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EFFECTS OF AQUEOUS SEED EXTRACT OF *GARCINIA KOLA* ON SPRAY PAINT-INDUCED HISTOLOGICAL CHANGES IN THE LUNGS OF WISTAR RATS

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ABSTRACT

The most common cause of lung diseases among people with spray paint-related occupations is hydrocarbon poisoning which has significant morbidity and mortality if left untreated. This study aimed to investigate the effects of aqueous seed extract of Garcinia kolaon spray paint-induced interstitial pneumonitis in the adult Wistar rats. Thirty adult Wistar rats weighing between 240 g and 270 g were divided into fivegroups of six rats each. Animals in group A were placed on feed and water only. Animals in group B were exposed to 100 ml of spray paintfumes via inhalation. Animals in group C received 500mg/kg body weight per day of Garcinia kola. Animals in group D were exposed to 100 ml of spray paint fumes via inhalation and received 250mg/kg body weight per day of Garcinia kola. Animals in group E were exposed to 100 ml of spray paint fumes via inhalation and received 500mg/kg body weight per day of Garcinia kola. The extract was administered for 30 consecutive days via an orogastric tube. Groups A, C, D and E revealed normal histoarchitecture of the lung; normal alveoli and normal interstitial space. There were observable histological variations in the lung tissues of Group B rats which include severe alveolar dilation, interstitial haemorrhage, interstitial infiltrates of inflammatory cells, severe vascular ulceration and congestion (evidence of interstitial pneumonitis). It is concluded that Garcinia kolahasan ameliorative effect on spray paint-induced histological changes in the lungs of Wistar rats.

Keywords: *Garcinia kola*, histoarchitecture, Spray paint, Interstitial pneumonitis, Lungs.

INTRODUCTION

Spray paint is a type of paint that is dispensed in a fine mist from a can or container (Ojo, 2020). Spray paint is typically made up of a mixture of pigments, solvents, propellants and additives (Ibrahim et al., 2016). Painting and coating workers in the architectural field, automotive workers, including those in automotive refinishers, marine workers who paint and seal ocean and freshwater vessels, industrial wood workers who stain, paint, laminate and coat wood building materials and metal coil coating workersare often exposed to spray paint fumes daily for hours during the course of their work. Spray paint contains organic chemicals, viz., toluene, ethylbenzene, propylene glycol, methyl isocyanate and acrylic acid as the active ingredients which are potentially deadly to humans, requiring timely treatment to prevent severe illness and death (Ojo, 2020). The most reported occupational

hazards for spray painters are respiratory allergy, shortness of breath and other respiratory illnesses (Ibrahim et al., 2016). Signs and symptoms of spray paint poisoning include headache, dizziness, fatigue, coughing, chest pain, wheezing and tachypnea (Mark et al., 2016).

Respiratory diseases represent a major health challenge among individuals with occupational exposure to spray paint fumes (Ojo, 2020; Iyawe et. al., 2016). Spray paint-related diseases that are mainly developed because of occupational exposures include respiratory allergies, asthma, bronchiolitis, chronic obstructive pulmonary disease, interstitial pnemonitis and a lot of other respiratory diseases (Harrison, 2018)..Interstitial pneumonitis is a condition characterized by inflammation and damage to the interstitial tissue of the lungs (Moore and Daley, 2014).Itoccurs when an irritating substance e.g., spray paint fumes causes an inflammation and scarring of the lung tissue. Prolonged scarring associated with interstitial pneumonitis can lead to reduced lung function, resulting in life-threatening complications like pulmonary fibrosis, pulmonary hypertension and respiratory failure (Mark et al., 2016; Ross and Wilson, 2018).

