University of Benghazi
Faculty of Education Al marj
Global Libyan Journal

جامعة بنغازي
كلية التربية المرج
المجلة الليبية العالمية

العدد الثامن - يوليو 2016

Antioxidant properties of indole-3-carbinol on hepatotoxicity induction in experimental animals

Dr. Eda M.A. Alshailabi.

(Zoology Department, Science Faculty, Omar AL-Mukhtar University, El-Beida, Libya)

University of Benghazi
Faculty of Education Al marj
Global Libyan Journal

جامعة بنغازي
كلية التربية المرج
المجلة الليبية العالمية

العدد الثامن - يوليو 2016

Antioxidant properties of indole-3-carbinol on hepatotoxicity induction in experimental animals

الملخص:

استهدفت الدراسة تقييم النشاط الوقائي للاندول-3- كربنول علي تلف الكبد المستحدث بالأسبرين في ذكور الجرذان البيضاء لمدة 7 أيام. وقد تم تقسيم التجربة الي 4 مجاميع (6 جرذان في كل مجموعة) المجموعة الأولى (مجموعة الكنترول) أعطيت الماء المقطر لمدة 7 أيام ، المجموعات الأخرى (2-4) أعطيت الأسبيرين (500 مجم/كجم من الجسم) والاندول-3- كربنول (20مجم/كجم من الجسم) كلا على حدا أو معا ، مرة واحدة في اليوم لمدة 7 أيام متتالية . وقد أظهرت النتائج في هذه الدراسة ان المجاميع المعاملة بالاندول-3- كربنول أنتجت تأثيرا وقائيا بقلة التأثيرات الهستوباثولوجية وظهور الشكل الطبيعي لخلايا الكبد ,حيث قام الاندول-3- كربنول بدور وقائي لأحتوائه على خصائص مضادات الأكسدة بحماية الخلايا الكبدية من التأثير الضار للأسبيرين في الجرذان البيضاء.

Abstract:

Indole-3-carbinol was found to have possible anticarcinogenic, antioxidant and antiatherogenic effects on the organism. The aim of the present study investigation is to evaluate the protective effect of indole-3-carbinol (I3C) against aspirin (ASA) induced effects on rat liver. Male albino rats were randomly divided into four groups of 6 animals in each. The control rats (group 1) were orally administration of distilled water for seven days and the experimental rats (groups 2-4) were treated with 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, once daily for seven consecutive days. Results of the present study showed that groups treated with indole-3-carbinol possessed protective activity possibly as evidenced by the reduction of histopathological alteration and showed normal histological structure of liver. This work suggest that I3C possesses significant hepatoprotective and antioxidant properties on hepatotoxicity induction by ASA in male albino rats.

Key words: Liver, Histopathological, Aspirin, Hepatotoxicity, Indole-3-carbinol, Rats.

Introduction:

A number of natural products found in fruits and vegetables are known to possess anti-mutagenic and anti-carcinogenic properties **(Boone et al., 1990 and Ahmad et al., 2011).** Cruciferous vegetables are a rich source of many phytochemicals, including indole derivatives, dithiolthiones, and isothiocyanates. 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* including broccoli, brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard greens, radish, rutabaga and turnip. These vegetables, are rich in glucosinolate, one of the anti-cancer agents in cruciferous vegetables **(Holst and Williamson, 2004).** Glucosinolate is hydrolyzed by myrosinase (also known as thioglucoside glycohydrolase). Since myrosinase localizes inside plant cells, the cutting and chewing of vegetables release the enzyme and enhance the hydrolysis of glucosinolate. Epidemiological studies suggest high dietary intake of cruciferous vegetables is associated with lower cancer risk, and it is possible that the chemopreventive properties are in part attributable to I3C **(Verhoeven et al., 1996).** Indeed, it has been demonstrated that I3C is an effective chemopreventive agent in rodents, active against a number of carcinogen-induced and spontaneous tumors in multiple tissues, including mammary gland, liver, lung, tongue and nasal mucosa, and endometrium. Many studies have reported that administration of glucosinolate metabolites (isothiocyanate and indol derivatives) and brassica plant extract elevates the phase-I and phase-II enzymes in the rat livers **(Anderton et al., 2004).**

