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Effects of Aqueous Leaf Extract of *Psidium guajava* on Formaldehyde-induced Interstitial Pneumonitis in Adult Wistar Rats

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ABSTRACT

The most common cause of lung diseases among people with formaldehyde-related occupations is formaldehyde poisoning which has significant morbidity and mortality if left untreated. This study was aimed at investigating the effects of aqueous leaf extract of *Psidium guajava* on formaldehyde-induced interstitial pneumonitis in adult Wistar rats. Thirty (30) adult Wistar rats weighing between 240 g and 270 g were divided into five (5) groups of six (6) rats per group. Group A rats were administered feed and water only; Group B rats were exposed to 40% formaldehyde via inhalation; Group C rats received 500 mg/kg body weight per day (BWT/D) of *Psidium guajava*; Group D rats were exposed to 40% formaldehyde via inhalation and received 250 mg/kg BWT/D of *Psidium guajava*; Group E rats were exposed to 40% formaldehyde via inhalation and received 500 mg/kg BWT/D of *Psidium guajava*. The dosages of the extract were given for 30 consecutive days via an orogastric tube. Groups A, C, D, and E revealed normal histoarchitecture of the lung; normal alveoli, patent bronchiolar lumen, and normal arteries. There were observable histological variations in the lung tissues of Group B rats which include severe bronchiolar mucosal ulceration, interstitial infiltrates of inflammatory cells, interstitial congestion, and interstitial hemorrhage. These injuries are consistent with usual histological findings in interstitial pneumonitis. It was concluded that *Psidium guajava* had an ameliorative effect on formaldehyde-induced interstitial pneumonitis in Wistar rats.

Keywords: *Psidium guajava*, formaldehyde, interstitial pneumonitis, lungs

INTRODUCTION

Formaldehyde is an organic chemical reagent commonly used in disinfectants, embalming, and medical laboratories[1,2]. Nurses, medical technicians, and laboratory scientists are often exposed to formaldehyde daily for hours. Nurses may be exposed to formaldehyde on the job through their work with formaldehyde-based products, medical equipment that uses formaldehyde for disinfection, and other sources. Breathing the fumes of formaldehyde can occur while working directly with formaldehyde, or using equipment cleaned with formaldehyde. Formaldehyde contains an organic chemical, methanol as the active ingredient which is toxic to humans with significant morbidity and mortality if left untreated[3]. Previous studies have shown that exposure to formaldehyde fumes, a severe respiratory and skin irritant, causes respiratory allergy and shortness of breath in experimental animals[1]. Signs and symptoms of formaldehyde poisoning include coughing, chest pain, wheezing, tachypnea, and inflammatory reaction[4].

Interstitial pneumonitis is a lung disorder characterized by inflammation in the structural spaces of the lungs, in between the alveoli[4]. It occurs when an irritating substance causes an inflammation of the lung interstitium which then makes it difficult for oxygen to pass through the alveoli into the bloodstream[5]. Besides formaldehyde fumes, other airborne particles such as gasoline, silica, and coal dust have been linked to interstitial pneumonitis[5].

Psidium guajava, popularly called 'poor man's apple' belongs to the family, *Myrtaceae*[6]. It is a species of flowering plant that is cultivated in Asia, China, South East Asia, and the Tropics[7]. *Psidium guajava* plant has up to 6 leaves per rosette. Mature leaves are dark green, elliptical, oval, and characterized by their obtuse-type apex and usually range between 30 and 35cm in length and 5 to 7cm in width[8]. The plant is cultivated mainly as a commercial fruit crop widely in both tropical and subtropical regions of the Indian subcontinent. It is also cultivated as medicinal for the treatment of malaria, cough, catarrh, and allergy[6].

Psidium guajava contains the toxic alkaloid cucuvesine, which has been found to also have anti-allergenic, antipyretic, and anti-inflammatory effects[9]. Phytochemical constituents of *Psidium guajava* include flavonoids, saponins, phenols, steroids, coumarine, curcubitane, triterpenoids, and fatty acid[9].

Literature reports that *Psidiumguajava* leaves can be utilized in the therapy of malaria fever, asthma, chest pain, cough, and catarrh[9]. Scientists have opined that the active principles that confer antipyretic, anti-inflammatory, antitussive, and soothing effects on the plant are the curcubitanes, triterpenoids, and flavonoids[9]. Hence, the present study investigates the effects of aqueous leaf extract of*Psidium guajava*on formaldehyde-induced interstitial pneumonitis in the lungs of adult Wistar rats. Evidence from the literature reveals that pneumonitis is associated with a range of anatomical changes including inflammation and oedema in the lung parenchyma, alveolar damage and interstitial haemorrhage, vascular ulceration and congestion, and interstitial thickening and fibrosis[4].

