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Aqueous extract of *Daucus carota* exerts a protective effect on the Renal, Hepatic and Duodenal mucosal histology in Diclofenac Induced Tissue Injury

Research Article

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Abstract

Objective: The use of ethnobiology in treatment of many diseases especially in rural residents with limited access to medical technology, treatment and equipment is beneficial and necessary. *Daucus carota* (DC) root extract was used as pre-treatment in adult Wistar rats exposed to diclofenac sodium (DF) to investigate a protective effect on the histology of liver, kidney and duodenum. Materials and Method: Twenty-five adult Wistar rats were used for this study and were randomly divided into 5 groups of 5 rats each, which included: Group A - Normal control; Group B – 50mg/kg DF control; Group C – 50mg/kg DC + 50mg/kg DF, Group D – 100mg/kg DC + 50mg/kg DF; and, Group E – 140mg/kg DC + 50mg/kg DC. Results and Discussion: The results showed a reduction in damage to the hepatocytes, maintenance of sinusoidal integrity and reduction in the number of inflammatory cells in the hepatic parenchyma, In the kidney tissue, the extract preserved the glomerular capillary tuft, renal tubular epithelial cells, conserved Bowman's space and lining epithelium of the capsule in a dose dependent manner. The intestinal mucosa in groups treated with higher doses of the extract were completely preserved and intact with minimal erosion of epithelial lining along with preservation of lamina propria and intestinal glands. Conclusion: Pre-administration of DC, preferably at concentrations of 100mg/kg and 140mg/kg reduced hepatotoxicity, renal tissue and mucosal layer damage in the duodenum following administration of DF. This preservation of tissues improved as concentration of the extract increased verifying that its efficiency was dose dependent.

Key Words: *Daucus carota,* Diclofenac, Duodenum, Ethnobotany, Glomerulus, Hepatotoxicity.

Introduction

Ethnobotany is a plant science that studies historical and current application of medicinal plants (1). The use of medicinal plants is important for the preservation of traditional medicinal plant resources, and also, may be useful to the health professionals, scientists and scholars working the field of pharmacology and therapeutics to develop evidence based alternative medicine sources to cure different kinds of diseases in man and animals (2). Furthermore, local and/or rural residents with limited access to medical technology, treatment and equipment may benefit from traditional remedies and medicinal plants, which has been known to form an effective native healthcare system relied on by members of that populace (1). The use of traditional medicinal plant species, often leads to the discovery of new drugs, and contributing to the growth of local economy as well as improving the standard of living of residents.

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Currently, millions of people in the developing world rely on traditional medicinal plants for primary healthcare, skin care, economic benefits, and cultural development (1). *Daucus carota* (DC), commonly known as carrot, is a popular medicinal plant with several pharmacological activities mentioned in traditional and contemporary phytotherapy including antidiabetic (3,4), antioxidant (5,6,7), analgesic (8), antimicrobial, anti-inflammatory (8), antihyperlipidemic (7), antifungal, diuretic, lithontripic, emmenagogue, intra occular hypotensive, gastroprotective, hepatoprotective (9), aphrodistic, nephroprotective, has anti-calcifying effect in urolithiasis (10), antispasmodic, anticancer, anti-ulcer (11), adverse effect on sperm activities and sperm morphology (12), antiestrogenic (13), oestrogenic (14), cardioprotective and muscular contraction regulation effects (15), can improve antioxidant status by inhibiting peroxidation activity in liver tissue (16) and wound healing activities. No serious adverse events have been recorded after ingestion of carrot except for some cases of photosensitivity (13).

Diclofenac sodium (Voltaren) is a potent and widely used non-steroidal anti-inflammatory and analgesic compound (17). Diclofenac and its metabolites are excreted in both urine and bile. Diclofenac sodium has been used in several experimental studies (18, 19, 20, 21) to induce

hepatotoxicity. The present study investigates the histological effects on the kidney, liver and duodenum following pre-treatment with aqueous extract of *Daucus carota* (DC) in adult Wistar rats exposed to diclofenac sodium (DF).

Materials and Methods

Plant Authentication, Extraction and Storage

Daucus carota (carrot) was purchased from Tashan Bama Market in Maiduguri, Borno State. The root vegetable was easily identified and authenticated by a Botanist in the Department of Biological Sciences, University of Maiduguri. The plant extraction procedure was carried out according to the methods employed by (22,23,24). 453.6g of carrot tubers was grated into smaller pieces and then allowed to dry in a shade, the dried pieces were then crushed into powder mechanically. The dried powder obtained was then sieved to obtain fine agranular powder which was immersed in two liters of absolute ethanol and allowed to stand for exactly twenty-four (24) hours with minimal agitation. This solution was allowed to cool under room temperature and then strained and the ethanol allowed to escape. The residual extract was stored in a refrigerator prior to use.