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 **(Abatan et al., 2006).** Furthermore, at least three different types of nephrotoxicity have been associated with NSAIDs administration **(Bach, 1997 and Whelton and Watson, 1998).** 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 **(Abatan et al., 2006).** Aspirin (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 **(Scheon and Vender, 1989).** ASA is known to be rapidly hydrolyzed to salicylate by esterases in the gastrointestinal tract and liver and to a lesser extent in plasma. Aspirin often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication. Aspirin also has an antiplatelet or anti-clotting effect and is used in long-term at low doses to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots **(Julian et al., 1996).** Despite the cardiovascular benefits of aspirin, a potential gastrointestinal harm has been noted in several clinical and preclinical studies. The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. The induction of aspirin is characterized by infiltration of neutrophils, growth factor inhibition and elevation of cytokines, which is produced by activated macrophages **(Konturek et al., 2004 and Sanchez-Fidalgo et al., 2004).** Moreover, many experimental data show a decrease in activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and an increase in production of nitric oxide (NO) after ASA treatment **(Polat and Emre, 2006).**

Aim of study:

The aim of this work is to assess the potential medicinal value of indole-3-carbinol as one promising anticancer agent, hepatoprotective and cytoprotective naturally occurring compound found in vegetables of the *Brassica* genus on hepatotoxicity induction by ASA in male albino rats.

Material & Methods:

Drugs:

1- Indole-3-carbinol (I3C) was purchased from Sigma-Aldrich Chemical Company U.S.A. (Cairo, Egypt).
2- Aspirin (ASA) tablets (Bayer AG, Germany) was obtained from pharmacy.

Experimental animals:

Male albino rats (*Rattus norvegicus*) weighing about 140-160 g were used throughout the experiment. The animals were housed in polypropylene cages with sterile, inert husk materials as bedding. The experimental animals were maintained under controlled environment conditions of light and dark cycle (light 12 h: dark 12 h, temperature 23±2 ˚C). They were allowed to acclimatize for 10 days and were provided a free access to standard pellet diet and water *ad libitum*. Animals were fasted about 24 hour with free access to drinking water before starting the experiment. The experimental protocols were approved by the animal house of Medical Research and Bilharizia center, Faculty of Medicine, Ain Shams University (Cairo. Egypt).

Treatment protocol:

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 seven consecutive days.

Group (2):- ASA group. Rats were administered an oral dose of ASA (500 mg/kg/body weight), once daily for seven consecutive days.

Group (3):- I3C group. Rats were administered an oral dose of I3C (20 mg/kg/body weight), once daily for seven consecutive days.

Group(4):- ASA+I3C group. Rats were administered an oral dose of ASA (500 mg/kg/body weight with I3C at a dose of 20 mg/kg/body weight), once daily for seven consecutive days.

Histopathological examination:

Pieces of the liver were immersed in 10% formalin solution, embedded in paraffin. The sections were made at a thickness of 5*μm* and stained with hematoxilin and eosin **(Lillie, 1954).** Histopathological examination was performed under light microscopy.

Results:

The present study demonstrated that rats treated with aspirin at a signal oral dose (500 mg/kg/body weight) for 7 days showed oedema with few inflammatory cells infiltration in the portal area as well as hyperplasia in bile ducts with congestion in the portal vein (Fig.2&3) compared with control rats that showed normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded in (Fig.1).

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

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 **(Hawkey, 2000).** Aspirin (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 **(Matzke, 1996).**

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 **(Tan et al., 1993).** 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 **(Sathish et al., 2011).**

In this study, oral administration of ASA for 7 days caused oedema with few inflammatory cells infiltration in the portal area as well as hyperplasia in bile ducts with congestion in the portal vein. These results were found to be in accordance with **(Souza et al., 2003 and Odashima et al., 2007).** They explained that, inflammation and inflammatory cells infiltration are also important in the pathogenesis of tissue damage induced by ASA. Similarly, **(Kobayashi, et al., 2001)** suggested that, neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide, and myeloperoxidase derived oxidants as a result they mediate lipid peroxidation. These reactive oxygen species are highly cytotoxic and can induce tissue damage. Furthermore, inflammation in tissue by aspirin is accompanied by increased production of tumor necrosis factor α (TNFα), which augments neutrophil-derived superoxide generation and stimulates production of interleukin-1 (IL-1) leading to neutrophil accumulations **(Kokura et al., 2000)**.

