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Mitigation of Diclofenac Sodium–Induced Hepatic Injury and Enteropathy in Rats by Vanillin

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Abstract

This study aimed to evaluate the potential protective effects of Vanillin (VA) against Diclofenac sodium (DFNa)-induced liver toxicity and enteropathy. A total of sixty male rats were divided into six groups; a control group received only saline, a DFNa-group received DFNa (9 mg/kg), 3rd, 4th, and 5th groups received DFNa along with escalated doses of VA (50, 100, 200 mg/kg). After five days of treatment, blood and tissue samples were collected for biochemical and histopathological analysis. According to our findings, administration of DFNa led to a substantial decrease in the final body weight and a considerable deterioration of hematologic parameters. Regarding biochemically, DFNa-induced a significant elevation in liver enzyme activities with a significant decrease in total protein and albumin levels, indicating liver intoxication. VA maintained normalized body weight and prevented DFNa-induced adverse effects on hematologic and liver parameters. Additionally, histopathological examinations revealed that DFNa showed perivascular inflammation and cellular infiltration, along with congestion and dilation of the central vein and induction of both types of cell death (necrosis and apoptosis). In the intestinal sections, DFNa resulted in necrotic enteritis, desquamation, and sloughing of the mucosa, as well as considerable congestion and dilation of the blood vessels with prominent submucosal edema. It is intriguing to note that the VA-treated animals demonstrated a significant protective effect against the deleterious effects of the DFNa on the liver and intestine. As a result of our study, VA could be used as an adjuvant agent as part of various regimens containing DFNa.

Keywords: Diclofenac, Vanillin, Liver, Intestine, Hemato-biochemically, Histopathology.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), including DFNa, are widely used both in human and animal medicine (Brogden et al., 1980; Gomaa and zoology, 2018) for anti-inflammatory, analgesic, and antipyretic purposes (Mazumdar et al., 2006; Novartis Pharma, 2006). While DFNa has numerous therapeutic benefits, large doses or therapeutic doses over an extended period are always associated with liver and gastrointestinal (GI) side effects (Brater, 2002; Tomic et al., 2008), which are manifested by GI bleeding, ulceration (Shin et al., 2017), fulminant hepatic injuries, hepatitis and aplastic anemia (Bessone, 2010).

The use of NSAIDs, specifically DFNa, may lead to hepatic injury, but these dysfunctions are usually transient and reversible (Rodríguez et al., 1994). On the other hand, these adverse effects are more prevalent among patients exposed to long-term DFNa to treat rheumatoid conditions such as arthritis (O'Connor et al., 2003).

Because of their chemical characteristics, NSAIDs are associated with an increased risk of acid-mediated damage to gastric and intestinal epithelial mucosa, impaired ability to repair damaged tissue, and GI injuries. A wide range of adverse effects was reported, ranging from mild erosion to severe peptic ulceration, and they occurred mainly if NSAIDs were taken for long-term (Gan, 2010). VA, 4-hydroxy-3-methoxybenzaldehyde, is the main active ingredient of Vanilla that is extracted, particularly Vanilla species; particularly *Vanilla pompon*, *Vanilla tahitensis*, and *Vanilla planifolia* (Divakaran et al., 2016). In the beverage and food industries as well as in the pharmaceutical market, such as the manufacture of cosmetics and perfumes, VA has been widely used as a flavoring agent (Bezerra-Filho et al., 2019).

Many studies have shown that VA holds a therapeutic potential against chemical-induced hepatotoxicity and enteropathy (Makni et al., 2011). Therefore, the primary purpose` of this study was to determine whether VA can alleviate the injury to the liver and intestines caused by DFNa.

Materials and methods

Drugs and Chemicals

Diclofenac sodium (Voltaren) ampoules containing 75 mg and Vanillin were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). Albumin and total protein kits were obtained from Diamond, Heliopolis, Cairo, Egypt. All other chemicals were of the highest-analytical grade commercially available.

Experimental Animals

Eight-week-old male rats (200 ± 20 g), Egyptian Company for Production of Antisera, Vaccines, and Drugs, Helwan, Egypt) were used in the study. During the experiment, rats were housed in polyethylene cages with a 12-hour light/dark cycle in a temperature-controlled and illuminated room, which was maintained with a constant temperature and constant lighting. The rats were given water and a balanced commercial diet as part of the ad libitum program. The experiments were carried out in accordance with the guideline for the care and use of laboratory animals and in compliance with the Research Ethics Committee at South Valley University, Qena, Egypt. Prior to the beginning of the experiment, the rats were given free access

to water but were deprived of food for 12 hours.

