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# Histopathological changes in rats administered acetylsalicylic acid and ameliorative effect of indole-3-carbinol.

**Eda M.A. Alshailabi**

*Zoology Department, Science Faculty, Omar AL-Mukhtar University, El-Beida, Libya*
*E-Mail: Zudi_2007ly@yahoo.com.*
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## Abstract:

Indole-3-carbinol (I3C) is a major derivative of glucobrassicin (3-indolylmethyl glucosinolate), a plant product common to vegetables of the class Cruciferae. I3C is both an anti-initiator and a promoter of carcinogenesis depending on the timing and dose of administration. This work aimed to evaluate the protective effect and curative role of indole-3-carbinol against acetylsalicylic acid (ASA) induced effects on rat kidney. Male albino rats were divided into four groups of six animals in each group. They were given different experimental inductions of ASA at a dose of 500 mg/kg/body weight and I3C at a dose of 20 mg/kg/body weight either alone or in combination with each other orally for four weeks. The first group (control group) was administrated distilled water for 4 weeks, the second group was administrated ASA, third group was received I3C and fourth group was administrated ASA+I3C. The results of the present study showed that I3C possessed protective activity as evidenced by the inhibition or reduction of histopathological alteration and showed normal histological structure of the glomeruli and tubules at the cortex. The present work provides a strong evidence of I3C produced a permanent protection from ASA induced kidney damage in animal models .

***Key words:*** Acetylsalicylic acid, Histopathological, Indole-3-carbinol, Kidney, Rats**.**

## Introduction:

Acetylsalicylic acid (ASA) is a widely used non-steroidal anti-inflammatory drug (NSAID), but it can damage the gastrointestinal mucosa and may reduce the incidence of thrombotic occlusive events in myocardial infarction and stroke. ASA is known to be rapidly hydrolyzed to salicylate by esterases in the gastrointestinal tract and liver and to a lesser extent in plasma. Non steroidal anti-inflammatory drugs are extensively used as analgesics and anti-inflammatory agents and produce their therapeutic effects through the inhibition of prostaglandin synthesis [1]. Furthermore, at least three different types of nephrotoxicity have been associated with NSAIDs administration [2,3]. These include acute renal failure which occur within hours of a large dose of a NSAID analgesic nephropathy which occurs from chronic consumption of NSAIDs [1]. and interstitial nephritis which is characterized by a diffuse interstitial edema with infiltration of inflammatory cells [3].

A number of natural products found in fruits and vegetables are known to possess anti-mutagenic and anti-carcinogenic properties [4,5]. Cruciferous vegetables are a rich source of many phyto-chemicals, including indole derivatives, dithiolthiones, and isothiocyanates. Indoles are natural compounds that are found in many plants but particularly associated with cruciferous vegetables such as broccoli, cauliflower, cabbage and brussels sprouts. All compounds that contain an indole ring system are indoles. Chemically, they are aromatic heterocyclic organic compounds that have a bicyclic structure consisting of a six-membered ring fused to a five-membered nitrogen-containing pyrrole ring. A beneficial effect of high dietary intake of fruits and vegetables against carcinogenesis is known [6] and an inhibitory effect of indoles and cruciferous vegetables against tumorigenesis and risk of cancers has also been demonstrated [7,8].

Indole-3-carbinol (I3C) is an indole found in some fruits and vegetables, including members of the cruciferous family and, particularly, in members of the genus *Brassica*. I3C is derived from the hydrolysis of glucobrassicin, a glucosinolate, which is predominant in *Brassica* vegetables including broccoli, brussels sprouts, cabbage, cauliflower, collard greens,

kale, kohlrabi, mustard greens, radish, rutabaga and turnip. The stability of glucosinolates is strongly influenced by the presence of external factors, thus the amount of I3C formed from glucobrassicin in foods is variable and depends on the processing and preparation of those foods. I3C is synthesized from indole-3-glucosinolate by the action of enzyme myrosinase [9].

**Aim of study:**

The aim of this work is to assess the potential medicinal value of indole-3-carbinol as one promising anticancer agent, cytoprotective naturally occurring compound found in vegetables of the *Brassica* genus on the histopathological alterations of kidney rats treated with ASA.

