IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 7 Ver. V (July. 2014), PP 53-58
www.iosrjournals.org

Histological Effect of Oral Administration of Ccl_4-Induced Stomach and Duodenum Damage and the Protective Effect of Nigella Sativa in Adult Wistar Rats

[1]J. Danladi, [1]Akpulu S. P., [2]Owolagba G. K., [1]Afuwai V. K., [1]Hadiza R. A., [1]T. Murdakai

[1]*Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria*
[2]*School of Medical Laboratory Sciences, Ahmadu Bello University Teaching Hospital Zaria, Nigeria*

Abstract: *Nigella sativa is a herbal plant commonly used for the treatment of several diseases including gastric ones. This study investigates the histological effects of cool extraction of Nigella sativa oil against CCl4-induced stomach and the duodenum damage in adult Wistar rats of both sexes. 35 adult Wistar rats of both sexes were equally divided into 5 groups (5 rats). Group 1 rats were administered normal Saline (volume per body weight) orally. Group 2 rats were administered olive oil 4ml/kg body weight orally. Group 3 rats were administered 4ml/kg body weight of N. sativa oil orally for 2 weeks. Group 4 rats were administered 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally. Group 5 rats were administered 4ml/kg body weight of N. sativa oil plus 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally. Histopathological changes were observed. The stomach and the duodenum in group 1, 2 and 3 showed normal stomach and the duodenum architecture. There was a widespread degeneration and ulceration in the stomach and the duodenum in the epithelium of group 4 rats. N .sativa oil showed protective effect on the histological section of the stomach and the duodenum in groups 5. From the results it is suggested that cool extraction of Nigella sativa oil extract has the ability to protect the stomach and the duodenum histological damages, possibly through antioxidant effects of its bioactive compounds.*

Keywords: *CCl_4, stomach, duodenum, histological, Nigella sativa*

I. Introduction

Herbal medicines are widely perceived by the public as being free from side effects. However, herbal therapies have not been effusively researched or standardized to enable clinical application1. Indeed, some of the herbal agents are unknown in the Western world and concerns regarding side effects in modern evidence-based medicine practice are major (Pieroni et al., 2008). Hence, safety of herbal medicines is still an issue worldwide (Calapai, 2008; Pieroni et al., 2008).

The seed of Nigella sativa (NS), an annual Ranunculaceae herbaceous plant, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma. NS contains more bthan 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. Phytochemically, the volatile oil has been shown to contain 18.4-24% thymoquinone and 46% many monoterpenes such as p-cymene, and α-pinene (El Tahir et al., 1993). Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, antiinflammatory and anti-tumour, antibacteria agents (Rogozhin et al., 2011; Alam et al., 2010; Houghton et al., 1995; El Daly et al., 1998; El-Kadi et al., 1987).

Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals (Tsukamoto et al., 1990). This model has been used in various studies on examined the deposition of extracellular matrix in the fibrotic and cirrhotic liver (Hernandez-Munoz et al., 1994; Muriel et al., 1998). CCl_4 is a selective hepatotoxic chemical agent. CCl_4- induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation. A number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals (Parola et al., 1992). Production of reactive oxygen species and lipid peroxidation induced by iron overload (Bacon et al., 1990), cholestatic injury (Parola et al., 1996) and intoxication by ethanol (Kamimura et al., 1992) and CCl4 (Parola et al., 1992) is associated with liver fibrosis and cirrhosis. These effects are partially prevented by antioxidant compounds including α-tocopherol (Parola et al., 1992; Halim et al., 1997), silymarin (Mourelle et al., 1989) and salvianolic acid (Hu et al., 1997).

In mammals including humans and rats, the digestive tract is composed of tubular organs that are joined together from the mouth, esophagus, stomach, small intestine (duodenum, ileum and jejunum) and large intestine to the anus. Along this continuum of the digestive tract, chemical digestion occurs minimally in the mouth, predominantly in stomach and duodenum, and is almost complete at arriving to the ileum. It is known that digestion of certain foods such as vegetables is often incomplete (Tovar et al., 1992). It is also known that

while food is emptied from the mouth and oesophagus as quickly as it is swallowed, emptying of the stomach and small intestine in digestive process is relatively slow and not consciously controlled. Yet, it is varied for different types of food. For instance, proteins are emptied slower that carbohydrates (Your digestive system and how it works. 2008). The implication is that any toxic content of food may have very limited time to affect the mouth and oesophagus, the potential risk on the stomach or small intestine is impacted by the relative longer time the food stays in these two regions. Therefore, the stomach and duodenum are logically the regions of the digestive tract that may be most affected by any toxic component of foods (Your digestive system and how it works. 2008).