Experimental Design

Sixty male rats weighing were divided into six groups of ten rats per group. group1 (control) received only saline by intraperitoneal injection (I.P), group 2 (DFNa-group) received DFNa (9 mg/kg, I.P.), group 3 received DFNa plus VA 50 mg/kg (DFNaVA50), group 4 received DFNa plus VA 50 mg/kg (DFNaVA100), group 5 received DFNa plus VA 50 mg/kg (DFNaVA200) and group 6 received only VA 200 mg/kg (VA200). Each rat in the 3rd, 4th, and 5th groups received DFNa (9 mg/kg, I.P.) after 2 h of VA administration (orally). A drug regimen was applied twice daily for five consecutive days. At 6th day after last treatment, rats were anesthetized with xylazine, and ketamine and blood were collected for biochemical parameter analysis. The rats were then sacrificed using cervical dislocation, and the tissues from different parts of the body were fixed in neutral buffer formalin 10% (NBF). Body weights from all groups were measured before and after the treatment period of 5 days.

Blood Sampling and Biochemical

After 24 hr from the last dose, two blood samples were collected from all rat's hearts by cardiac puncture under xylazine and ketamine anesthesia. The first sample was collected on anticoagulant (EDTA) coated tubes for hematologic studies. The second blood sample was used to collect sera from animals (sample were kept at room temperature for 30 min to complete clot formation and centrifuged @3000 rpm for 15 minutes) for serum separation. The obtained sera were kept at -20 ºC until used for biochemical analysis.

Hematologic Indices

Red blood cells (RBCs), hemoglobin (HB), white blood cell (WBC), platelet (PLT) counts and procalcitonin %(PCT) were determined using an autoanalyzer (SFRI blood cell counter, H18 light; Sean-Jeand'Illac, France).

Biochemical Analysis

An analysis of the serum levels of Alanine transaminase (ALT), aspartate transaminase (AST) and Alkaline phosphatase (ALP) was carried out by colorimetric methods using commercial kits (Dokki, Biodiagnostic, Giza, Egypt). Total protein and albumin levels were measured colorimetrically using the commercial kits method purchased from Diamond, Heliopolis, Cairo, Egypt. Globulin level was determined by subtracting the albumin value from the total protein value of the same sample.

Tissues Collection and Histopathological Assessments

A portion of each rat's liver lobe and intestine were carefully excised and fixed in NBF (10%) for histopathological analysis. Following dehydration in ascending concentrations of alcohol, clearing in xylene, and embedding in paraffin wax, specimens according to standard paraffin embedding techniques, sections of the tissue blocks were cut into 5 µm thickness and afterward stained with Haematoxylin and Eosin (H&E). The mucopolysaccharides demonstration was also conducted using Alcian blue and Periodic acid–Schiff (PAS) stains from other sections of the intestine. Histopathological scores were determined by analyzing stained hepatic and intestinal sections. There were three randomly selected fields in each section where lesions or injuries were graded as

absent (0, 0% alterations), mild (II, up to 30% alterations), moderate (II, 30-50 % alterations), or severe (III, >50 % alterations).

Statistical Analysis

Analysis of variance (ANOVA) and Tukey's multiple-comparison posttest was used to compare the results of different treated groups and controls. An analysis was considered significant when the P-value between the two groups was less than 0.05. The statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, California). We presented the data as mean ± SD. Body weights from all groups were measured before and after the treatment period of 5 days.

Results

Effect on Body Weights

A body weight measurement was taken before and after a five-day treatment period. One-way ANOVA was used to analyze body weight differences between experimental groups before and after treatment. A non-significant difference was found in initial body weight (before treatment) among all the experimental groups (Figure 1, A). Rats treated with DFNa for 5 days, twice daily, showed a significant decrease (P< 0.001) in both final (198 ± 2.3 gm, representing 17.5% loss) and delta body weights (20.67 ± 2.33 g), compared to the control group. Treatment DFNa-administered rats with VA caused a substantial increase in both final and delta body weights compared to DFNa-group (Figure 1, B-C).

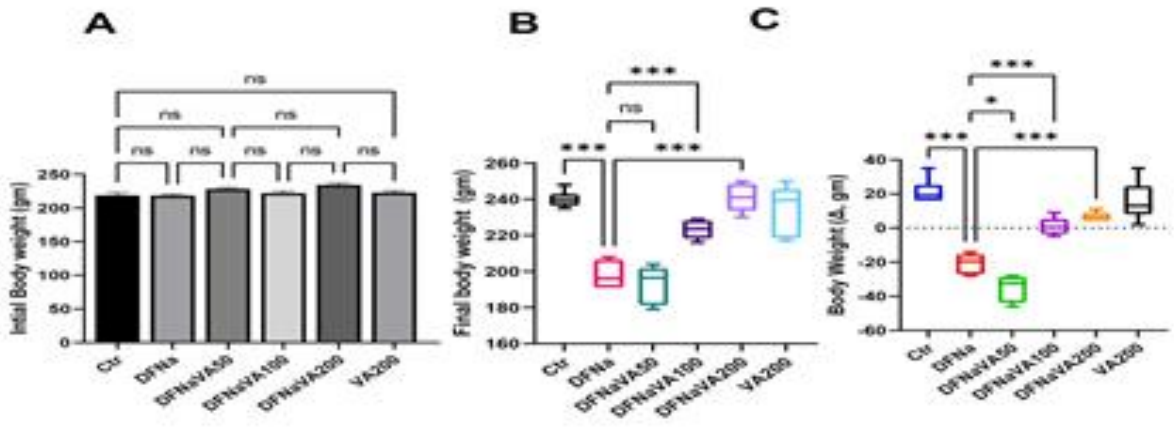

Figure 1: The effect of different drug regimens on body weight and liver weight. Each value represents the mean ± standard deviation of the mean; * P ≤ 0.05: ** P ≤ 0.01; ***P ≤ 0.001. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group; VA200, Vanillin at dose of 200 mg/kg.

