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RESEARCH ARTICLE

Novel Insights on the Pancreatic Toxicity Induced by Chronic Acesulfame-K Exposure in Rats

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A B S T R A C T

Acesulfame potassium (Ace-K) is one of the commonly used artificial sweeteners, keeping its sweetening property after heating or freezing encouraging its use in various products especially those consumed by children, and encounters a potential hazard for cumulative toxicity, therefore safety evaluation for its long-term exposure is necessary. A total of 90 mature and immature males Sprague Dawley rats was divided into six groups, 5 animals each: G1&G2 control untreated immature and immature rats, G3&G4 (immature and mature rats treated with Ace-k at dose of 15mg/kg. b.w) and G5&G6 (immature and mature rats treated with Ace-k at dose of 90 mg/kg. b.w), All treated rats received Ace-K via gastric intubation for 6 days per week for 10 weeks, at the end of experimental period blood samples were collected for the determination of serum amylase, lipase, and glycated hemoglobin, in addition, the pancreas was dissected for histopathological evaluation. Results revealed that chronic treatment with Ace-k induced a significant increase in weight gain in younger age treated groups compared with older ones. Pancreatic enzymes showed non-significant differences between control and treated groups at different ages while significant reduction in glycated hemoglobin was detected in older age receiving higher dose compared with other treated groups. Ace-K induced various pancreatic histopathological changes that exhibited dose dependent increase in lesions severity among treated groups. The present data revealed that chronic treatment of rat with Ace-K induced pancreatic injury. In addition, the histological hallmarks of pancreatic pathology induced by Ace-k give attention to the possible carcinogenic potential of Ace-k on pancreatic tissue.

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INTRODUCTION

Obesity is a complex multifactorial problem that could be considered a pandemic problem. Recent investigations confirmed a correlation between obesity and viral infections (Shirani *et al.,* 2017) Obesity increased the risk of chronic diseases, Over six hundred million people were obese in 2014, and it is assumed that the fifty one percent of humans will be obese by 2090 (Farshidi *et al.,* 2008).

Recently, artificial sweeteners become used greatly to manage overweight problems and prevent obesity (Benton, 2005). Artificial sweeteners are used as a tool to provide a sweet taste without the extra energy derived from foods and drinks containing caloric sugars (Bellisle and Drewnowski, 2007). Artificial sweeteners are commonly used in the pharmaceutical compounds, food, and beverages (Chattopadhyay *et al.,* 2014). There are two generations of artificial sweeteners. The first generation like Aspartame, Cyclamate, and Saccharin while the second one included Acesulfame-K, Neotame, Sucralose, and Alitame (Benton, 2005).

It was believed that artificial sweeteners are not harmful and aid in the reduction of body weight however recent studies clarified that using ~~of~~ artificial sweeteners alters glucose metabolism and induces metabolic disorders like diabetes mellitus and weight gain (Brown and Rother, 2012; Kundi *et al.,* 2019; Majeed *et al.,* 2021).

several toxicological studies were conducted for safety evaluation of artificial sweeteners and they found that Acesulfame-K, Saccharin, Sucralose, Neotame and Aspartame were safe with acceptable daily intake (Lorenzo *et al.,* 2015). On contrary byproduct of their

breakdown has a deleterious health effect include mutagenic and metabolic side effects (Shastry *et al*., 2012; Ebrahimzadeh *et al*., 2018**).** In addition, several side effect have been reported in animal studies conducted on artificial sweeteners, those include weight gain, bladder and brain neoplasia (Tandel, 2011; Ahmad *et al*., 2021).

Acesulfame potassium (Ace-K) is a potassium salt, white crystalline powder and an acidic cyclic sulphonamide derivative**.** The acceptable daily intake (ADI) of Ace-K is 15mg/kg of body weight per day (Kroger *et al*., 2006). Research on Ace-K toxicity are considered inadequate (Karstadt, 2010). Breakdown of Ace-k in the body produces acetoacetamide that was considered toxic at high doses (Helal *et al*., 2019). Studies have shown that acetoacetamide induced thyroid tumors in rats, rabbits, and dogs after administration of only 1% acetoacetamide in the diet for three months (Findikli and Turkoglu, 2014). Ace-k is an important low and no-calorie sweeteners -that extensively accumulated in the environment and found to be environmentally persistent (Belton *et al*., 2020).