Garcinia kola, popularly called 'bitter koa' is a traditional medicinal plant that is widely used for itsanti-inflammatory properties which can inhibit the production of pro-inflammatory cytokines and chemokines, and promote the production of anti-inflammatory cytokines (Olaleye et al., 2018). It is a species of flowering plant that grows in the rainforests of Central and Western Afirca and belongs to the family, *Cusiaceae* (Ibulubo et al., 2012). The plant has a long history of traditional medicinal use in West Africa. Phytochemical constituents of *Garcinia kola*include: phenols, steroid, coumarine, curcubitane, triterpenoids, fatty acid and a variety of antioxidants, such as flavonoids, tannins, and saponins which can scavenge free radicals and protect cells from oxidative damage (Okafor et al.,2020). Literature reports that *Garcinia kola* aqueous seed extract can be utilized in the therapy of respiratory allergy, asthma, chest pain, cough, catarrh and other respiratory diseases (Olaleye et al., 2018). Scientists have opined that the active principles which confer antitussive effects, antioxidant and anti-infammatory properties on the plant are the flavonoids, tannins and saponins. Hence, the present study investigated the effects of aqueous seed extract of *Garcinia kola* on spray paint-induced histological changes in the lungs and body weight ofadult Wistar rats.

MATERIALS AND METHODS

Plant Materials: The *Garcinia kola* seeds used in this study were collected from the University of Benin Farm Project, located in BeninCity, Nigeria. The plant material was authenticated by botanists at the Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen (voucher number: UB/MPI/008) was lodged at the department's herbarium for future reference.

Extract Preparation: *Garcinia kola* seeds were oven-dried at 40^0C after air-drying for 7 days. The dried seeds were then grounded using a 2018 model mechanical grinder (Dozenmann, U.S.A). The cold maceration method was used to extract the powdered material by soaking 500g of the powdered *Garcinia kola seeds* in 1litre of water for 24 hours at room temperature (Okafor et al., 2020). The soaked *Garcinia kola*was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath at a temperature of 60 ^{0}C to obtain 20 g concentrated jellylike extract of *Garcinia kola* seed. The aqueous extraction yielded a crude extract with a percentage yield of 10.2 % (w/w), which was further purified to obtain a yield of 3.5 % (w/w) aqueous extract of *Garcinia kola*. The 3.5 % (w/w) aqueous seed extract was then transferred into a sample bottle for storage in a refrigerator at 4^{o}C.

Experimental animals: Thirty (30) adult Wistar rats of 173-215g were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria. The animals

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were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed and clean water *ad libitum*.

***Ethical Consideration*:** Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (Approval number: CMS/REC/2012/302). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

***Induction of Interstitial pneumonitis*:** Interstitial pneumonitis was induced by exposing the test animals to 100 ml of spray paint via a fume-distributor glass-chamber (2006 model, manufactured by Hoddlier and Stoughton Group of Company, London) for 1 hour daily for 30 consecutive days Ojo et.al. (2016**).** A pilot study was done on of the 30th day of the experiment which revealed severe alveolar dilation, interstitial haemorrhage, interstitial inflammatory cell infiltrates, severe vascular ulceration and congestion (evidence of interstitial pneumonitis).

***Experimental design*:** In this study, 30 animals were divided into 5 groups comprising of 6 rats each. Animals in group A served as control, group B were exposed to 100 ml of spray paint fumes via inhalation, group C received 500mg/kg body weight per day of *Garcinia kola.* Animals in group D were exposed to 100 ml of spray paint via inhalation and received 250mg/kg body weight *of Garcinia kola* while the Group E rats were exposed to 100 ml of spray paint fumes via inhalation and received 500mg/kg body weight of *Garcinia kola*. The administration of the extract and exposure to the spray paint lasted for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken and recorded weekly and the difference between them and previous weights were noted.

