring 3.0 x 2.1mm.Areas of degeneration were observed on the lungs of more than 50% of the rat population. Whereas, in the rats administered DMSO (30 mg/kg). Hydrothorax was

observed in about all the rat population, the lungs were pale and shrunken. Stomach was full but intestine was empty. The heart was congested.

DISCUSSION

Induction of lung tumour associated with its biomarkers within 3 weeks using DMSO and anthracene in DMSO agrees with the report indicating that tumorigenesis of the lung can take place within 21 days (Neto*et al*., 2008). Decreased calorie, total oxygen consumption, oxygen consumption per kilogram, lung ventilation rate and tidal volume which are suggestive of dyspnea and hypoxemia observed in this study, agrees with the report recommending oxygen for lung cancer patients (Tiep*et al*., 2013). However, oxygen use is not associated with survival in patients with advanced lung carcinoma (Igarashi *et al*., 2020), but, new adjuvant chemotherapy could cause linear peak of volume of oxygen (Vo_2) in cancer patients (Fresard*et al*., 2016). Conversely, peak oxygen consumption is a predictor of lung cancer resected patient survival (Lindenman*et al*., 2020). Therefore peak Vo_2 is an independent predictor of survival in non-small cell lung cancer (Jones *et al*., 2010). Tidal volume is a predictor of pulmonary complication in lung cancer surgery (Licker *et al*., 2011). Increased and decreased heart rate observed in 50mg/kg and 25mg/kg anthracene in DMSO treated groups respectively agree with the report of Franklin *et al* (2016) indicating that heart rate is a predictor of survival in non-small cell lung cancer and as such represent a therapeutic target as observed in anthracene in fish (75mg/kg) treated group. Univariant analysis reveals that heart rate variability parameters are a predictor of poor survival in advanced non-small cell lung cancer (Kim *et al*., 2015) and brain metastasis (Wang *et al*., 2013). However, elevated heart rate could be a risk factor for cardiopulmonary complications

after resection of lung cancer (Fu *et al*., 2018), but asymptomatic sinus bradycardia could be caused by crizotinib in non-small cell lung cancer patients (Ou*et al*., 2011), which has 5 year survival in stage I of >21mm (Okada *et al.,* 2004). Staging of primary carcinoma of the lung acquires invasion of the elastic layer of viscera pleura (Khan and Lynch, 2021). Adenocarcinoma, squamous cell carcinoma and large cell carcinoma are the three main types of non-small cell lung cancer that affect 80 – 85% whereas small cell lung cancer which is neuro-endocrine affect 15 – 20% lung cancer patients (Perez-Moreno *et al.,* 2012).

The increased cancer antigen 125 (CA-125) and carcinoembryonic antigen (CEA) observed in all the treated groups confirm tumorigenic potential of DMSO and anthracene in the present study. CA-125 and CEA have been reported to be biomarkers of non-small cell lung cancer, lung tumour, lung adenocarcinoma, liver and ocular metastases secondary to lung cancer (Salfic*et al*., 2000; Diez*et al*., 1991; Bucheri and Ferrigno, 2002; Yang *et al*., 2018; Li *et al*., 2020; Zang*et al*., 2019; Isaksson*et al*., 2017; Wang *et al*., 2020) as indicated by gross pathology and histopathology of the lung lesions. The two antibodies could be used also for lung cancer screening, liver and ocular metastases secondary to lung cancer and as a predictor of resectable lung adenocarcinoma recurrence (Yang *et al*., 2018; Wang *et al*., 2020; Li *et al*., 2020; Isaksson*et al*., 2017).Therefore, oncologist should take cognisance of clinical relevance of anaemia in lung cancer (Pirker*et al*., 2003), because observed anaemia could be exacerbated by some anticancer drugs (Crawford *et al*., 2006). Decreased red blood cells distribution width (RDW) is a strong index of survival in lung cancer patients (Kos *et al*., 2016). The observed neutrophilia and neutropenia in the treated group agrees