Acesulfame has unique character encourage its use in a wide range of product as it can be heated and frozen without losing sweetening power. Acesulfame is used in the various product like ice cream, soda, candy, cookies, fruit juice drinks, and pharmaceuticals, thus children consumed Ace-k sweetened product at high range (Karstadt, 2010).

Ace-K is rapidly absorbed into the systemic circulation and distributed to tissues throughout the body. In rats, the highest tissue concentrations were observed in absorption and excretion organs (gastrointestinal tract, urinary bladder, and kidneys) as Absorbed ACK is excreted through the kidneys (Klug and Von, 2012). Previous research confirmed the role of Ace-K on induction of oxidative stress in common carp after Ace-K water exposure (Kroger *et al*., 2006; Cruz *et al.,* 2019). ATSDR (1998) stated that Acesulfame potassium contains the chemical methylene chloride, that with Long-term exposure induced hepatic and renal injury, in addition Eman *et al.* (2019) found that treatment of rats with ACE-K at dose of 15 mg/kg b.w induced elevation serum liver enzymes. No available literature concerning the effect of Ace-K exposure on pancreas either toxicity or carcinogenicity studies. The present study was designed to investigate the effect of long-term oral treatment of acesulfame k in male rats on pancreatic tissue with special emphasis on the age-related effect on the development of pancreatic pathology.

MATERIALS AND METHODS

Chemicals

Acesulfame–k with purity (>=99%) for food analysis was obtained from **Sigma-Aldrich** Company (Germany). It is available in the form of white crystals in 25 gm package. CAS no. (55589-62-3).

Ethics statement: The study procedures were approved by the Institutional Animal Care and Use Committee (Vet.CU.IACUC), Cairo University, Egypt (Approval number of ethics committee: Vet CU28/04/2021/286).

Experimental design: A total of 90 mature and immature males Sprague Dawley rats were obtained from vacsera. Mature ones are weighed between (170-220 gm) and immature ones between (50-70 gm), rats were kept in metal cage with 5 rats per cage and provided standard pelleted diet and tap water ad libitum and animals were kept for one week for acclimatization. The animals were classified according to age to two main groups (immature and mature) of 5 rats per each, two control untreated groups (immature and mature), and four Ace-k treated ones at doses of (15 and 90 mg/kg b.w) for immature and mature rats. The doses were selected according to Eman *et al*. (2019) and Parsapour *et al*. (2020).

The animals were observed for any abnormal clinical toxicity signs and weighed every two weeks during the experimental period. All treated animals were received the determined dose of Ace-K via gastric intubation for 6 days per week for 10 weeks. Animals were weighed every two weeks along the period of experiment. Collection of blood samples and tissue pancreatic specimens were performed at the end of the experimental period.

Determination of pancreatic function: Pancreatic amylase and lipase were assayed calorimetrically and glycosylated hemoglobin (HbA1C) was measured by high performance liquid chromatography.

Histopathological examination: Pancreatic tissue specimens were collected then fixed for in 10% neutral buffered formalin, dehydrated in ascending concentration of ethyl alcohol, cleared in xylene and embedded in paraffin for tissue sectioning and staining by routine (H&E) and Masson trichrome stain according to Bancroft *et al*. (2013).

Statistical analysis: The obtained values were presented as means ± SE of the mean. Comparisons between different groups were carried out by two-way analysis of variance (ANOVA) followed by LSD comparisons test. The level of significance was set at $P<0.05$ using SPSS software (version 16.0). ($P<0.05$).

RESULTS

Body weight gain: The determination of the difference between final and initial body weight was estimated as body weight gain and shown in Fig. 1. The results revealed that there was a significant increase in body weight gain in younger age treated groups compared to older ones and the increased values were dose dependent. while the increased weight gain was numerical but non-significant with increasing the dose of ACE-K.

Pancreatic functions: The analysis of serum amylase and lipase between different groups revealed non-significant differences between all treated groups and control ones at different ages.

The glycated hemoglobin level in serum showed a significant reduction in the older age group received higher dose compared with the control untreated one of the same age. While the younger age group treated with a lower dose showed a significant reduction in glycated

hemoglobin level compared with higher one of the same age. Data was shown in Fig. 2.