## MATERIALS AND METHODS

**Drugs:**

- Indole-3-carbinol (I3C) was purchased from Sigma-Aldrich Chemical Company U.S.A. (Cairo, Egypt). Animals were given (I3C) at a dose of 20 mg/kg/body weight dissolved in distilled water [10] orally.
- Acetylsalicylic acid (ASA) tablets (Bayer AG, Germany) were given to animals in this study at a dose of 500 mg/kg/body weight dissolved in distilled water [11] orally.

**Animals:**

The present work was conducted using healthy adult male albino rats (*Rattus norvegicus*) weighing 140+160 g. The animals were housed in the vivarium of the animal house of Medical Research and Bilharizia center, Faculty of Medicine, Ain Shams University (Cairo. Egypt). Animals housed under standard laboratory conditions with a 12:12 light/dark cycle and a temperature of 23-25ºC. Rats were fed standard laboratory diet and water *ad libitum* with fresh daily supplies. All procedures have been performed in accordance with national animal welfare legislation of Faculty of Medicine, Ain Shams University. They were allowed for 10 days in the pre-experimental period to adapt to the laboratory conditions.

**Experimental groups:**

Animals were fasted about 24 hour with free access to drinking water before starting the experiment. Rats were randomly divided into four experimental groups of six rats in each group as follows:

- Group 1:- Normal control group (received distilled water) orally for 4 weeks.
- Group 2:- ASA group (received ASA at a dose of 500 mg/kg/body weight) orally for 4 weeks.
- Group 3:- I3C group (received I3C at a dose of 20 mg/kg/body weight) orally for 4 weeks.
- Group 4:- ASA+I3C group (received ASA at a dose of 500 mg/kg/body weight with I3C at a dose of 20 mg/kg/body weight) orally for 4 weeks.

**Histopathological studies:**

Specimens of the kidneys from each group were fixed in 10% buffered formalin for 24 h and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 $\mu$ and stained with Hematoxylin and eosin for histological evaluation [12]. Sections were evaluated by light microscopy.

**Results:**

There was no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex were recorded in control group (Fig.1), throughout the whole experimental period, which was showed by hematoxylin &eosin technique (Figures 2,3, 4 and 5).

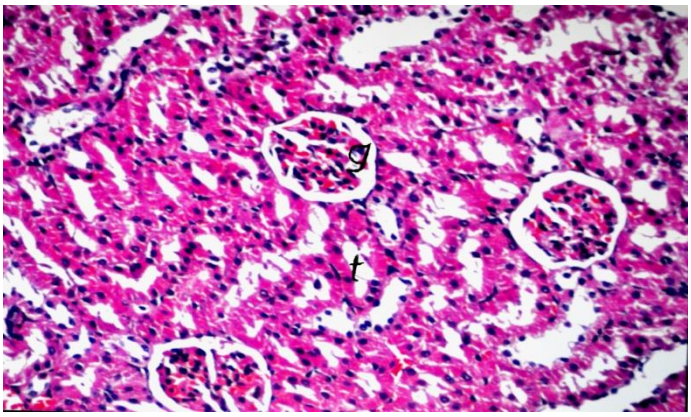


**Figure 1:**

Microscopic appearance of kidney in control group showing normal histological structure of the glomeruli (g) and tubules (t) at the cortex (H&E, x400).

In comparison with control, histopathogical examination of kidney tissue of rats administrating a signal oral dose of ASA for 4 weeks produced sever congestion in the cortex associated with focal fibrosis with inflammatory cells infiltration and eroded areas were noticed (Fig.2). The corticomedullary portion showed focal haemorrhage (Fig.3).