with the report indicating that neutrophils could be used as biomarker of cancer detection. Tumour- associated neutrophilia (TANs) could be controlled by tumour microenvironment and aid in tumour progression. So TANs could be detrimental and beneficial (Uribe-Querol and Rosales, 2015). However, neutrophilia is a negative prognostic factor in lung cancer patients, but other white blood cells do not affect patient survival (Kohutek and Bystricky, 2019). Nevertheless, leucocyte count is independent on physical activity in lung cancer patients (Sprague *et al*., 2008), as shown by increased heart rate and respiration rate in the present study. Suggesting that leucocytosis is an important biomarker for increased lung cancer risk (Wong *et al*., 2019). Hence, tumour-related leucocytosis is related importantly to non- small cell carcinoma and it is an ominous prognostic sign (Kasaga*et al*., 2001). The swelling of the right vena cava of all the rats administered 50mg/kg of anthracene in DMSO is similar to superior vena cava syndrome in cancer, thoracic malignancies, thyroid metastasis and non-small cell lung cancer, as position is dependent onperiorbital oedema, in a patient with advanced lung cancer that can respond to chemoradiation, and can be treated with implantation of intravascular stent and chemotherapy, angioplasm, anticoagulation, raising the head, and its proposal classification maybe based on algorithm of management (Coiffard*et al*., 2014, Kinnard 2012; Lepper*et al*., 2011; Pose *et al*., 2017; Brzeniak*et al*., 2017; Rajagopal and Simala, 2014; Ngugen*et al* ., 2018; Hinlon*et al*., 2018; Chong *et al*., 2005; Cirino*et al*., 2005; Yu *et al*., 2008). Induction of lung tumour as shown by histological slides is via histochemical enzymes (Suzuki, 1966).

Tumor makers are quite diagnostic, prognostic and for monitoring cancer initiation and progression (Faulkner and Meldrum, 2012). Therefore tumor makers

including CEA are used for population screening, diagnosis, tumor detection and staping. They are proteins, antigens, glycoproteins, hormones and enzymes. Monoclonal antibody is commonly used for identification of specific markers in urine, blood and tissue samples (Kumar *et al*., 2014). CA-125 of molecular weight 200KD identifies ovarian carcinoma and it is prognostic after chemotherapy, whereas CEA which comprises 45-60% carbohydrate (180KDa) is used for monitoring gastrointestinal colorectal, lung and mammary tumors (Kumar *et al*., 2014). High CEA levels have been observed in epithelial tumors and 40-80% of non-small cell lung cancer with higher sensitivity in advanced cancer (Fletcher, 1986; Hansen, 1991). Hence numerous biomarkers are required for detection of lung tumor at early stage (Ferrigno and Buccheri, 1995). Interstitial fibrosis, emphysema and entrapped pneumocytes show predominance of squamous cell carcinoma (Hayashi *et al.,* 2013). Suggesting that natural products may be used to fight both phenotypic and genetic components of oncogenesis (Bertrand *et al*., 2014) together with analogous patient-derived immune cell vaccines, peptide vaccine and tumor antigen-expressing recombinant vaccine (Thomas and Prendergast, 2016). Hydration and nutrition provide benefit for cancer patients (Arends*et al*., 2017). Overweight and obesity increase the risk of colorectal, oesophageal, breast, endometrial and kidney cancers. Fruits and vegetables reduce the risk of oesophageal, colorectal, stomach and oral cavity cancers. The ameliorative effects of fish in the present study disagrees with the report indicating that China-style salted fish increases the risk of nasopharyngeal cancer, as red meat increases the risk by colorectal cancer (Key *et al*., 2004). However low protein diet is associated with low incidence of cancer risk and mortality, hence it can be used in treatment and prevention

of cancer (Yin *et al*., 2021) as done in the present study. Fish oil is a promising intervention for lung cancer (Kiss, 2016). Vitamin E increased incidence of lung cancer (Slative*et al*., 2008). Defensive micronutrients, carcinogens and mutagens may adapt cancer development in genetically susceptible individuals (Patel *et al*., 2018). Early nutritional intervention improves health status in cancer patients (Richards *et al*., 2020).