MATERIALS AND METHODS

Extract preparation

Psidium guajava leaves were oven-dried at 40^{o}C after air-drying for 7 days. Alternatively, a gentler drying method, such as freeze-drying or vacuum drying, to minimize the risk of protein denaturation could be used. The dried leaves 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 500 g of the powdered *Psidium guajava* leaf in 1 litre of water for 24 hours at room temperature[10]. The soaked *Psidium guajava* was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath to obtain 20 g concentrated jellylike extract of *Psidium guajava* leaf which was then transferred into a sample bottle for storage in a refrigerator at 4^{o}C. The acute oral toxicity of the extract was evaluated.

Experimental animals

Thirty (30) adult Wistar rats of 240-270 g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria, and were utilized for this experiment. The animals 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 following approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research [11].

Experimental design

In this study, 30 animals were divided into 5 groups comprising 6 rats per group. Group A rats which served as control received standard feed and clean water *ad libitum*. Group B rats were exposed to 40% formaldehyde via inhalation. Group C rats received 500 mg/kg body weight per day (BWT/D) of *Psidium guajava.* Group D rats were exposed to 40% formaldehyde via inhalation and received 250mg/kg BWT/D of *Psidium guajava*. Group E rats were exposed to 40% formaldehyde via inhalation and received 500 mg/kg BWT/D of *Psidium guajava.* The dosages of the extract were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken weekly and the difference between them and previous weights were noted.

Induction of interstitial pneumonitis

Interstitial pneumonitis was induced in the test animals by exposing them to 40% formaldehyde via a fume distributor glass-chamber (FDGC) for one (1) hour daily for 30 consecutive days[1]. At the end of the 28th day of exposure, a pilot study was done. One animal from each of the test groups was euthanized under chloroform anaesthesia; a midline incision was made through the ventral wall of the thorax of the rats to access the lungs which were harvested and immediately fixed in 10% formal saline for 24 hours before the histological procedures. The tissue sections were cut in the sagittal plane, with a thickness of 5µ (micrometer), using a microtome and processed according to the method of Drury and Wallington [10]. The trimmed tissues were histologically processed using the method of fixation, embedding, and tissue staining for microscopy. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at a magnification of x40 and x100 which showed interstitial haemorrhage, severe bronchiolar ulceration, and heavy interstitial infiltrates of inflammatory cells (evidence of interstitial pneumonitis) in the rat's lungs exposed to 40% formaldehyde only (Group C, Figures 5 and 6).

Method of sacrifice and sample collection

On the 30th day of exposure to formaldehyde, the animals were weighed and subsequently euthanized under chloroform anesthesia. and the lung of each rat was excised and fixed in 10% formal saline for 24 hours before the histological procedures. Following euthanasia, the lungs were harvested, fixed in 10% formal saline for 24 hours, and then processed for histological examination. The tissue sections were cut in the sagittal plane, with a thickness of 5µ (micrometer), using a microtome and processed according to the method of Drury and Wallington [10, 12]. Consistency was ensured by using a precision microtome with a digital micrometer, allowing for precise control over section thickness. The trimmed tissues were histologically assessed using the method of fixation, embedding, and tissue staining for microscopy. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x40 and x100.

*Statistical analysis***:** Statistical analysis was carried out with Statistical Software Package, Microsoft Excel, 2010, and Statistical Package for Social Sciences (S.P.S.S.) version 20. Results were presented as Mean (X) ± Standard error of mean (SEM). The one-way analysis of Variance (ANOVA) was used to determine the significance of the difference in mean at 95% confidence interval. P≤0.05 was considered significant.

RESULTS

Changes in body weights of the animals in all the experimental groups are presented in Table 1. Body weight analysis revealed a significant increase in all the groups, but the magnitude of weight gain was significantly reduced (P<0.05) in the formaldehyde-exposed groups compared to the control group, suggesting formaldehyde's inhibitory effect on weight gain.

Significant findings from the study for histological investigation of the group exposed to 40% formaldehyde only include alveolar dilation, vascular ulceration and congestion, interstitial hemorrhage, and interstitial inflammatory cell infiltrates. These histomorphological changes indicate diseases and pathological symptoms of a variety of maladies including interstitial pneumonitis. The findings from the study for histological investigation were almost consistent in the various groups exposed to 40% formaldehyde and administered 250 mg/kg body weight and 500 mg/kg body weight of the extract respectively and they include normal vascular architecture, florid activation of the bronchiolo-alveolar lymphoid aggregates, vascular congestion and bronchiolar dilation resulting in a protective effect against the formaldehyde-induced lung injuries that were observed in the exposed group.