Effect on Hematological Parameters

There was a significantly decreased (P <0.01) RBCs count (6.631 ± 0.24 x10^6/l) and HB concentration (12.20 ± 0.39 g/dl) in the DFNa-treated group compared to the control group (7.834, ± 0.22 x 10^6/l, 14.15 ± 0.19 g/dl), representing a decrease of

15% and 14%, respectively. Compared to the DFNa control group, significant increases in RBCs count and HB concentrations were detected in VA-pretreated groups in a dose-dependent manner (Figure 2, A-B).

DFNa-administration showed a significant decrease ($P < 0.001$) in WBCs count (reduction of 53%) when compared to the control group. Compared to the DFNa-group, there were marked increases in WBCs count in VA-pretreated groups (Figure 2, C). Additionally, in comparison to normal control animals, animals treated with DFNa exhibited significant increases ($P < 0.001$) in the PCT%. VA-pretreated groups showed significant decreases in PCT% Compared to the DFNa-treated group. Moreover, a substantial increase ($P < 0.001$) in PLT count was noted in the DFNa-treated group compared to the control group. Compared to the DFNa-treated group, significant decreases in PLT count have been reported in VA-pretreated groups in a concentration-dependent manner (Figure 2, D-E).

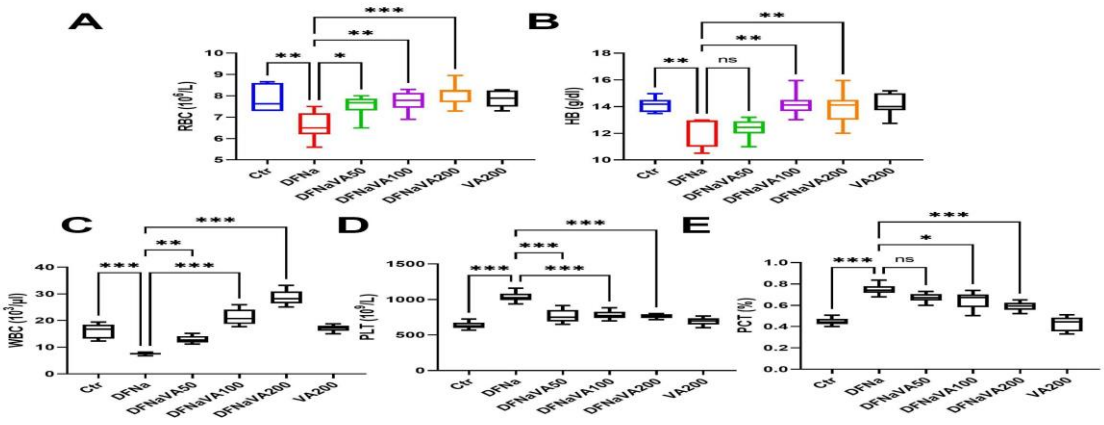

Figure 2: The effect of different drug regimens on hematologic parameters. Each value represents the mean ± standard deviation of the mean * P≤ 0.05: ** P ≤ 0.01; ***P ≤ 0.001. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group; VA200, Vanillin at dose of 200 mg/kg.

Hepatic Biomarkers

We assessed the enzyme activity of the ALT, AST, and ALP enzymes in order to evaluate the therapeutic potential of VA agonists-induced hepatotoxicity caused by DFNa. As shown in Figure 3, DFNa-treated animals exhibited a significant elevation ($P< 0.001$) in the enzyme activity of ALT (65.8 ± 1.6 IU/L, $P< 0.05$), AST (162.2 ± 9.9 IU/L, $P< 0.05$), and ALP (576.3 ± 46.3 IU/L, $P< 0.001$), which represents an increase of 140%, 132% and 198%, respectively, in comparison to control group (Figure 3, A-C). As expected, treatment of animal, challenged with DFNa, with VA significantly reduce the activity of hepatotoxic enzyme markers (ALT, AST ALP) (Figure 3, A-C). Additionally, DFNa-administration disturbed the normal hepatic functions; inducing a significant reduction ($P< 0.01$)

in level of both albumin; total protein (5.517 ± 0.166 gm/dl), that representing a decrease of 18% when compared to control one (6.783 ± 0.204 gm/dl), Albumin (2.983 ± 0.125 gm/dl) which represents a decrease of 24% when compared to the control (3.933 ± 0.165 gm/dl). VA pretreatment groups showed significant elevation of both total protein and albumin blood levels (Figure 3, D-E. Globulins data analysis showed non-significant changes in all experimental groups when compared to the normal control group (Figure 3, F).