Tumor antigens could serve as basis for immunotherapy in the clinical oncology (Ribas*et al*., 2013). Cancer genomes could be rearranged with changes in copy number losses and gains, that deregulate tumor suppressors and oncogenes, which may acquire mutation and fusion genes may be created by rearrangement of genomes. Changes in chromatin structure and methylation could contribute to cancer development. Driver mutations contribute to tumorigenesis whereas passenger mutations are acquired during cancer evolution, but do not contribute to carcinogenesis (Lach and Adams, 2013). Changes observed in cardiorespiratory parameters agree with the report recommending physiotherapy for management of lung cancer (Granger, 2016). More so gene-specific cancer therapy is a function of oncogenes and tumor suppressor genes (Vogt, 1993). Methods used for stimulation of effective and lung-lasting immune responses have been part of focus in cancer vaccinology and immunology (Apostolopoulos, 2019), as cancer vaccine prevents cancer causing viruses, prevents oncogenesis and treats cancer (Arvind*et al*., 2010). Binding of oncogenes to DNA has been responsible for mutagenesis and carcinogenesis (Stark, 1980). Dendritic cells are vital to generation by immune responses, therefore represents vector and target for vaccination in cancer immunology (Palucka*et al*., 2011).

Anthracene has a characteristic environmental behaviour; at 20^0C, it is solid with very low volatility and low solubility. It volatilize moderately once dissolved and adsorbs very strongly to organic matter. When present in soil, anthracene will volatilize and solubilize very slowly. Once dissolved, it will either enter the groundwater table or migrate towards a waterway, where it will be diluted before partially volatilizing. Fragments of anthracene in the waterways will deposit at the bottom and will slowly dissolve. Even after the source is removed, adsorbed anthracene will take a very long time to disappear, thereby, liberating contamination in either the gaseous or dissolved state with relatively small size fumes. Information on the toxicity of anthracene exposure in humans is very limited. Evidence indicates that anthracene is absorbed following oral and dermal exposure. Targets for anthracene toxicity are the skin, hematopoietic system, lymphoid system, and gastrointestinal tract. Adverse dermatologic effects have been observed in humans and animals in conjunction with acute and subchronic exposure to anthracene. In humans, anthracene may cause acute dermatitis with symptoms of burning, itching, and edema. Prolonged dermal exposure produces pigmentation, cornification of skin surface layers, and telangiectasis (Volkova, 1983). There are little or no data on the tumorigenic effects of anthracene on the lungs following oral administration, although a large body of literature exists on the toxicity of PAHs, data for anthracene are limited. Carcinogenicity bioassays with anthracene generally gave negative results except in the present study. The U.S. Environmental Protection Agency has indicated that not enough information exists to classify anthracene as a cancer causing substance (USEPA, 1986).

CONCLUSION

The LD_{50} of anthracene in DMSO was estimated to be above 3000 mg/kg whereas that of anthracene in 10% acetone and alcohol was estimated at 151.4 – 223.6 mg/kg body weight respectively. Anthracene in DMSO at 50 mg/kg caused significant increased tumour parameters that were ameliorated by anthracene in fish (75 mg/kg). the increased parameters were principally of lung tumour. Macro-nodular lesions of 1-3 mm were also observed in the lung of affected rats as confirmed by histopathological lesions. Hence, acute oral ingestion of anthracene could pose risk of lung tumour. Haematological, cardio-respiratory, tumour kinetic and cancer biomarkers could be used for detection of anthracene induced lung tumour in rats.

ACKNOWLEDGEMENTS

The authors thank sincerely MrUpev Vincent, Mr Daniel Achanya and Mr David Orbunde of Physiology and Biochemistry laboratory and Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Makurdi, for their contributions in various capacities.

AUTHORS CONTRIBUTIONS

SaganuwamAlhajiSaganuwan and Patrick A. Onyeyili designed the study, whereas Miriam O. Johnson and Saganuwan A. Saganuwan carried out the study and statistical analysis. All the contributors proofread the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL CLEARANCE

Ethical clearance was obtained from College of Veterinary Medicine, Federal University of Agriculture, Makurdi.