Histopathological pancreatic alterations: The microscopic examination of pancreatic tissue of control young and old age untreated groups revealed normal histological architecture of pancreatic lobular structures (Fig. 3a & 3b).

The treatment of Ace-k induced various pancreatic histopathological alterations, the lesions severity varied among age groups and was dose-dependent. The pancreatic lesions in rats treated with lower dose were age dependent as the lesions were more severe in young age than old one. Concerning the younger age group received lower dose (G3), maintenance of lobular pancreatic structures, proliferation of periductular connective of the execratory duct were evident (Fig. 3c). The lesion was associated with mononuclear cell infiltration and hyperplasia of pancreatic duct (Fig. 3d). Concurrently, formation of fibrovascular tissue in interlobular area was observed, the lesion characterized by fibroblast proliferation, mononuclear cell infiltration and associated with formation of new pancreatic ductules that containing vesicular nuclei and oval shaped cells proliferation **(**Fig. 3e). The pancreatic acinar cells showed individual atrophy, apoptosis, necrosis and binucleated cells with karyomegalic nuclei. The pancreatic lesions were of random distribution (Fig. 3f).

On the other hand, the pancreatic lesions in older age group that received a lower dose of Ace-K (G4) were extremely mild and restricted to mild periductular fibroplasia with few mononuclear cell infiltrations.

The pancreatic lesions in rats that received higher dose of Ace-k were severe. concerning the younger age group (G5), periductal fibrosis with oval shaped cells proliferation, mononuclear cell aggregation and proliferation of ductular epithelium were detected. The intensity of fibrosis was more severe compared with G3 and associated with marked hyperplasia of execratory ductal and ductular epithelium (Fig. 4a).

Chronic pancreatitis was evident in this group, the lesion ranged from smaller mild locus of pancreatic necro apoptosis with mononuclear cells infiltration to more severe areas of marked histological alterations (Figs. 4b & 4c). There was a discrete area of pancreatic acini showed diffuse vacuolization, necro apoptosis and increased number of binucleated epithelium with karyomegaly and appearance of scattered acini with ductal metaplasia while the interstitial tissue displayed mononuclear cells infiltration and fibroblast proliferation (Figs. 4d & 4e). The pancreatic tissue adjacent to the area of inflammation showed an increased number of autophagic vacuoles, apoptosis, and pyknosis (Fig. 4f).

The microscopic examination of the older age group that received the higher dose of Ace-K (G6) showed severe pancreatic lesions compared with other groups. The lesions comprising the execratory ductal and ductular epithelium were more like G5, but the intensity of fibrosis was higher (Fig. 5a) chronic pancreatitis was also evident but histologically differ from the previous group with complete loss of lobular structure, marked loss of zymogen granules, autophagy, karyomegaly with intense interstitial mononuclear cells infiltration and fibroplasia were evident (Fig. 5b). There was an increased number of acinar cells that exhibited ductal metaplasia compared to the previous group. The histological hallmarks of differentiated acinar cells with ductal metaplasia included loss of zymogen granules, increased luminal size, reduction

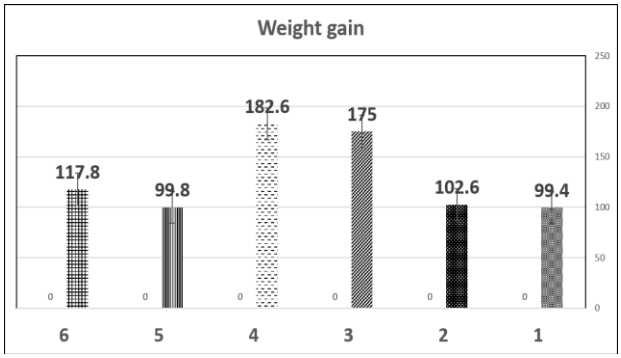

Fig. 1: Chart illustrating the weight gain in different treated groups.

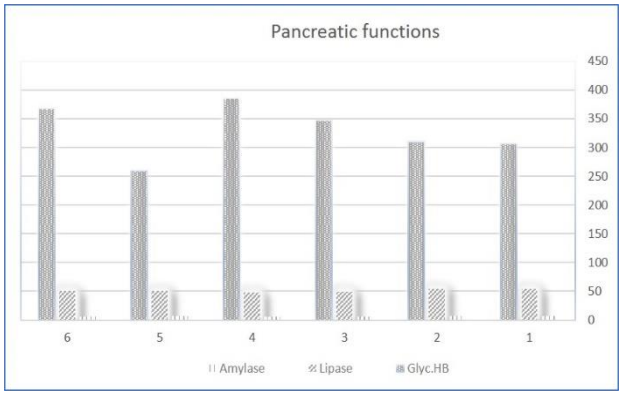

Fig. 2: Chart illustrating the pancreatic functions in different treated groups.