Table 1: Changes in body weights of the rats in all the experimental groups

GROUPS	Initial body weight	Final body weight	Weight Difference	P-value
Group A (Control)	5.60 ± 0.68	7.74 ± 0.60	0.94 ± 3.34	0.000
Group B (Formaldehyde Exposure only)	0.60 ± 0.19*	0.18 ± 0.09*	0.80 ± 0.14	0.000
Group C (Extract only)	3.42 ± 0.16*	5.36 ± 0.10*	0.68 ± 2.20	0.000
Group D (Low dose of extract + Formaldehyde)	0.38 ± 0.16*	0.04 ± 0.18*	0.58 ± 0.10	0.000
Group E (High dose of extract + Formaldehyde)	0.28 ± 0.16*	0.02 ± 0.116*	0.50 ± 0.04	0.000

n=6; Values are Mean ± S.E.M. * significant difference between initial and final weights (P≤0.05)

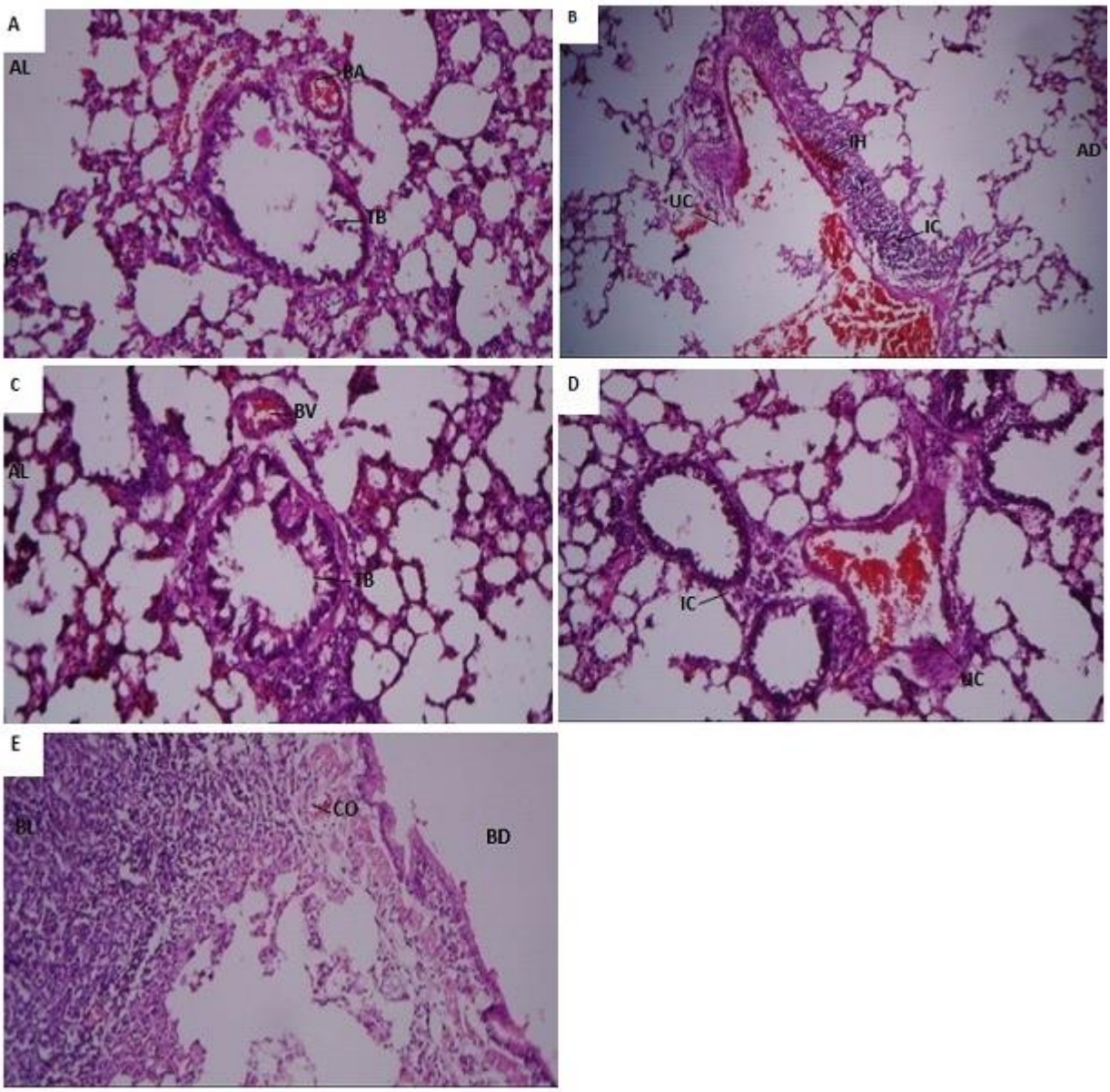

Figure 1: Photomicrographs of a section of lungs of the control group (A) showing normal alveoli **'AL'**, interstitial space **(IS)**, bronchial artery **(BA),** and terminal bronchiole **(TB)**. Group B **(B)** exposed to 40% formaldehyde only shows severe alveolar dilation **(AD)**, severe vascular ulceration and congestion **(UC)**, interstitial haemorrhage **(IH),** and interstitial inflammatory cell infiltrates (IC). Group C **(C)** administered 500mg/kg body weight of *Psidium guajava* only shows the normal architecture of alveoli **(AL)**, normal bronchial vein **(BV),** and normal architecture of terminal bronchiole **(TB).** Group D **(D)** exposed to 40% formaldehyde and administered 250mg/kg body weight of *Psidium guajava* shows normal architecture of vascular ulceration and congestion **(UC)** and interstitial infiltrates of inflammatory cells **(IC)**. Group E **(E)** was exposed to 40% formaldehyde and administered 500mg/kg body weight of *Psidium guajava* shows florid activation of the bronchioloalveolar lymphoid aggregate, **(BL)**, bronchiolar dilation **(BD)** and active vascular congestion **(CO)**. (H&E; 100×).

DISCUSSION

Psidium guajava has been reported to have various medicinal uses. Literature reports that it has analgesic, anti-tussive, and anti-inflammatory properties. Against this background, this study was conducted to evaluate the effects of aqueous leaf extract of *Psidium guajava* on formaldehyde-induced interstitial pneumonitis in Wistar rats.

Body weight analysis showed a significant increase in all groups, but the magnitude of weight gain was significantly lower ($P<0.05$) in the formaldehyde-exposed groups compared to the control group, suggesting a negative impact of formaldehyde on weight gain.