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Figures

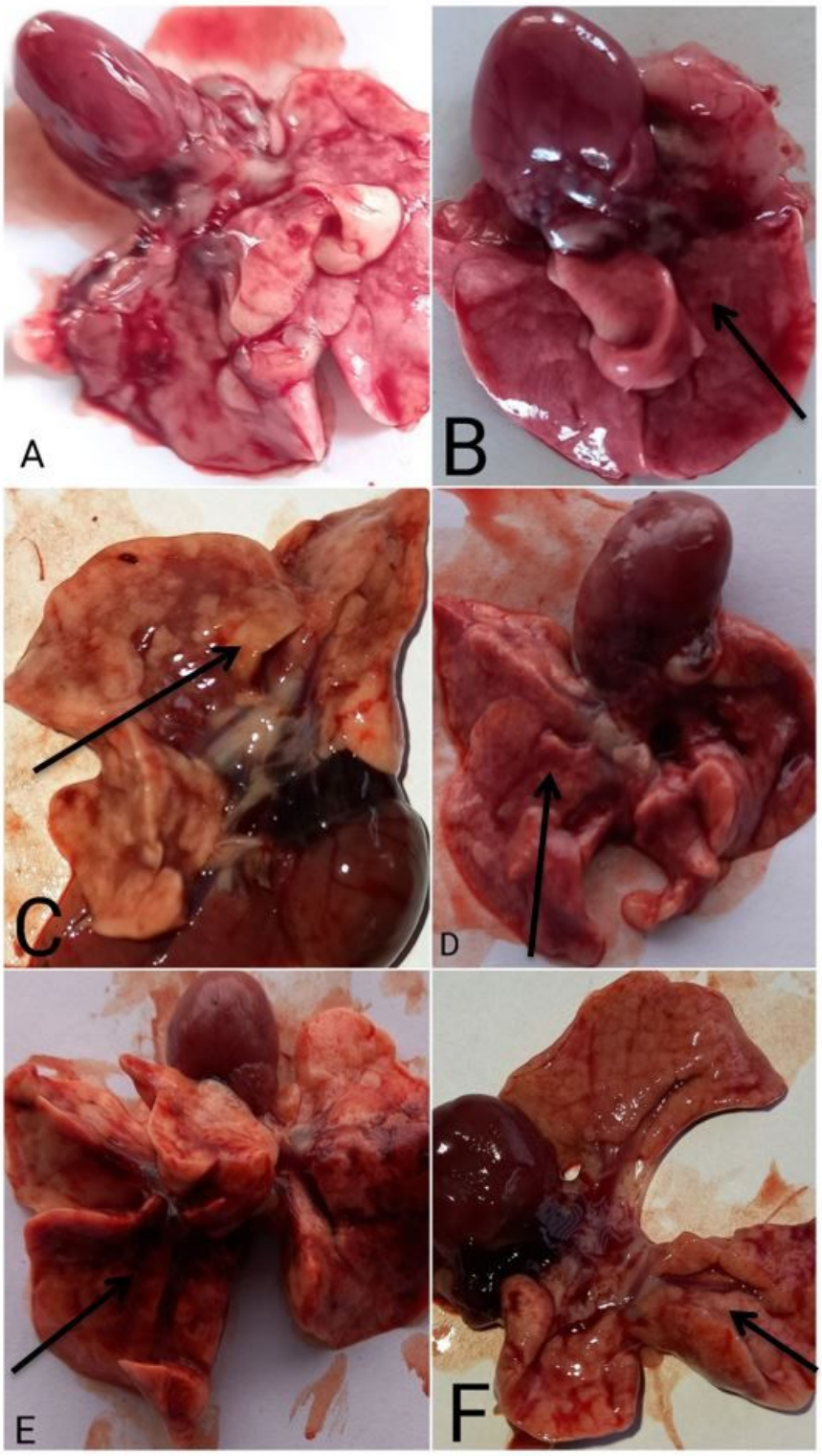

Figure 1

Shows lung and heart of rat administered water (A), anthracene in fish; 75 mg/kg (B), anthracene in DMSO; 50 mg/kg, 25 mg/kg, 12.5 mg/kg (C, D and E respectively), and DMSO; 30 mg/kg (F) for a period of 21 days. Arrows show affected tumour lesions.