II. Materials And Methods

Collection of Plant Nmaterial

The plant was obtained at Sabon Gari market, Zaria in November, 2012. The plant was identified and authenticated in the herbarium section in the Department of Biological Science, Ahmadu Bello University, Zaria. And then processed and extracted at National Reseach Institute of Technology (NARICT).

Preparation of extract

Cool Water extraction of N. sativa seed was prepared. N. sativa seeds were collected, and pulverized with pestle and mortar. About 100 g of the powder was mixed with 50mls of water. The mixture was made in to several mold and dried in a dessicator for 10 minutes. Each mold was rap and tied in white pieces of cloth and then subjected to pressure using hydrolic machine. The oil was collected in air-tight container and stored in the refrigerator prior to the commencement of the experiment.

Experimental Animals

A total number of 25 young adult Wistar rats of both sexes were purchased from the Department of Pharmacology, Ahmadu Bello University Zaria. The animals were housed in the animal house of the Department of Human Anatomy, Ahmadu Bello University Zaria. The animals were between the ages of six and seven weeks and weighed between 130-180g. The animals were kept and maintained on standard laboratory condition of room temperature, humidity and under twelve hours dark – light cycle. The animals were fed with standard pellet diet and water. The animals were allowed to acclimatize to their new condition for two weeks before the commencement of the experiment.

Experimental Protocol

The 25 animals were randomly divided in to five groups. Each group comprised of 5 rats and each rat in every group was marked for identification. The experimental animals weighed between 130-180 g and were randomly divided in to seven groups. Each group comprised of 5 rats. Group 1 rats were administered normal Saline (volume per body weight) orally. Group 2 rats were administered olive oil 4ml/kg body weight orally. Group 3 rats were administered 4ml/kg body weight of N. sativa oil orally for 2 weeks. Group 4 rats were administered 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally. Group 5 rats were administered 4ml/kg body weight of N. sativa oil plus 4ml/kg body weight CCl4 (30% CCl4 in 70% olive oil) orally.

Sacrifice Of Animals

On day 15, all animals were humanely sacrificed. The stomach and the duodenum were immediately dissected out and fixed in 10% formaldehyde- saline for routine histological technique.

Histological Analysis

The stomach and the duodenum placed in 10% formalin solution, and processed routinely by embedding in paraffin. Tissue sections (4-5 μm) were stained with haematoxylin-eosin and examined under light microscope (Celestron dgital microscope).

III. Result

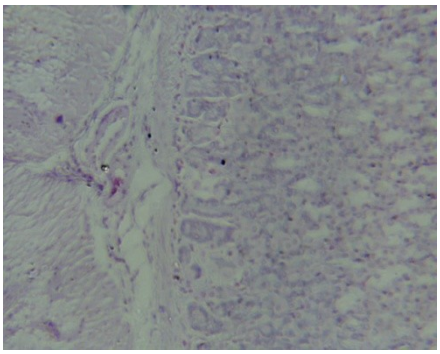

Plate 1:The photomicrograph of the stomach section From the control group showed normal histological Features with the mucosa lined with simple columnar the stomach was well noticed in the control section (H and E x 100).

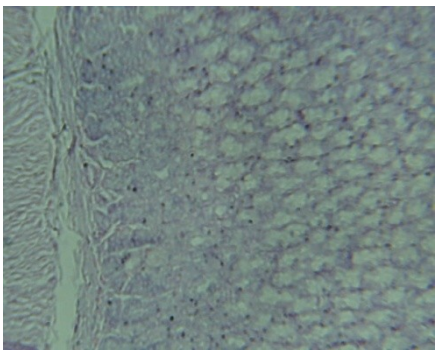

plate 2The photomicrograph of the stomach section from the group 2 (4ml/kg body weight olive oil) showed normal histological features as that of Glandular secretory cells. The muscularis mucosa of the control (H and E x 100). Epithelial cells, lamina propria with some packed

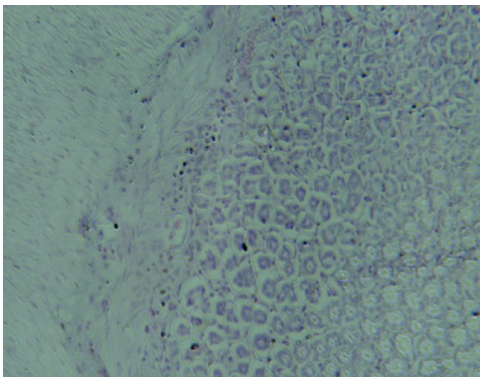

Plate 3: The photomicrograph of the stomach section from the group 3 (4ml/kg body weight of N. sativa) normal histological Features as that of control (H and E x 100).