Animal Husbandry

Twenty-five Albino rats were obtained from the Department of Human Anatomy, Faculty of Basic Medical Science, Ahmadu Bello University, Zaria and housed in the animal house of the Faculty of Science of the University of Maiduguri for a period of two weeks prior to the start of the experimental study to allow acclimatization. The accommodations provided a favorable atmosphere which provided satisfactory room temperature, humidity, and automatic 12 hours light, 12 hours dark cycle which were suitable for the welfare of the rats. All animals were housed in standard cages and fed with rat chow and tap water *ad libitum.*

Experimental Design

The rats were weighed and their weights varied between 90g and 162g at the start of the experimental study. The rats are grouped randomly into five (A, B, C, D and E) with each group consisting of five (5) rats each. Group A - Normal control; Group B – DF control; Group C - Low dose DC + DF, Group D – Medium dose DC + DF, Group E – High Dose DC + DF. The rats in Group A were administrated physiological saline at a concentration of 50ml/kg body weight via the oral route while Group B rats were administered 50 mg/kg body weight of DF serving as the negative control group. Group C rats were administered 50 mg/kg body weight of DF and 50mg/kg of DC both via the oral route, while the rats in Groups D were administered DF (50 mg/kg body weight) and 100 mg/kg body weight of DC also via the oral route, rats in Group E were treated with 50 mg/kg body weight of DF and 140mg/kg of DC both via the oral route respectively. The ethanolic extract of DC was administered to the rats for a period of 7 days. This extract was given an hour before administration of Diclofenac sodium (DF).

Experimental hepatoxicity was induced using 50mg/kg Diclofenac sodium in a method as indicated by (19, 20, 24).

Animal Sacrifice

Twenty-four hours after the last dose was administered, the rats in all groups were sacrificed. The rats were euthanized using Ketamine hydrochloride obtained from Ralington pharma LLP (India). The injection was given to the left thigh of the rats and this induced sleep. A laparoscopic procedure was performed with a horizontal midline incision to expose abdominal organs. The duodenum, liver and kidneys were quickly harvested and washed in saline solution to remove excess debris and blood and in preparation for further histopathologic evaluation.

Tissue Processing

The tissues were thereafter fixed in 10% neutral buffered formalin and dehydrated in graded series of alcohol (Sigma-Aldrich, USA), cleared in xylene (Veckridge Chemicals, New Jersey), and embedded in paraffin wax (Lodha Petrol, India). The tissues were sectioned at 5 µm using a rotary microtome (Leica RM2125 Rotary Microtome) and stained with Hematoxylin and Eosin (Abbey Colour, Philadelphia).

The sections were then photographed using an Amscope light microscope (MBJX-ISCOPE, Los Angeles) with a digital camera (M500, X 64, version 3.7) under X40 and X100 magnifications. Images of the histological sections were photographed using 10X objective lens and these images are presented as results.

Compliance with Ethical Standard

The experimental procedures were conducted in accordance with the University of Maiduguri

Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was also conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Results

Histological Observations in the Micrograph of the Liver

The micrograph of the liver is represented in Figures 1A-E. The liver in the control group showed normal hepatic architecture with cords of hepatocytes radiating as a bicycle spoke towards the central hepatic vein. The sinusoidal spaces were clear and were defined between the hepatocytes. The hepatocytes were clearly defined and had prominent nucleoli which was darkly stained. The cytoplasm was granular and eosinophilic (Figure 1A).

Rats that were administered with 50mg/kg DF showed distorted hepatocytes and reduced sinusoidal spaces. The lining endothelium of the central vein was discontinuous and the central vein showed an accumulation of red blood cells which suggested hepatic congestion (Figure 1B).

The rats that were treated with 50mg/kg DF + 50mg/kg of DC showed a hepatocyte with granulated cytoplasm. The sinusoids were occupied with inflammatory cells and the spaces were narrowed. The endothelial lining of the central vein was continuous and the central venous space clear (Figure 1C).

The micrograph of rats treated with 50mg/kg DF + 100mg/kg of DC exhibited characteristics similar to the group treated with a lower dose as there were lymphocytes observed infiltrating the sinusoidal spaces, however, more numerous than in the group treated with the low dose of the extract. The hepatocytes were intact and not distorted and the endothelium lining the central vein was continuous and few red blood cells were found in the lumen (Figure 1D).