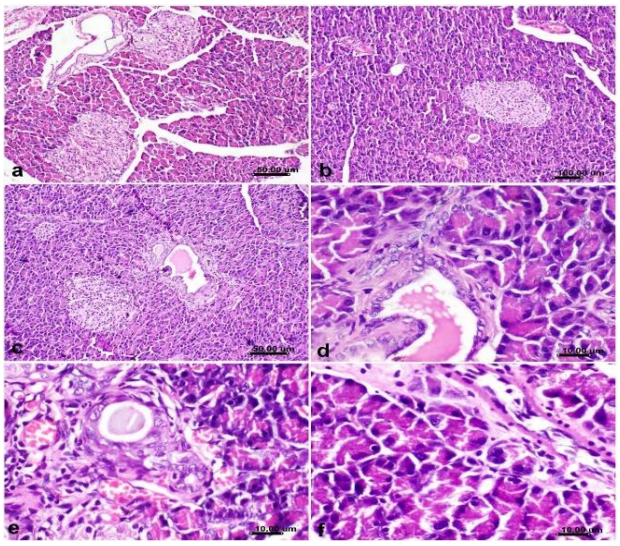

Fig. 3: Histological section of pancreas stained with (H&E). (a and b) control young and old rats showing normal histological pancreatic lobular structure. (c-f) young rats treated with 15mg/kg b.w. (c) periductal fibrosis with hyperplasia of duct epithelium (d) higher magnification showing the hyperplastic epithelium with vesicular nuclei associated with proliferation of oval cells in the vicinity of fibroplasia. (e) fibrovascular tissue proliferation in periductular area with mononuclear cells infiltration. (f) intralobular fibrous tissue proliferation with atrophy and pyknosis of pancreatic acinar epithelium with presence of binucleated acinar cells.

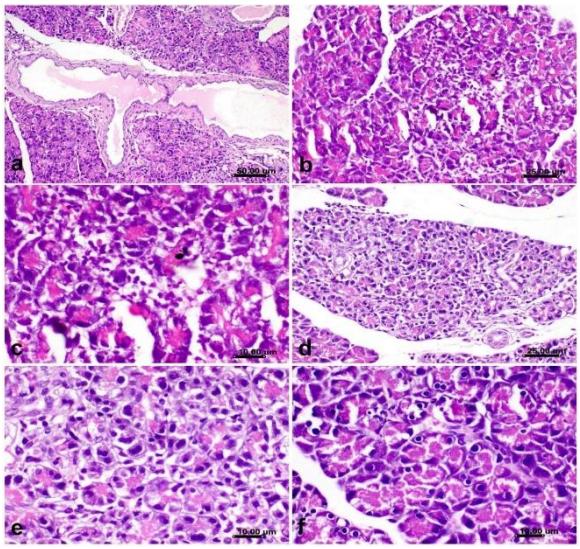

Fig. 4: Histological section of pancreas stained with (H&E). (a-f) young rats treated with 90mg/kg b.w showing: (a) marked hyperplasia of exocrine pancreatic duct periductal fibrosis with mononuclear cells infiltration (b) exocrine pancreatic acini showing maintained lobular architecture with mild inflammatory reaction. (c) Higher magnification showing vacuolization of pancreatic acini, pyknosis and apoptosis note the circumscribed eosinophilic apoptotic body. (d) complete disruption of exocrine pancreatic lobular architecture with fibroinflammatory and necrotic reaction. (e) higher magnification showing necroapoptosis pancreatic acinar epithelium, vaculolation, marked reduction of zymogen granules, increased binuclear acinar cells with fibroplasia and mononuclear cells infiltration in interstitium. (f) acinar epithelium showing autophagosomes and pyknosis of nuclear elements of pancreatic acinar cells.