Observations based on photomicrography show that formaldehyde caused severe alveolar dilation, severe vascular ulceration and congestion, interstitial hemorrhage, and interstitial infiltrates of inflammatory cells (evidence of interstitial pneumonitis) in the exposed rats. There was florid activation of the lung tissue of the rats that were exposed to formaldehyde alone which occurred as a result of the body sensing a foreign body (excess accumulation of formaldehyde fumes) leading to the activation of lymphoid tissues to get rid of it. *Psidium guajava* had no negative effects on the histology of the lungs as the alveoli, interstitial space, bronchial artery, and terminal bronchioles were found to be histologically normal in the rats that were administered only the extract. Low doses of *Psidium guajava* caused normal architecture of the alveoli, bronchial artery, terminal bronchiole, and activated cells of the mononuclear phagocyte system. *Psidium guajava* showed a protective effect against formaldehyde-induced lung injuries. Interstitial pneumonitis was completely prevented and the accumulated formaldehyde particulate matters were cleared. The phytochemicals present in *Psidium guajava*, particularly flavonoids, saponins, and alkaloids, likely contributed to its protective effects against formaldehyde-induced lung damage. Flavonoids, for instance, may have reduced inflammation by inhibiting pro-inflammatory cytokines and scavenging free radicals, while saponins may have modulated immune responses and reduced oxidative stress[13,14].

The key phytochemicals in *Psidium guajava*, including flavonoids and saponins, have been shown to exhibit anti-inflammatory and antioxidant properties, which may have contributed to the observed protective effects. Our findings are consistent with previous studies that have demonstrated the protective effects of *Psidium guajava* against toxicant-induced lung damage. For example, a study by Weli *et al.*[9] found that *Psidium guajava* extract reduced lung inflammation and oxidative stress in mice exposed to cigarette smoke.

The phytochemicals in *Psidium guajava* may interact with specific cellular pathways or molecular targets to reduce lung inflammation, including the Nuclear Factor Kappa B (NF-KB) and Nuclear Factor erythroid 2-related factor 2 (Nrf2 pathways, which regulate inflammatory responses and antioxidative defenses. Further studies are needed to elucidate the precise mechanisms involved.

The different doses of *Psidium guajava* extract used in this study may have influenced the effects on the outcome measured by modulating the bioavailability and pharmacokinetics of the phytochemicals. Lower doses may have resulted in greater bioavailability and enhanced protective effects. Our findings suggest that *Psidium guajava* extracts may have potential therapeutic applications in treating or preventing lung diseases caused by formaldehyde exposure.

Conclusion

Psidium guajava has ameliorative effects against formaldehyde-induced bronchiolar ulceration, interstitial hemorrhage, inflammation of the lung interstitial space, and activation of lymphoid tissue. *Psidium guajava* is therefore valuable in combating interstitial pneumonitis.

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