The group that received 50mg/kg DF + 140mg/kg of DC showed hepatic architecture that was similar to the control group with fewer lymphocytes observed in the sinusoidal spaces and hepatic parenchyma, the central vein was clear and devoid of blood cells and the hepatocytes were also granular and eosinophilic. The hepatic parenchyma was well preserved in this group with the radiating cords of hepatocytes arranged towards the sinusoids (Figure 1E).

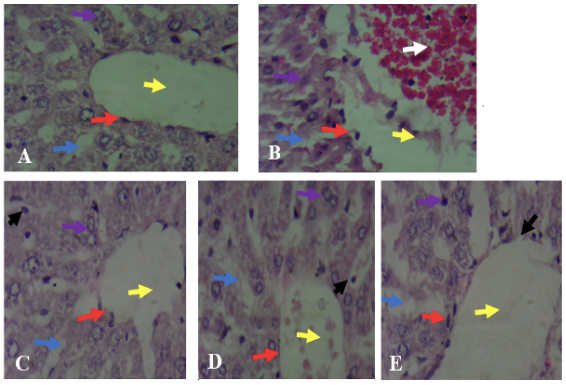

Figure 1 A-E showing the micrograph of the liver in all groups with yellow arrow showing the central vein which is suffused with blood represented with a white arrow in Figure 1B. The hepatocytes (purple arrow) and are bracketed on both sides with venous sinusoids (blue arrow) which are also distorted in Groups B and C. The endothelium lining the central vein (red arrow) is also discontinuous in Group B. Inflammatory cells are represented by a black arrow in Groups C, D and E. H and E X400

Histological Observation in the Micrograph of the Kidney

The micrographs representing the kidney of rats treated in the present study are shown in Figures 2A-E.

The micrograph of the control group showed a tuft of glomerulus encapsulated in Bowman's space. The space was clear and lined by the epithelial cells of Bowman's capsule. This epithelium was also continuous and the squamous cells that formed this layer had their nuclei bulging into Bowman's space. The renal tubules were intact, consisting of simple cuboidal epithelium resting on a basement membrane. The apical borders of the epithelial cells had a brush border appearance in this group and the cytoplasm was homogenous and eosinophilic (Figure 2A).

The negative control group that received 50mg/kg DF showed renal tubular cells that were distorted and wider in diameter than the control group. Bowman's space was reduced and blood cells were seen to be present in this space. The epithelium lining Bowman's capsule was thickened with more cytoplasm observed in these cells than in the control group (Figure 2B).

Figure 2C shows the micrograph of the kidney in rats that were treated with 50mg/kg of DF + 50mg/kg DC and the glomerulus was dispersed in Bowman's space almost obliterating this space. The lining epithelial cells were flattened and their nuclei bulged into the reduced Bowman's space. Renal tubules were intact in the renal parenchyma and the distal convoluted tubules were low cuboidal in the distal convoluted tubules and high cuboidal with a brush border in the proximal convoluted tubules.

The rats that were treated with 50mg/kg of DF + 100mg/kg DC showed a Bowman's space that was restored, the lining epithelium of Bowman's capsule showed flattened cells with nuclei bulging into the space. The cells of the renal tubules were simple cuboidal with the apical parts exhibiting a brush border appearance (Figure 2D).

The rats that were treated with 50mg/kg of DF + 140mg/kg of DC exhibited a micrograph of renal tissue that was similar to the control group. The glomerulus was not dispersed, obliterating Bowman's space, but the space was continuous around the capillary tuft, with no blood cells observed in this space. Bowman's capsule was also continuous around Bowman's space and the capillary tuft of the glomerulus. The renal tubular cells were intact and eosinophilic and granular, indicating numerous cellular organelles typical of an active cell. The apical borders also had a brush border appearance (Figure 2E).