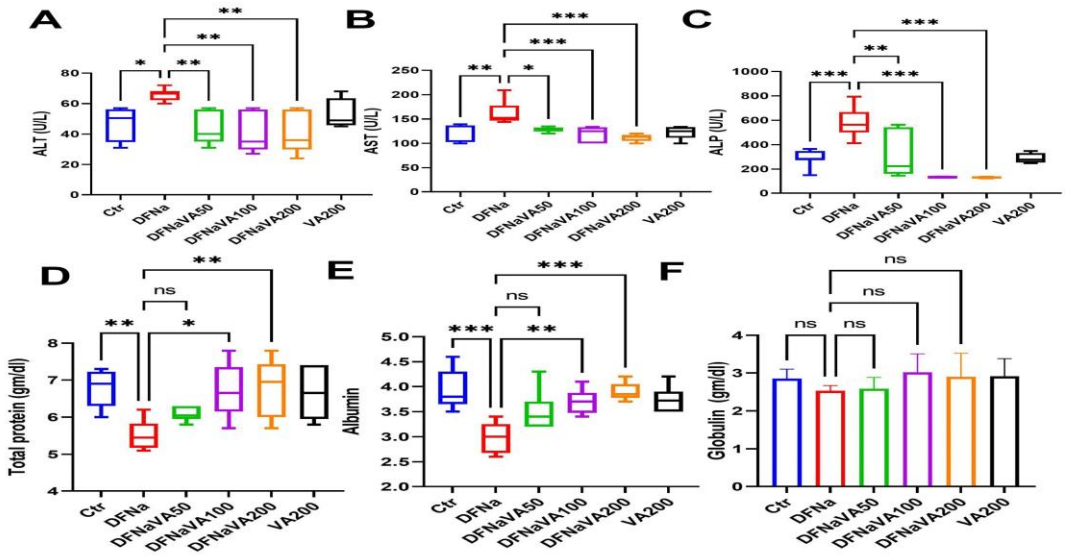

Figure 3: The effect of different drug regimens on serum biochemical parameters related to liver function and integrity. Each value represents the mean ± standard deviation of the mean * P ≤ 0.05: ** P ≤ 0.01; ***P ≤ 0.001. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group; VA200, Vanillin at dose of 200 mg/kg.

Histopathological Studies

Hepatic H&E Staining (Morphological Assessments)

The Control Liver section showed normal hepatic architecture and normal hepatocyte morphology. DFNa-group demonstrated remarkable congestion and dilatation of the central vein, and perivascular inflammation with cellular infiltration. overall, VA treatment showed a concentration-dependent reduction in histopathological/inflammatory score, 19%, 60%, 85%, respectively. In addition, there are cytoplasmic vacuolization, hyaline degeneration, focal hepatocyte necrosis, and apoptosis (Figure 4), with moderate histopathological (necrotic and apoptotic) score (Figure 4, B-D), were detected. Interestingly, even low concentration of VA, DFNaVA50 exhibited noticeable improvement of the histopathological changes. While tissue section of DFNaVA100 revealed minor pathological changes, alongside the promising level of protection against DFNa-induced hepatotoxicity (Figure 4,

A), with mild histopathological score (Figure 4, B-D, 68% and 73% reduction of necrosis and apoptosis, respectively). In parallel of the control group, DFNaVA200 group showed apparent normal liver morphology (Figure 4).