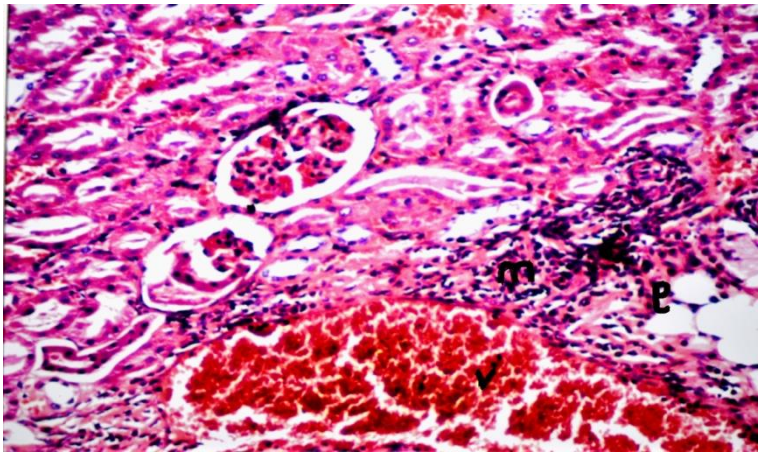


**Figure 2:**

Microscopic appearance of kidney treated with ASA at a dose of 500 mg/kg/body weight for 4 weeks showing sever congestion in cortical blood vessels (v), focal inflammatory cells infiltration (m) with fibroblastic pnlyeration in cortical portion and eroded areas (e) (H&E, x400).

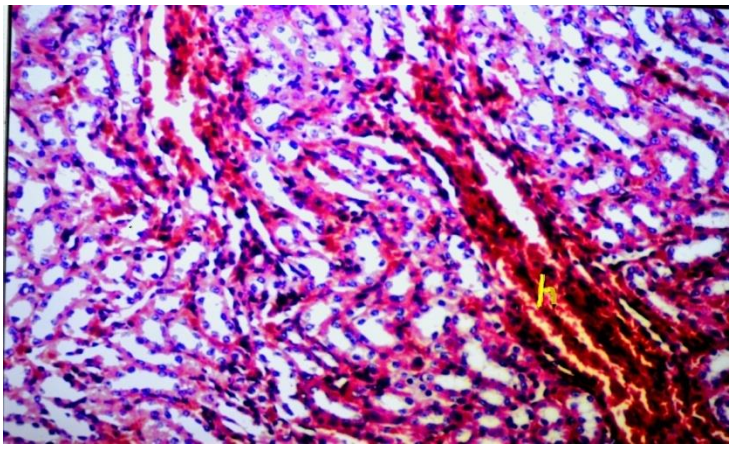


**Figure 3:**

Microscopic appearance of kidney treated with ASA at a dose of 500 mg/kg/body weight for 4 weeks showing focal haemorrhage (h) in corticomedullorry potion (H&E, x400) .

On the other hand, kidneys of rats given I3C showed normal histological structure of the glomeruli and tubules at the cortex and no histopathological alteration (Fig.4). In addition, no evident histopathological changes were seen in kidney of rats treated with ASA + I3C as compared to control group (Fig.5).

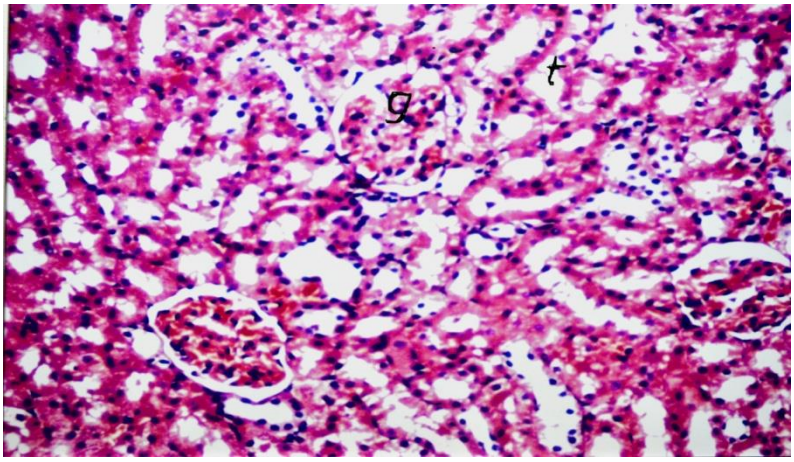


**Figure 4:**

Microscopic appearance of kidney treated with I3C at a dose of 20 mg/kg/body weight for 4 weeks showing normal histological structure (H&E, x400).