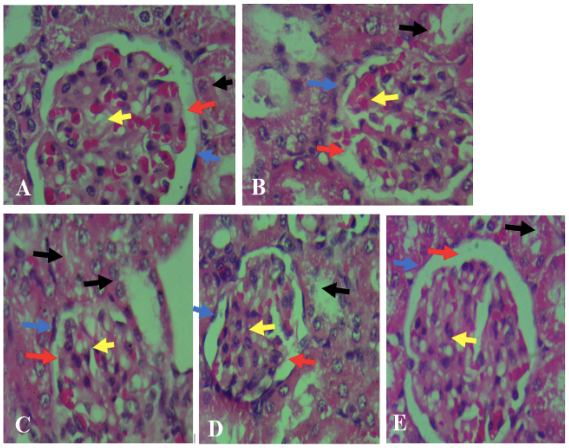

Figure 2 A-E representing the micrograph of the renal cortex in all groups. The glomerular tuft (yellow arrow) is encircled with Bowman's space (red arrow) which is inundated with blood in Groups B and C. the lining epithelium of Bowman's capsule (blue arrow) is continuous in all groups. The renal tubules (black arrow) shows different stages of distortion in all experimental groups. H and E X400.

Histological Observation in the Micrograph of the Small Intestines

The histology of the small intestine is observed in Figures 3A-E. The small intestine in the rats that were treated with 50mg/kg of saline solution was arranged in layers from the luminal surface going outwards. The luminal surface was thrown into finger-like villi which were lined with columnar epithelial cells interspersed with unicellular glands (goblet cells) which are observed light, rounded spaces in the epithelial lining. The core of the villi contained connective tissue (lamina propria) which was continuous at the base of the villi. At the base of adjourning villi were simple tubular

glands (crypts of Lieberkuhn) which opened up into the luminal surfaces of the small intestine bringing with it secretions of the intestinal glands. The mucous layer was separated from the submucous layer by a layer of smooth muscle (muscularis mucosa) which contracts to eject the secretions of the intestinal glands in lamina propria. The submucosal space was areolar and contained blood vessels. The muscular layer was evenly thick and continuous at the base of the submucous layer (Figure 3A).

The intestine of rats that received 50mg/kg of DC showed a damage to the mucous layer with the arrangement of the villi disorganised as the epithelium was eroded. The epithelial cells were lost and/or reduced with very few goblet cells observed. Lamina propria was also reduced, as well as the intestinal crypts of Lieberkuhn were reduced and also eroded. Muscularis mucosa was robust and formed a continuous ribbon of muscle below the lamina propria. The submucosa and muscularis externa layers remained unchanged in this group (Figure 3B).

The micrograph of rats treated with 50mg/kg of DF and 50mg/kg of DC also had an eroded mucosal layer with reduced epithelial and goblet cells and reduced lamina propria as well. Muscularis mucosa was also continuous and the muscular layers and submucosa was also unchanged in this group (Figure 3C).

Figure 3D shows the micrograph of rats treated with 50mg/kg of DF and 100mg/kg of DC. The epithelial layer was intact, with the enterocytes interspersed with light staining goblet cells. Crypts of Liebekuhn were also preserved and found at the base of intestinal villi. Lamina propria was also continuous within the intestinal villi. Muscularis mucosa, submucosa and the muscular layers were also same as observed in other groups (Figure 3D).

The group that received 50mg/kg of DF and 140mg/kg of DC showed normal intestinal architecture with numerous intestinal villi, which extended into the lumen of the small intestine. The goblet glands were also numerous and studded the epithelial lining. The lamina propria core extended to the base of the villi and intestinal crypts lay at the base of adjacent villi. Lying also at the base of the crypts was a continuous band of muscularis mucosa. Submucosal and muscular layers were also continuous and similar to that observed in other groups (Figure 3E).

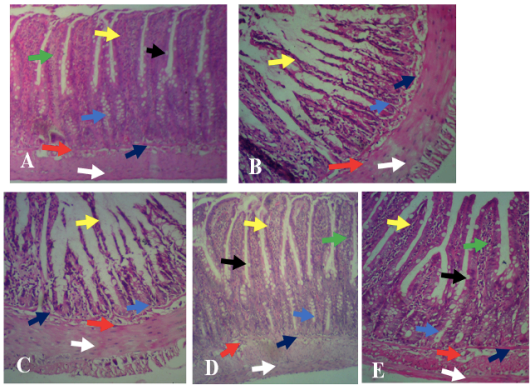

Figure 3 A-E showing the micrograph of the proximal gut showing the layers. The intestinal villi is lined with epithelium (yellow arrow) which is intact in Groups A, D and E and eroded in Groups B and C. Intestinal crypts of Lieberkun and intestinal tubular glands (blue arrow) are also eroded with the mucosal lining in Groups B and C. The submucosal (red arrow) and muscular layers (white arrow) remains relatively same in all groups.Unicellular mucous glands (black arrow) are present in the mucous layer of Groups A, D and E. Dark blue arrow – muscularis mucosa, green arrow – lamina propria. H and E X 200