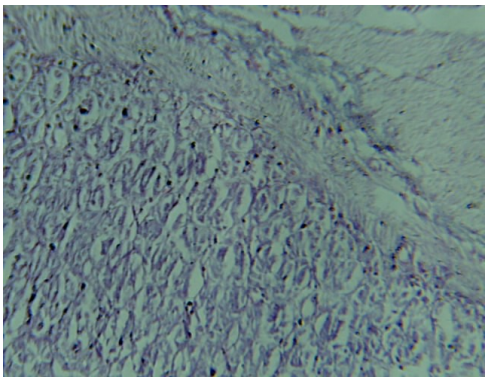

Plate 4: The photomicrograph of the stomach section from the group 4 (30% CCl4 in 70% olive oil) showing high degree distortion in the epithelial Layer, proliferation, ulceration and atrophic Changes (H and E x 100).

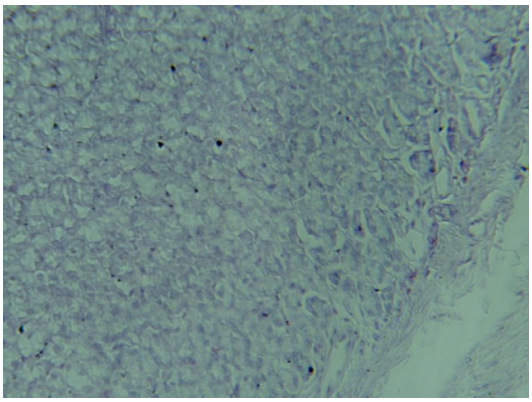

Plate 5: The photomicrograph of the stomach sectio From group 5 (4ml/kg N. sativa + 4ml/kg CCl_4) showed normal histological Features with the mucosa lined with simple columnar Epithelial cells, lamina propria with some packed Glandular secretory cells. (H and E x 100).

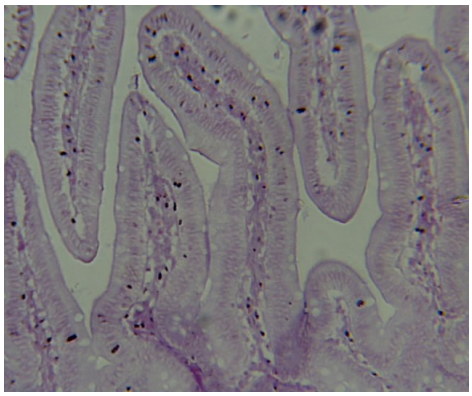

Plate 6: The duodenum section from the control (Control). Showing the presence of group 1 Brunner's gland and other cells lining the Duodenum (H and E x 100)

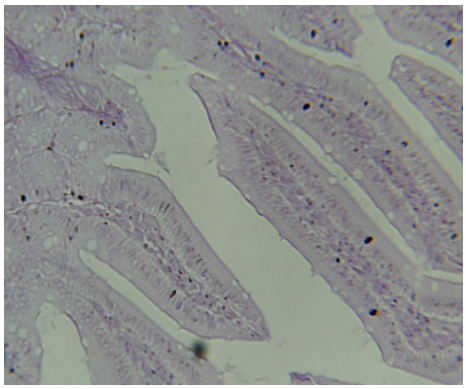

Plate 7: Duodenum section from the control group from the group 2 (4ml/kg body weight olive oil). with the presence of Brunner's gland and other cells lining the duodenum (H and E x 100).