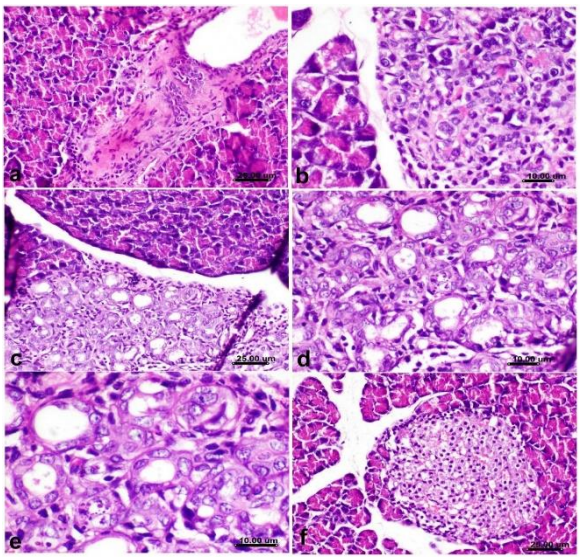

Fig. 5: Histological section of pancreas stained with (H&E). (a-f) old rats treated with 90mg/kg b.w showing: (a) marked periductal fibrosis of exocrine pancreatic duct with mononuclear cells infiltration. (b) two exocrine pancreatic lobules, left one with normal pancreatic architecture and the right one showing marked disruption of lobular structure and severe histological alterations including severe reduction of zymogen granules, vacuolation and necrosis of acinar epithelium with interstitial fibrosis with mononuclear cells infiltration. (c) the lower pancreatic lobule showed marked disruption of pancreatic lobular architecture with marked fibroinflammatory reaction involving pancreatic interstitium. (d) higher magnification showing ductal metaplastic changes of acinar epithelium that characterized by increased luminal size, reduction in cytoplasm and complete loss of zymogen granules. (e) pancreatic acini showing ductal metaplasia with nuclear changes including karyomegally, anisokaryosis, prominent nucleoli and marked apoptosis. (f) Individual endocrine pancreatic islets showing scarce aggregation of mononuclear cells surrounding the islet.

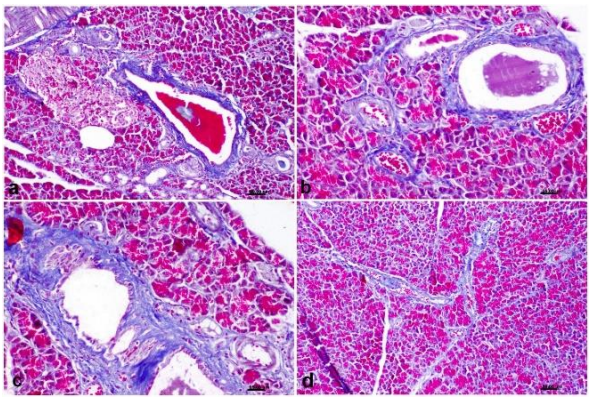

Fig. 6: Histological section of pancreas stained with (MTC) showing the intensity of periductal and interlobular fibroplasia among different treated group G3 (a), G5 (b), and G6 (c and d).

in cytoplasmic volume, the nuclei showed anisocytosis, karyomegaly and containing prominent nucleoli, increased fibroinflammatory reaction and apoptosis were evident (Fig. 5c-5e). Individual pancreatic islets showed few aggregations of mononuclear cells (Fig. 5f).

The histological pancreatic sections that were stained with MTC showed an increased in collagen intensity in periductal and periductular tissue that increased in intensity with advancing dose (Figs. 6a-6f).

DISCUSSION

The artificial sweeteners are widely used for controlling the obesity and reducing the body weight. Although several studies on the safety of artificial sweeteners have been conducted, the adverse effects of chronic long-term exposure to their byproducts in the animal body are unclear. The present study investigated the effect of long-term treatment of Ace-K at different doses in rats and determined the difference in pancreatic pathology in relation to age. The present data showed that chronic exposure with Ace-K affects body weight gain that differs between different age groups. Our findings revealed that exposing of young aged rats to Ace-k induced a significant increase in weight gain compared to the adult group, confirming that exposing young rats to Ace-k increases the risk of obesity. Similar results were clarified by Ebrahimzadeh *et al*. (2018) who found that chronic exposure to artificial sweeteners increased body weight. Similarly, Swithers *et al*. (2009) stated that male rats that were fed on an ACK or saccharin-sweetened yoghurt diet showed more weight gain compared with rats fed with a glucose- sweetened yoghurt diet. On contrary Belton *et al*. (2020) stated that rats showed no health hazard when they were fed diet containing 3% Ace-k for 24 months with no effect on body weight.