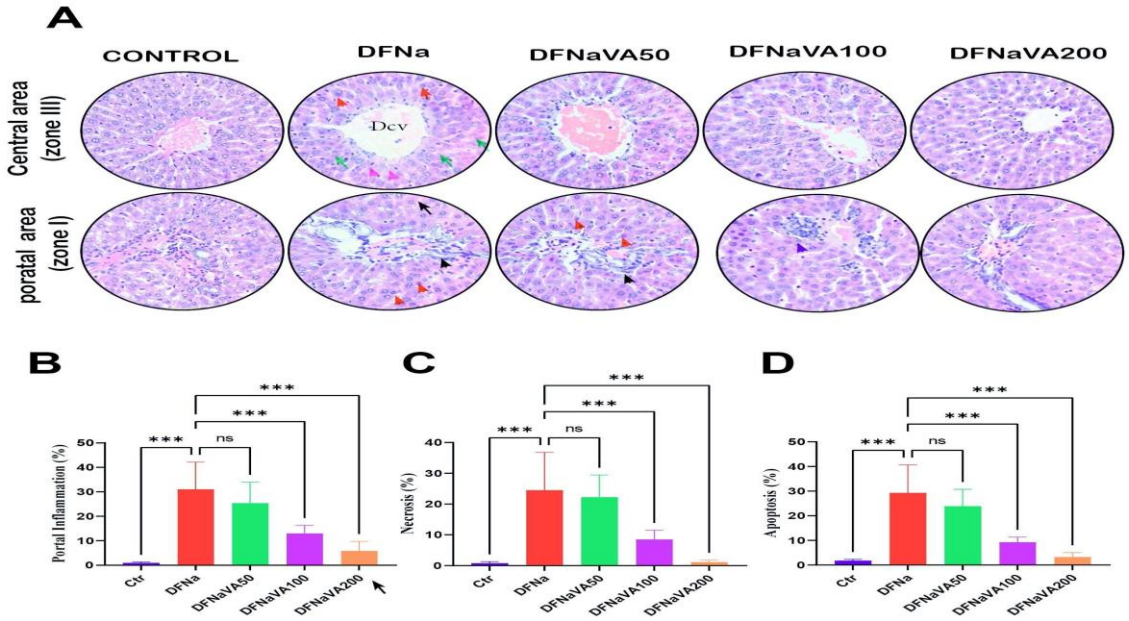

Figure 4: The effect of different drug regimens on hepatic architecture, photomicrographs of different liver sections. A) Liver sections stained with hematoxylin and eosin (X200). Control Liver showed normal hepatic architecture and normal hepatocytes. DFNa-group shows remarkable cellular hyaline, vacular degeneration, blood congestion, necrosis, apoptosis, and lymphocytic infiltration. VA50-VA200; Escalated doses of VA show concentration-dependent mitigation of DFNa-induced hepatic injury. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group; VA200. keys: black arrowhead; Lymphocytic infiltration, blue arrowhead; focal cellular infiltration, red arrowhead; apoptotic cells, black arrow; vacular degeneration, cyano-arrow head; necrotic cell, green arrow; hyaline degeneration, Dcv, congested-dilated CV.

Intestinal H&E Staining (Morphological Assessments)

As shown in Figure 5A, the control group exhibits an intact squamous mucosa constituting squamous epithelium, lamina propria, and muscularis mucosa. DFNa-group tissue section showed sloughing and desquamation of the mucosa and villi, together with severe congestion and dilation of blood vessels and accumulation of inflammatory cells. Whereas DFNaVA50 revealed a moderate degree of necrotic enteritis characterized by slight aggregation of inflammatory cells. DFNaVA100 exhibited minimal inflammatory cell infiltration. At the same time, DFNaVA200 and VA200 demonstrated intact intestinal layers (Figure 5).

Overall, DFNa revealed a high inflammatory score (Figure 5, B-C), a significant decrease in villi length (Figure 5, D), and severe submucosal edema (Figure 5, E). therapeutically, VA

pretreatment protective intestinal mucosa from DFNa-induced injury, in a concentration-dependent manner (Figure 5, B-E).