Following the end of 30th day exposure, the animals were weighed, euthanized under chloroform anaesthesiaand a midline incision was made through the ventral wall of the thorax of the rats to access the lungs. The harvested organs were immediately fixed in 10% formal saline for 24 hours to prevent tissue degradation and autolysis before the histological procedures. The tissues were sectioned into about 3-5 micrometer thick sections and processed according to the method of Drury and Wallington, (1980).The thin tissue sections were histologically processed using the methods of fixation, embedding and tissue staining for microscopy. Histological sections were examined under a Leica DM750 light microscope with an attached digital camera (Leica ICC50). Photomicrographs of the tissue sections were taken at magnification of x100.

*Statistical analysis***:** Data were analyzed with a Statistical Software Package and, Microsoft Excel, 2010. The results were presented as Mean ± Standard error of mean (SEM). The data were subjected to using the student's- t-test. Level of significant was set at $P \leq 0.05$.

RESULTS

Body Weight Findings

Changes in body weights of the animals in all the experimental groups are presented in Table 1. The results showed that there was no significant increase ($P \leq 0.05$) in body weight of the animals at the end of the study.

Histological Findings

The photomicrograph of the control group (group A), shows normal features of the lungs such as alveoli, interstitial space, terminal bronchiole and bronchial artery (Figure 1A). In the rats exposed to 100 mls of spray paint fumes (group B), there were severe alveolar dilation, interstitial haemorrhage, interstitial inflammatory cell infiltrates, severe vascular ulceration and congestion, (Figure 1B). The group that was given 500mg extract only (Group C), presented normal architecture of alveoli, bronchial vein and terminal bronchiole (Figure 1C). In the animals given 250mg/kg extract and exposed to 100 mls of spray paint fumes (group D), there were interstitial

infiltrates of inflammatory cells, vascular ulceration and congestion. (Figure 1D). The group that was given 500mg/kg body weight extract and exposed to100mls of spray paint fumes (Group E; Figure 1E) presented active vascular congestion, bronchiolar dilation and florid activation of the bronchioloalveolar lymphoid aggregates.

Table 1: Change in Body Weights of the Animals in all the Experimental Groups

Groups	**Initial Body Weight**	**Final Body Weight**	**P-value**
Control (Group A)	168.00±19.90	195.00±19.22	0.158
Spray paint exposure only (Group B)	214.67±13.30	211.67±17.46	0.580
Extract only (Group C)	184.00±18.04	192.00±16.70	0.062
Spray paint + low dose (250mg/kg body weight) of extract (Group D)	176.67 ±11.86	179.67±16.75	0.605
Spray paint + high dose(500mg/kg body weight) of extract (Group E)	173.67 ± 8.41	188.33 ±7.45	0.137