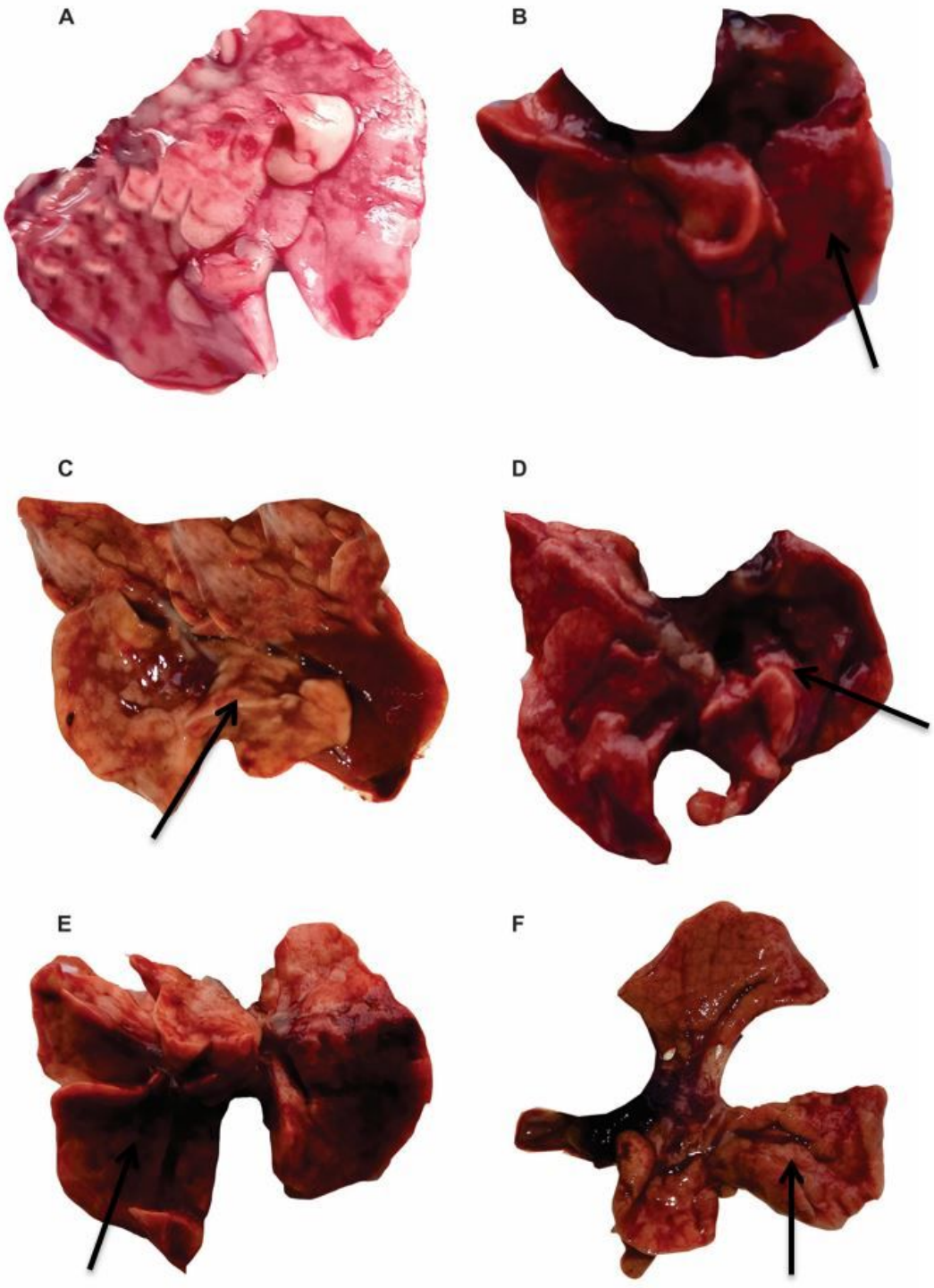

Figure 2

Shows lung of rat administered water (A), anthracene in fish; 75 mg/kg (B), anthracene in DMSO; 50 mg/kg, 25 mg/kg, 12.5 mg/kg (C, D and E respectively), and DMSO; 30 mg/kg (F) for a period of 21 days. Arrows show affected tumour lesions.