Discussion

Diclofenac sodium (DF) is a well-known anti-inflammatory, antipyretic drug which is safe in therapeutic doses but can produce serious hepatic necrosis in man and animals with toxic doses (25, 26). In the present study, a single dose of DF (50mg/kg intraorally) confirms the damage to the hepatic, renal and intestinal tissue. This was evidenced by disorganised arrangement of the hepatocytes, presence of lymphocyte aggregation in the hepatic parenchyma, alteration in the integrity of the sinusoids and a pooling of blood in the central vein with disruption of endothelial cells lining the central vein. In the renal tissue, there was also damage to the renal tubular cell arrangement, narrowing of Bowman's space and thickening of Bowman's capsule and bleeding into Bowman's space. The mucosal lining in the intestines were eroded and the epithelial tissue was lost. This is consistent with studies carried out by (27) who discovered characteristic histopathological deposition of white crystals on the heart, liver, kidneys, spleen, lungs and joints of research animals treated with varying doses of DC. Histopathological studies revealed dilation of central vein and hepatic sinusoids, infiltration of hepatic parenchyma with reticulo-endothelial cells, cloudy swellings in the liver, in the spleen, there was degenerative lymphoid follicles and shrunken glomeruli along with infiltration of leucocytes in inter-tubular spaces of the kidneys. The results obtained from that study indicated that diclofenac sodium had hepatotoxic, nephrotoxic, and visceral gout-inducing potentials in the research animals. Also in agreement with the present study is another study conducted by (28), histopathological examinations of diclofenac exposed fish revealed alteration of the kidney such as hyaline droplets, increasing of interstitial cells, shrinkage of glomeruli, and the presence of melano-macrophages and necrosis of epithelial cells of renal tubules. In the liver, there were melano-macrophages in hepatocytes, degeneration and vacuolation and necrosis of hepatocytes.

Daucus carota (DC) showed protective effect in the histology of the selected tissues by preserving tissue integrity. In the liver, this was observed as a reduction in the damage to the hepatocytes, maintenance in the integrity of the sinusoids and reduction in the number of inflammatory cells in the hepatic parenchyma. (29) investigated the hepatoprotective effect of kaempferol (100 and 200 mg/kg body weight) which was isolated from DC leaves was tested in acetaminophen-induced liver damage of albino rats. Oral treatment with kaempferol reversed all the serum and liver parameters in a dose-dependent manner as observed in the current study.

In the kidney tissue, the extract also preserved the glomerular capillary tuft, renal tubular epithelial cells, conserved Bowman's space and the lining epithelium of the capsule as these structures were similar to the control group showing that the extract had protective effect on the renal histology as well. (30) investigated the nephroprotective effects of ethanolic root extract of *Daucus carota* (200 and 400 mg/kg, po) against

gentamicin-induced nephrotoxicity in albino rats. Gentamicin caused elevated biochemical parameters which were significantly lower in groups receiving DC dose-dependently. The nephroprotective effects of DC were further confirmed by histological observations of the kidney tissue.

The *Daucus carota* plant is traditionally used as an antiulcer agent in several regions of the world (31). The intestinal mucosa in the groups treated with higher doses of the extract were completely preserved and intact with minimal wearing away of the mucosal layer in the group that were treated with the lowest dose of the extract. The result obtained above proves the hepatoprotective, reno-protective and intestinal cytoprotective effect of DC extract. (11) investigated the gastric antisecretory and gastric cytoprotective effects of DC fruit compared with the antiulcer drug pantoprazole. The methanolic and aqueous extract of DC was found to possess a cytoprotective effect in the gastric region against gastric ulcers in rats induced by ethanol damage (32). The roots are also proved to have an antisecretory, gastroprotective, and antacid capacity using experimental rats with acidic levels dropping at doses of 100mg/kg and 200mg/kg. Histological assessment of gastric mucosa by (33) showed that DC protected gastric mucosa by inhibiting congestion, oedema, haemorrhage, and necrosis. In the current study, it showed the same protective effect on the mucosa of the intestine.

Conclusion

Daucus carota L. (Carrot) is a popular vegetable known throughout the world. DC has been used medicinally for the treatment of a spectrum of diseases and in the present study, it was found to restore the histology of the parenchyma of the liver, kidney and preserved the mucosa of the intestine in rats that were administered diclofenac sodium to induce damage in these tissues in a dose dependant manner. The higher the dose of DC administered, the more preserved the tissue was as observed in the micrographs of the tissue.

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Conflict of Interest

The Authors have no conflict of interest to declare

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