n=6; Values are Mean ± S.E.M

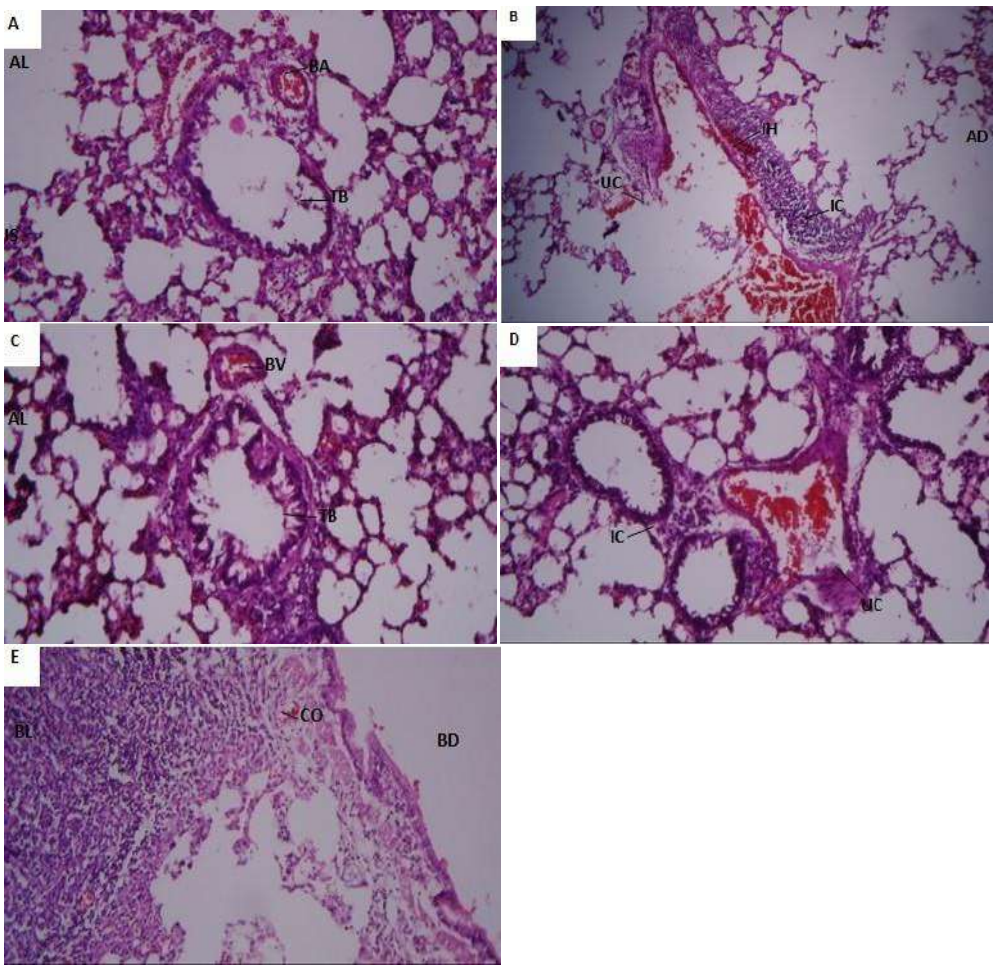

Figure 1: Representative photomicrographs ofH&E sections of the lungs of the experimental animals at the end of study: 'A' shows the Group A (Control group) showing normal alveoli**'AL'**, interstitial space**'IS'**, normal bronchial artery**'BA'**and terminal bronchiole**'TB'**. 'B' shows Group B exposed to 100 mls of spray paint only via inhalation daily for 30 days showingsevere alveolar dilation**'AD'**, severe vascular ulceration and congestion**'UC'**,interstitial haemorrhage**'**IH**'**and interstitial inflammatory cell infiltrates (IC): 'C'shows Group C administered 500mg/kg body weight per day of *Garcinia kola* aqueous seed extract daily for 30 days showing normal architecture of alveoli**'AL'**, normal bronchial vein**'BV'** and normal architecture of terminal bronchiole**'TB'.** 'D' shows Group D exposed to 100 ml of spray paint fumes via inhalation and received 250mg/kg body weight of *Garcinia kola*aqueous seed extract daily for 30 days showing normal architecture of vascular ulceration and congestion**'UC'** and

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interstitial infiltrates of inflammatory cells**'IC'**. 'E' shows Group E exposed to 100 ml of spray paint fumes via inhalation and received 500mg/kg body weight of *Garcinia kola* aqueous seed extract daily for 30 days showing floridactivation of the bronchioloalveolar lymphoid aggregate, **'BL'**, bronchiolar dilation**'BD'** and active vascular congestion**'CO'**.

DISCUSSION

*Garcinia kola*has been reported to have various medicinal uses. Literature reports that it has anti-tussive, anti-inflammatory and anti-histamine properties (Olaleye et al., 2018). Against this background, this study was conducted to evaluate the effects of aqueous seed extract of *Garcinia kola* on spray paint-induced interstitial pneumonitis in adult Wistar rats.

As shown in Table 1, there was no significant difference in the body weight of the rats in the various groups exposed to spray paint. The results of the findings showed that *Garciniakola* aqueous seed extract had no effect on body weight of the animas that were exposed to spray paint (Groups B, D and E) which concurs with previous works (Ibrahim et. al., 2016; Ojo et al., 2020). This is an essential observation, as it indicates that the treatments did not lead to gross physical changes in the animals during the study. Consequently, there must be a way of the animals expelling the toxic organic chemicals in the spray paint from the adipose tissues that is not available to the respiratory tissues.