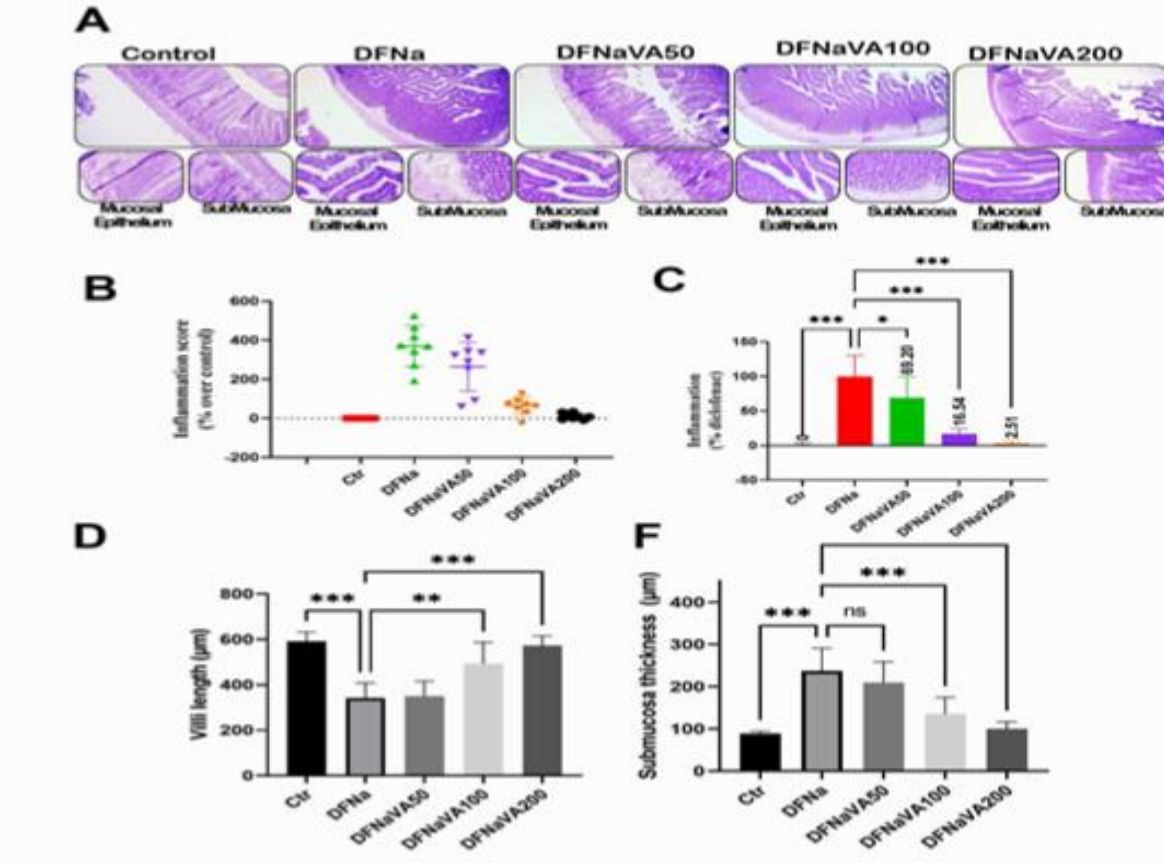

Figure 5: The effect of different drug regimens on intestinal architecture, photomicrographs of different intestinal sections. Intestinal sections stained with H&E (X200). The control intestine shows intact squamous mucosa overlying a submucosa, a dense muscular and serosa layer. The DFNa-group shows severe congested and dilated blood vessels, inflammatory edema, and necrotic enteritis. Escalated doses of VA (50 – 200 mg/kg) show concentration-dependent protection against DFNa-induced intestinal, these groups have mild to moderate necrotic enteritis characterized by slight aggregation of lymphocytes and minor submucosal edema. A higher concentration of VA shows minimal cellular infiltration with fully intact intestinal layers. Each value represents the mean ± standard deviation of the mean * $P \leq 0.05$: ** $P \leq 0.01$; ***$P \leq 0.001$. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group.

Intestinal Alcian Blue Staining

Mucopolysaccharides play a vital role in preventing the damaging effect arising from DFNa therapy. The control group showed a significant distribution of mucous characterized by a heavy bluish stain. On the other hand, in DFNa-group, there was discontinuous staining of the goblet cells with faint blue color. However, DFNaVA50 were manifested by detecting acceptable areas of mucous infiltration with intense bluish coloration.

Furthermore, DFNaVA100, DFNaVA200 and VA200 revealed deep bluish discolorations that demonstrated the abundant distribution of mucous (Figure 6).

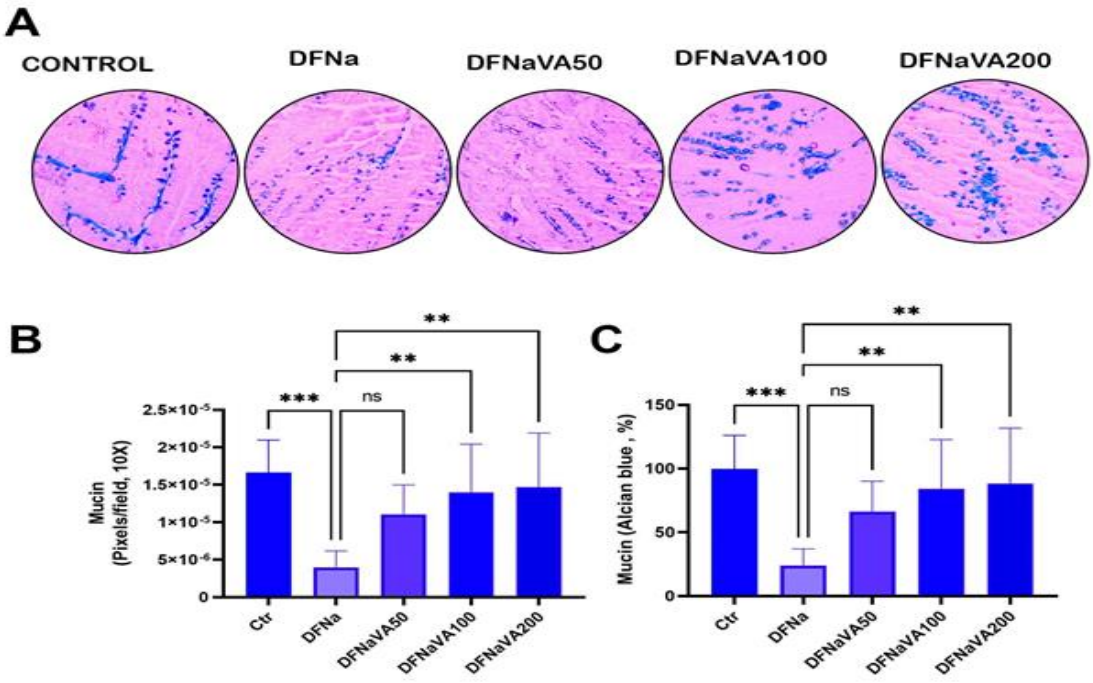

Figure 6: The effect of different drug regimens on intestinal tissue stained with Alcian blue stain (X200). Control group shows extreme mucin staining that expressed as deeply bluish coloration. DFNa-group shows a significant decrease in mucin level in the intestinal section, represented as discontinuous staining of the goblet cells with faint blue color. DFNaVA50 exhibit minor elevation of mucin when compared to DFNa-group. DFNaVA100 and DFNaVA200 show profound increase in mucin when compared to DFNa-group, represented as heavy blue colorations manifested by abundant mucous infiltration. Each value represents the mean ± standard deviation of the mean * P≤ 0.05: ** P ≤ 0.01; ***P ≤ 0.001. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg;