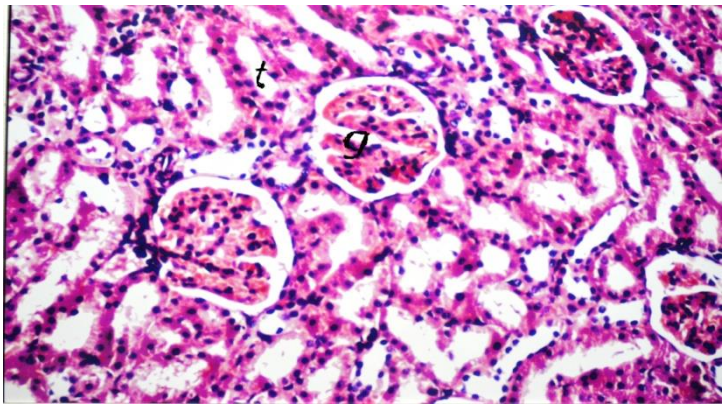


**Figure 5:**

Microscopic appearance of kidney treated with ASA at a dose of (500 mg/kg/body weight) + I3C at a dose of (20 mg/kg/body weight) for 4 weeks showing normal histological structure and no histopathological alteration (H&E, x400).

## Discussion:

The use of non-steroidal anti-inflammatory drugs (NSAIDs) still represents a serious problem due to their side effects, particularly those affecting the gastrointestinal tract. Despite this fact, NSAIDs are widely accepted in daily practice worldwide [13]. Acetylsalicylic acid (ASA), one of the widely used NSAIDs, is probably one of the most highly consumed pharmaceutical products in the world. It has gained greater importance not only as analgesic but also as a cardio-protective drug. However, the use of ASA is also associated with significant morbidity and mortality due to its adverse effects on multiple organ systems [14].

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. So it will be pertinent to examine the possible role of 'free radicals' in disease and 'antioxidants' in its prevention, especially the current status of the subject matter and future prospects. Free radicals are produced basically during cellular metabolism and some functional activities and have essential roles in cell signaling, apoptosis and gene expression. On the other hand, excessive free radical attack can damage DNA, proteins and lipids,

resulting very important diseases. Antioxidants can decrease the oxidative damage by reacting with free radicals or by inhibiting their activity [15]. Moreover, Antioxidants could help to protect cells from damage caused by oxidative stress and enhanced the body's defense systems against degenerative diseases. Administration of antioxidants inhibits ASA-induced tissue injury in rat [16].

The major finding of this study are that administration of ASA for four weeks caused sever congestion in the cortex associated with focal fibrosis with inflammatory cells infiltration and focal haemorrhage in the corticomedullary portion of kidney. These results were found to be in accordance with [17,18]. They explained that, NSAIDs lead to inflammatory cells infiltration which is a major source of a superoxide radical anion that reacts with cellular lipids, forming lipid peroxides metabolized to malondialdehyde (MDA). Also and supporting this findings, [19,20] demonstrated that, inflammation and inflammatory cells infiltration are also important in the pathogenesis of tissue damage induced by ASA.

The results of this study indicated that the effect of I3C with ASA for 4 weeks showed normal histological structure of kidney tissue and no histopathological alteration. These findings strongly support the hypothesis that I3C attenuates ASA-induced inflammatory cells accumulation by inhibiting production of proinflammatory cytokines [21]. The ability of I3C to enhance antioxidant enzymes demonstrates its possible preventative value in the inhibition of free radical reactions. This may be possible by blocking oxidative damage through lipid peroxidation. In addition, I3C prevents loss of membrane permeability and dysfunction of cellular proteins, leading to survival of the functionally active cells [22,23]. I3C could have a unique capacity to block this oxidative damage similar to that shown by $H_2O_2$ scavenger, catalase, indicating its potent antioxidant role to protect DNA from the attack of reactive oxygen species (ROS). Furthermore, protective mechanism of action of I3C as an antiinflammatory drugs is by acting on first phase by inhibiting the mediator of inflammation, probably by inhibiting the platelets activating factor receptors present in the proinflammatory cells like mast cells and neutrophils [24]. I3C treatment was found to preserve the functional cytoarchitecture of the entire kidney tissue. These findings confirm the cytoprotective nature of I3C.

## Conclusions:

In conclusion, it was found demonstrated that rats treated with indole-3-carbinol (20 mg/kg/body weight) manifested no abnormal signs. I3C could significantly protect the kidney tissue against ASA induced injury by the inhibition or reduction of histopathological alteration and showed normal histological structure. This study provides evidence that I3C possesses as an antiinflammatory drugs effect.

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