The histological examination of the lung tissue in the control group (Group A) revealed normal lung histoarchitecture, viz., normal alveoli, terminal bronchioles, interstitial space, and bronchial artery (Figure 1A), while there wasinterstitial infiltrate of inflammatory cellsin the lungs of the rats that were exposed to spray paint alone (Group B) which occurred as a result of the body sensing a foreign body (excess accumulated spray paint fumes) leading to the activation of lymphoid tissues to get rid of it.Severe alveolar dilation and congestion, severe vascular ulceration and interstitial haemorrhagewere also observed in the group that were exposed to spray paint alone (Group B) which concur with previous work (Pronk et al., 2006).

The severe alveolar dilation, also known as emphysema observed in the exposed animals is a sign of lung dysfunction. It implicates reduced lung elasticity and compliance, increased lung volume and hyperinflation, decreased gas exchange and oxygenation and increased risk of respiratory failure (Drake et al., 2010; Mark et al.,2016). Understanding their implications is essential for developing effective treatments and therapies. The observed vascular ulceration and congestion in the exposed animals may cause significant chest pain, recurrent pulmonary infections, airway obstruction, impaired lung function, chronic cough, dyspnoea and haemoptysis. The interstitial haemorrhage observed in the exposed animals may cause asphyxiation due to airway obstruction or blood filling the interstitial space while the observed interstitial infiltrate of inflammatory cells in the exposed animas may cause disruption of the normal functioning of the muscularis layer and underlying tissues leading to impaired barrier function and increased susceptibility to infections (Mark et al., 2016). The histological findings are consistent with the results of a similar study by Poinen-Rughooputh et al., (2016) which used silica dust to induce bronchitis.

As shown inGroup C, *Garcinia kola* showed no negative effect on the histology of the lungs as the alveoli, bronchial vein and terminal bronchioles were found to be histologically normal in the animals that were administered only the extract. For the rats in Group D, at low doses, *Garcinia kola* showed a protective effect against spray paint-induced interstitial pneumonitis. Interstitial pneumonitis was completely prevented and the accumulated particulate matters were cleared allowing the flow of oxygen into the alveoli and release of carbon dioxide from the blood. The group treated with 500mg/kg body weight of *Garcinia Kola* + Spray Paint demonstrated an intriguing pattern of histopathological changes. This group displayed florid activation of the bronchioloalveolar lymphoid aggregates, bronchiolar dilation, and active

vascular congestion. These observations suggest that the combination of *GarciniaKola* extract and spray paint exposure may have led to unique pathological responses, potentially indicating a protective effect of the extract on lung tissues. The protective effect of *Garcinia kola* seed aqueous extract against spray paint-induced lung damage may be attributable to its antioxidant and anti-inflammatory properties and concurs with a similar work done by Ibulubo et al., 2012.

To minimize cement dust exposure risks, it is essential to adhere to proper safety precautions, such as wearing personal protective equipment (PPE) like face masks, gloves, and coveralls, conduct regular medical checkups, particularly among workers in the painting and coating industries, educate the public about the health risks and hazards of associated with spray paint through regular awareness campaigns and implement modern machines and techniques in spray booth, especially in developing countries, to reduce environmental pollution.By adopting these measures, we can effectively mitigate the pulmonary toxicity and complications associated with spray paint exposure, ensuring a safer working environment and improvesd public health.

CONCLUSION

Garcinia kola demonstrated a dose-dependent protective effect against spray paint-induced vascular damage, congestion, haemorrhage, and inflammation in the lungs. The extract's ameliorative effects were more pronounced at higher doses. These findings suggest that *Garcinia kola* could be a potential valuable natural remedy for combating interstitial pneumonitis and related vascular complications.

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