Intestinal Periodic Acid–Schiff Stain (PAS) Staining

PAS staining is used to detect polysaccharides such as glycogen, glycoproteins and mucin in tissues. Where, Control group showed a strong positive reaction of magenta red coloration. While in DFNa-group showed significant depletion of cytoplasmic mucopolysaccharides occurred, which displayed by a faint color (magenta) when compared to the control group. In addition, in DFNaVA50, mild changes in the amount of cytoplasmic mucopolysaccharides were observed by the moderate positive reaction when compared to DFNa-group. As well, DFNaVA100, DFNaVA200 and VA200 revealed sharp magenta red discolorations that demonstrated abundant distribution of mucous (Figure 7).

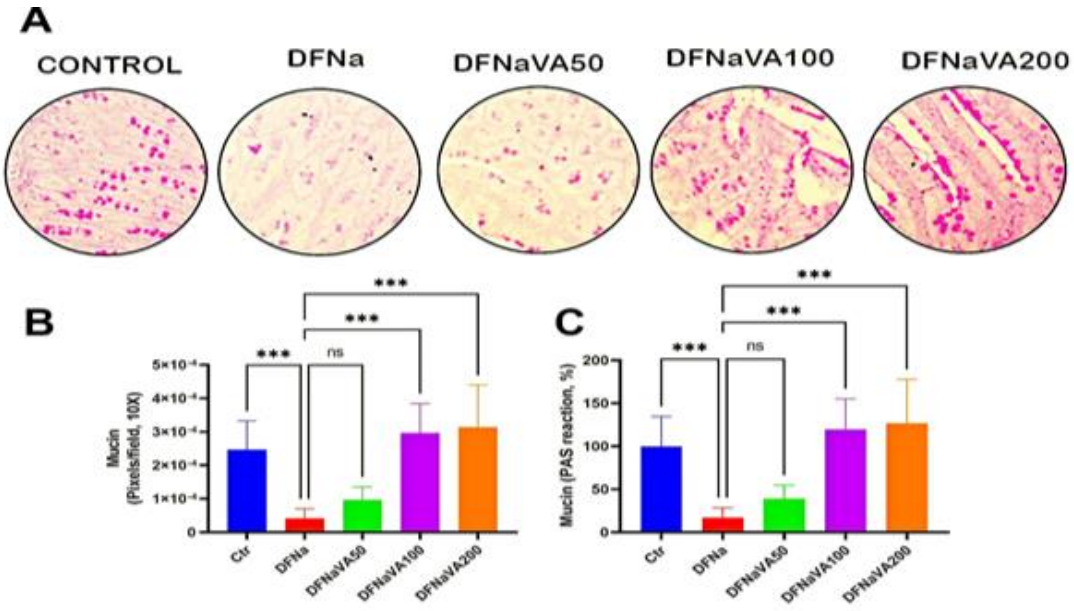

Figure 7: The effect of different drug regimens on intestinal tissue staining with PAS. PAS staining (X200). Control group shows an intense PAS reaction (positive PAS staining). DFNa-group shows negative PAS staining. DFNaV50 minor increase in PAS reaction when compared to DFNa-group. DFNaVA100 and DFNaVA200 show an intense and significant increase in PAS reaction compared to DFNa-group. Each value represents the mean ± standard deviation of the mean * $P \leq 0.05$: ** $P \leq 0.01$; ***$P \leq 0.001$. Abbreviations: Ctr, control; DFNa-group, Diclofenac sodium (9 mg/kg); DFNaVA50, Diclofenac sodium and Vanillin 50 mg/kg; DFNaVA100, Diclofenac sodium and Vanillin 100 mg/kg; DFNa VA200: Diclofenac sodium and Vanillin at dose of 200 mg/kg and group.

Discussion

There are a number of uses of DFNa, including the symptomatic relief of pain and the treatment of musculoskeletal inflammatory conditions (Gomaa and zoology, 2018). However, the administration of DFNa has been linked to severe gastrointestinal side effects and hepatotoxicity (Tomic et al., 2008). A variety of beneficial pharmacological bioactivities have been demonstrated for VA, including anti-inflammatory, anti-carcinogenic (Bezerra et al., 2016), antioxidant (Shyamala et al., 2007), and hepatoprotective effects (Kamat et al., 2000). In this study, we explored the possibility that VA could protect against hepatic and intestinal injury associated with DFNa.

The current study confirmed that treatment with DFNa led to a reduction in the mean of final body (BW) weight, which might be correlated due to tissue damage, as it was previously mentioned that significant decreases in BW were observed in all DFNa-treated animals in a concentration-dependent manner, indicating its dose-dependent toxicity of DFNa (Hussain et al., 2008). In our study DFNa treatment induced a significant weight loss associated with histological alterations in intestinal tissue such as edema, erosion, ulceration, necrosis, and fibrosis. These findings support the previous studies that found that NSAIDs can cause enteropathy, which is characterized by increased intestinal permeability, inflammation with blood, and protein loss (Davies et al., 1996),

consequently contributing to a decreased body weight.