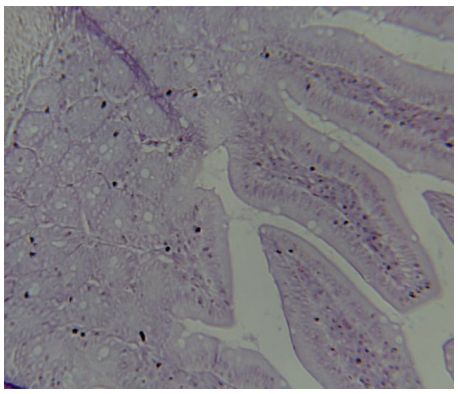

Plate 8: The photomicrograph from the duodenum of from group 3 (4ml/kg body weight of N. sativa). Showing the Brunner's gland and other cells of the duodenum (H and E x 100)

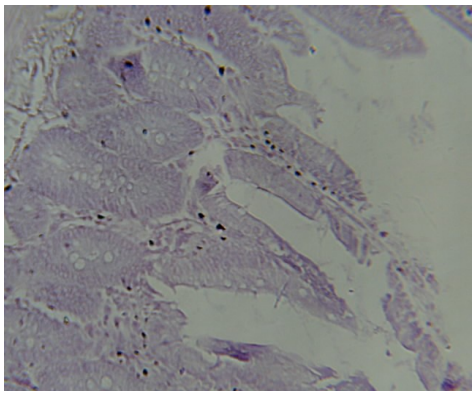

Plate9: The photomicrograph from the duodenum group 4 (30% CCl4 in 70% olive oil). Showing some distortion and disruption in the epithelial lining,which may be suggestive of atrophic(H and E x 100)

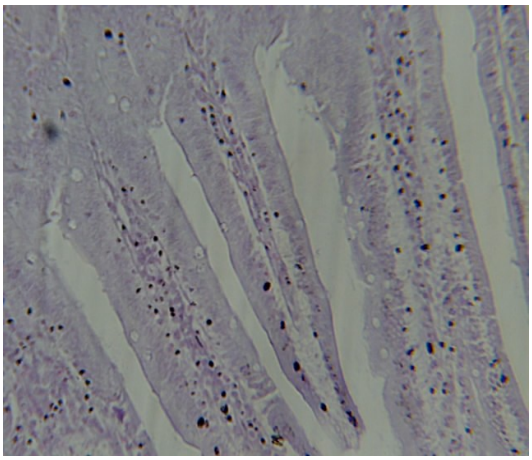

Plate 10: The photomicrograph of the duodenum section From group 5 (4ml/kg N. sativa + 4ml/kg CCl_4) showed Mnormal histological Features with the mucosa lined with simple columnar Epithelial cells, lamina propria with some packed Glandular secretory cells. (H and E x 100).

IV. Discussion

Treatment of animals with CCl_4 is known to cause severe hepatic injury (Terblanche and Hickman 1991).

It has been earlier reported in the literature that decreasing the metabolic activation of carbon tetrachloride, the antioxidant activity, prevention of generation of reactive oxygen species and scavenging of generated free radicals or by combination of these which causes tissue damage are important mechanisms in the protection against CCl_4-induced organ damage (Yutin et al., 1990). Although the antioxidant status of the extract in the stomach and duodenum was not investigated in the current study. The histological photomicrograph of the stomach section from the groups, 1, 2, 3 and 5 showed normal histological features with the mucosa lined with simple columnar epithelial cells, lamina propria with some highly packed glandular secretory cells. The muscularis mucosa of the stomach was well noticed in the control section (plate 1, 2, 3 and 5). The sections of the stomach treated with CCl_4 group (4) revealed some varying degree of distortion in the epithelial layer. There were obvious sign of proliferation, and atrophic changes in the treated stomach sections (plate 4).The duodenum section from the control group 1, 2, 3 and 5 also evidenced normal histological features. There were indications of the presence of Brunner's gland and other cells lining the duodenum (plate 6, 7, 8 and 9). The duodenum treated with P amarus showed signs of erosion and inflammation, which are suggestive of ulcerations. The photomicrograph revealed some distortion and disruption in the epithelial lining, which may be evidence of atrophic duodenitis (plate 10).

The histological findings thus obtained suggest that the extract may be exerting its protective effects by decreasing the metabolic activation of carbon tetrachloride, or by acting as a chain-breaking antioxidant for scavenging free radicals that may cause tissue damage or by a combination of these effects. The results obtained in this study suggest that the extract possesses protective effect on the deleterious effect of CCl_4. Maximum protection was offered by the extract when it was administered at the single, daily oral dose of 4ml mg/kg body weight for 14 days along side with CCl4.

V. Conclusion

It was found that the This study presents histological evidence supporting that N. sataiva oil administration orally has potential protective effect on the microanatomy of the stomach and the duodenum.

Acknowledgment

I wish to acknowledge the technical assistance of the laboratory staff of the Department of Human Anatomy and Pathology, Ahmadu Bello University, Zaria, Nigeria.

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