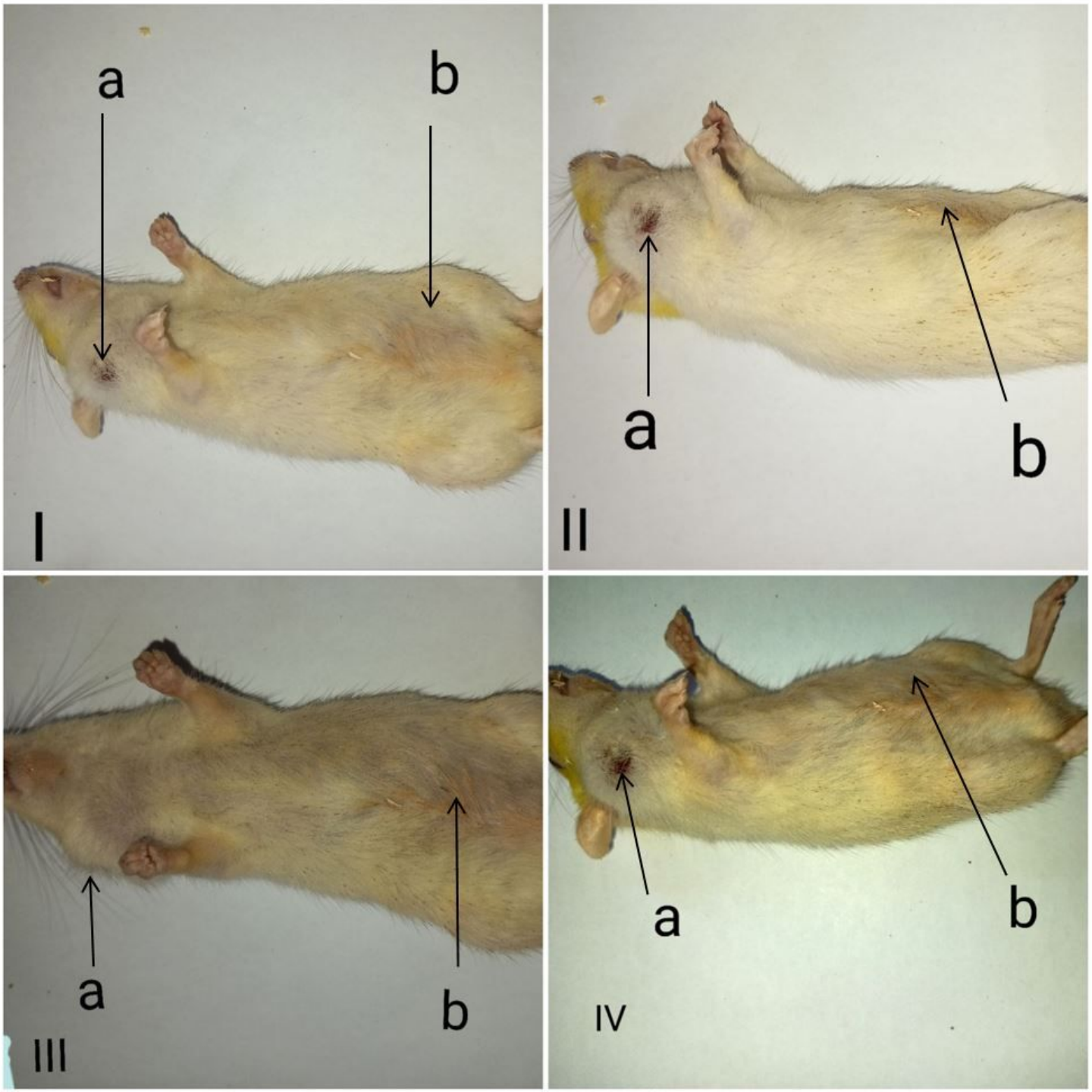

Figure 3

Rats I – IV administered 50 mg/kg body weight of anthracene in DMSO for 21 days, showing edema of superior vena cava (a) and cyanosis (b).