The present data showed that chronic Ace-k exposure did not affect adversely the exocrine pancreatic functions, and this was attributed to the random distribution of chronic neuroinflammatory reactions involving the pancreatic tissue as the pancreas has great functional reserve as confirmed by Völzke *et al*. (2005) and Brown & Rother (2012). In addition, Domínguez *et al*. (1993) found that serum pancreatic enzymes have limited usefulness in the diagnosis of chronic pancreatitis (CP). In addition, Usborne *et al*. (2014) found no correlation

between increased amylase and lipase values and the increased severity of exocrine injury.

Glycated hemoglobin level was higher in the younger age group that was treated with 90 mg/kg b.w compared with control one, while non-significant difference in glycated hemoglobin values was observed in other treated groups compared with control one. These results come on contrary to those observed by Ebrahimzadeh *et al.* (2018) who stated that artificial sweetener induced impairment of glucose and insulin homeostasis. In addition, Malaisse *et al.* (1998) found that ACK induced insulin-secretion and considered as an insulinotropic agent and this may reflect the lower glycated hemoglobin in rats that received higher doses of Ace-k in the present study. The histopathological pancreatic alterations induced by chronic Ace-k treatment denoted the picture of chronic pancreatitis (CP). Apoptosis and the formation of autophagosomes were evident in exocrine pancreatic acini, the necro apoptosis assumed to be as a result of Ace-k induced oxidative stress as shown by Kroger *et al*. (2006) who found that long treatment with artificial sweeteners may induce increasing oxidative stress and nucleic acid oxidation. Zhang *et al.* (2014) stated that autophagy of pancreatic acini was identified as a contributing factor in caerulein-induced pancreatitis. Previous researchers found that autophagy has reemerged as a novel theory for induction or initiation of pancreatitis, especially through intracellular activation of trypsinogen to trypsin that leads to escalating protein degradation and further enzyme activation (Ohmuraya and Yamamura, 2008; Fortunato and Kroemer, 2009).

In addition, nuclear changes of metaplastic pancreatic acini were more severe in older age and included karyomegaly and anisokaryosis. These nuclear alterations of pancreatic acinar epithelium proved that Ace-k affects DNA and may progress to precancerous lesions as discussed by Zhang *et al*. (2014). The necro inflammatory reactions were evident and increased with advancing dose. The lesions were associated with presence of oval shaped cells proliferation, Vogelmann *et al*. (2001) described the pancreatic stellate cells (PCs) in mice with chronic pancreatitis and fibrosis and they stated that PCs were localized as oval shaped cells in fibrotic pancreatic tissue. Masamune *et al*. (2009) found that pancreatic stellate cells (PSCs) played a key role in physiopathological mechanisms underlying the development and progression of pancreatitis and cancer and their activation was related to occurrence of chronic pancreatitis. Other theories of chronic pancreatitis and fibrosis was clarified by Min Xua *et al*. (2016) who stated that oxidative stress was implicated in progression of inflammation and fibrosis. The role of Ace-k in the induction of oxidative stress was confirmed by Cruz-Rojas *et al.* (2019) who found that exposure of common carp to Ace-k in water induced tissue changes involving several organs and was mediated by oxidative stress.

Conclusions: The present data revealed that chronic treatment of rat with Ace-k induced pancreatic injury in dose dependent manner and affect drastically weight gain especially in young aged rats. In addition, the histological hallmarks of chronic pancreatitis induced by Ace-k with the progression of metaplastic changes of pancreatic acini associated with marked nuclear changes give an attention for possible carcinogenic potential of Ace-k on pancreatic tissue. So, further investigations are essential to completely elucidate the mechanism of Ace-k induced chronic pancreatitis.

Authors contribution: EGA run all experiments, statistical analysis and drafted the manuscript. FFM contributed to study design, interpreted histological results and drafted the manuscript. AMA contributed to study design, elucidated the toxicological results and drafted the manuscript. SSA contributed to study design, interpreted histological results and drafted the manuscript. All authors read and approved the final manuscript.

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