Our study revealed that DFNa-administration, BID, for 5 days, led to a decrease in the mean values of RBC, and HB, which agreed with Thanagari *et al* 2012 who reported that intramuscular s administration of DFNa (13.5 mg/kg) for 14 consecutive days to adult male rats induced a significant reduction on blood Hb, RBCs values compared to the control. Moreover, oral administration of DFNa (9.5 mg/kg) for 28 days in albino mice caused a profoundly significant decrease in HB value (Shridar and Narayanan, 2007). These alterations in the response indicated that DFNa-intoxication was dose- and time-dependent, regardless of the route of administration. Bone marrow toxicity associated with DFNa-administration was characterized by a reduction in RBC and HB counts (Selvaraj et al., 2017). In addition, the loss of blood during GI bleeding and the release of immature RBCs can also alter hematologic findings (Al-najjar, 2018; Thanagari et al., 2012).

According to Sanchez-Matienzo et al. (Sanchez-Matienzo et al., 2006), DFNa is more likely to cause liver disorders than any other NSAIDs and independent of animal species (Ahmad et al., 2012) that characterized by substantial serum elevation of ALT and AST values (Ahmad et al., 2012; Lar et al., 2016), and associated with high-profile hepatic necrosis (Lar et al., 2016). In the line of this report (Sanchez-Matienzo et al., 2006), our study revealed that DFNa-administration significantly increased levels of markers of hepatocellular injury (ALT, AST, and ALP), as well as a significant reduction of total protein and albumin values.

In terms of therapeutic effects, VA treatment increased body weight significantly and restored altered hematological and biochemical parameters induced by DFNa to normal values. Several studies have shown that VA acts as a cytoprotective in several tissues (Mohamed and Zoology, 2019), restoring levels of ALT, AST, and ALP (Kumar et al., 2010), while also restoring tissue antioxidant/oxidant status by enhancing antioxidant tissue levels as well as maintaining normal levels of oxidant markers (Mostafa et al., 2021).

Histopathological investigations confirmed serum elevation of hepatic enzymes and showed that DFNa-group induced remarkable morphological alterations. The study of Klatskin et al. can explain this alteration, they reported that hepatocellular damage is initiated by dilatation and congestion of hepatic blood vessels as a result of direct toxics (Klatskin and Conn, 1993). As these alterations progressed, hepatocellular damage was augmented by mitochondrial dysfunction (Mossa et al., 2013). It has been reported that DFNa-induced tissue damage is associated with induction of both cell death, necrosis, and apoptosis, downstream player of execution of oxidative stress (Galati et al., 2002; Hassan et al., 2021). Our study represents that VA could be allivated DFNa-induced hepatic alterations, including necrosis, apoptosis, and inflammation, for the first time. This ameliorative property of VA is due to its anti-inflammatory, antioxidant activities (Bezerra-Filho et al., 2019; Bezerra et al., 2016; Makni et al., 2012; Makni et al., 2011; Mohamed and Zoology, 2019; Shyamala et al., 2007)

As a result of the GI damage caused by NSAIDs, these drugs are often poorly tolerated and have limited clinical utility (Scarpignato and Hunt, 2010). In the DFNa-group, necrotic enteritis resulted in squamous epithelium desquamation and submucosa edema. There was a marked depletion of mucopolysaccharides in the DFNa-treated group, possibly contributing to intestinal damage. In comparison with the DFNa-treated groups, VA-treated groups had a significant increase in mucopolysaccharides concentrations.

It has been reported that DFNa treatment is linked to a gradual increase in intestinal epithelial permeability (Reuter et al., 1997), facilitating gram-negative infection and induction of peptic ulcers. Also high rates of acute erosions have been reported after DFNa treatment (Hawkins and Hanks, 2000; Reuter et al., 1997). It has been shown that DFNa can cause severe ultrastructural damage to the gastric surface epithelium, as well as subepithelial hemorrhages and erosions of the gastroduodenum within several hours after ingestion (Hawkins and Hanks, 2000). Interestingly, our study showed that VA administration could mitigate and enhance the restoration of typical structure in the liver and intestine after DFNa exposure, thereby improving the maintenance of liver and intestine histological changes. Myriad of studies has been documented that DFNa trigger induction of oxidative stress and tissue oxidant/antioxidant imbalance, consequently tissue damages (Galati et al., 2002; Hassan et al., 2021; Masubuchi et al., 2002). Thus the protection that VA provides may be attributed to its antioxidant and anti-inflammatory properties that protect cells and tissues from the oxidative damage created by the generation of reactive oxygen species (Bezerra-Filho et al., 2019; Kamat et al., 2000; Makni et al., 2012).

By attenuating the hepatic and intestinal damages caused by DFNa regimen, VA could improve the therapeutic impact of DFNa. Therefore, we recommend using VA as an adjuvant agent with DFNa treatment.

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