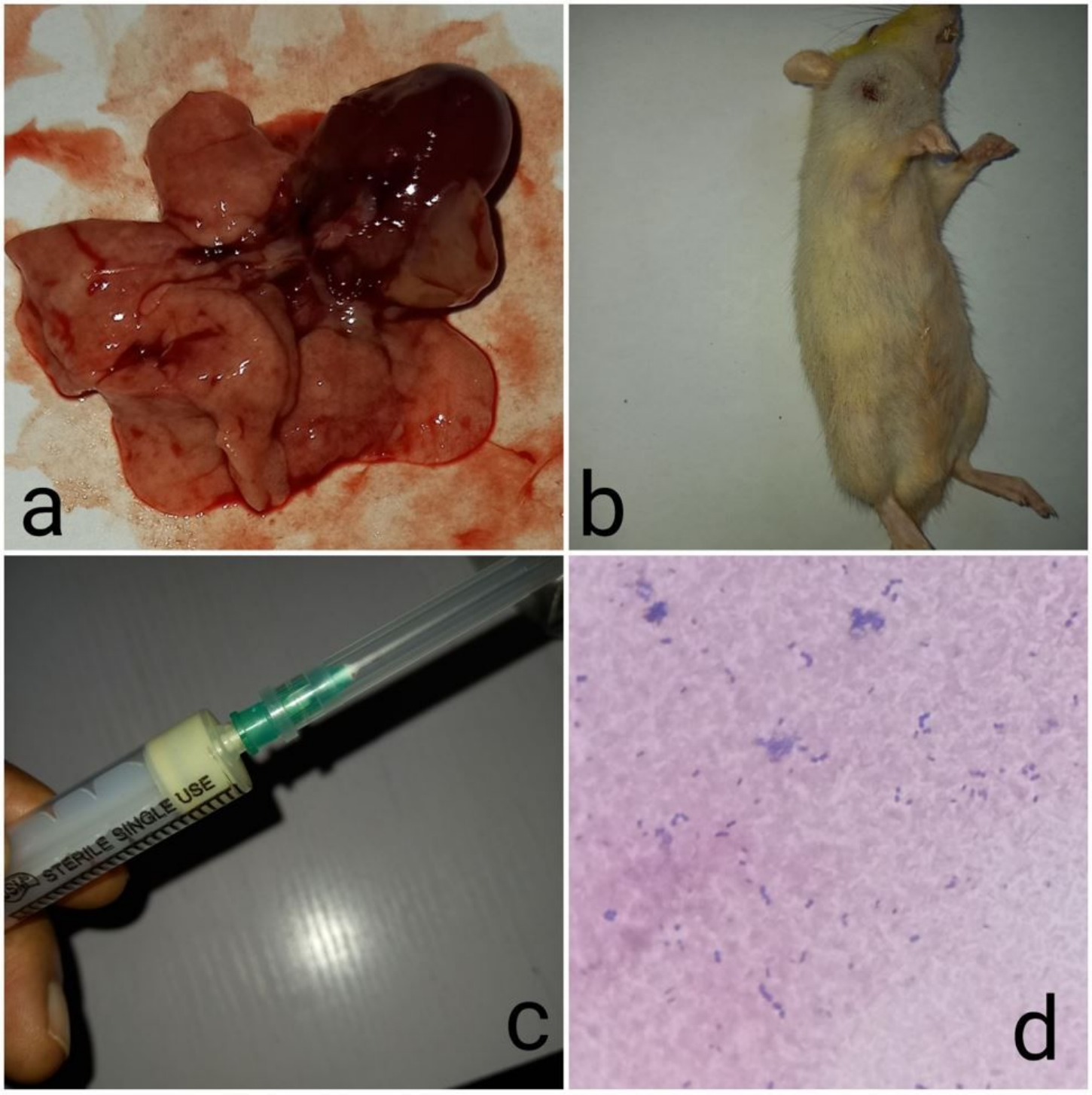

Figure 4

Showing lung and heart of rat (a), with superior vena cava (b), purulent exudates collected from superior vena cava (c) and Gram staining reaction (gram negative bacilli and yeast cells) from aspirates (d) of rats administered 50 mg/kg anthracene in DMSO.

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