The experimental results of this study indicated that the effect of I3C with ASA for seven days showed normal histological structure of liver 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 **(Jainu et al., 2006).** Indole-3-carbinol (I3C) is found in cruciferous vegetables, including

cabbage, broccoli, cauliflower, Brussels sprouts, Napa cabbage, kale, and mustard. These cruciferous vegetables lower the risk of cancer incidence, which may be due to their high contents of glucosinolates and their derivatives, including I3C **(Wang et al., 2012).** 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 **(Tsai et al., 1998 and Chen et al., 2003).** 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). Moreover, 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 **(Tour and Talele, 2011).** I3C treatment was found to preserve the functional cytoarchitecture of the entire liver tissue. These findings confirm the cytoprotective nature of I3C.

Conclusion:

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 liver tissue against ASA induced injury by the inhibition of histopathological alteration and showed normal histological structure. This study provides evidence that I3C possesses as an effective anti-inflammatory and antioxidant activities against aspirin-induced hepatotoxicity in rats.

References:

Abatan, M.O., Lateef I., and Taiwo V.O., (2006). Toxic effects of non-steroidal anti-inflammatory agents in rats. *African Journal of Biomedical Research.* **9:** 219- 223.

Ahmad, A., Sakr, W.A., and Rahman, K.M.W., (2011). Novel targets for detection of cancer and their modulation by chemopreventive natural compounds. *Bioscience.***7:**100-106.

Anderton, M. J., Manson, M. M., Verschoyle, R. D., Gescher, A., Lamb, J. H., Farmer, P.B., Steward, W. P., and **Williams, M .L., (2004).** Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. *Clinical Cancer Research.* **10:**5233-5241.

Bach, P. H., (1997). The renal medulla and distal nephron toxicity. *Comprehensive Toxicology*.**7:** 279-298.

Boone, C.W., Kelloff, G.J., and Malone, W.E., (1990). Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials. *Cancer Research*. **50:** 2-9.

Chen, Y.H., Dai, H.J., and Chang, H.P., (2003). Suppression of inducible nitric oxide 330 production by indole and isothiocyanate derivatives from *Brassica* plants in 331 stimulated macrophages. *Planta Medica.* **69:** 696-700.

Hawkey, C.J., (2000). Non-steroidal anti-inflammatory drug gastropathy. *Gastroenterology*. **119:** 521-535.

Heber, D., and Bowerman, S., (2001). Applying science to changing dietary patterns. *Journal of Nutrition.* **131:** 3078-3081.

Holst, B., and Williamson, G. (2004). A critical review of the bioavailability of glucosinolates and related compounds. *Natural Prod. Rep.* **21** : 425-447.

Jainu, M., Mohan, V., and Devi, C.S.S., (2006). Gastroprotective effect of *Cissus quadrangularis* extract in rats with experimentally induced ulcer. *Indian Journal of Medical Research.* **123:** 799-806.

Julian, D.A., Chamberlain, S.J. and Pocock, A. (1996). Comparison of aspirin and anticoagulation following thrombolysis for myocardial infarction (the aftrer study): A multicentre unblinded randomised clinical trial. *British Medicine Journal*.**313**: 1429-1431.

Kobayashi, H., Gil-Guzman, E., and Mahran, A. M. (2001). Quality control of reactive oxygen species measurement by luminoldependent chemiluminescence assay. *Journal of Andrology*. **4:** 568-574.

Kokura, S., Wolf, R.E., Yoshikawa, T. and Granger, D.N. (2000). T-lymphocyte-derived tumor necrosis factor exacerbates anoxia-reoxygenation-induced neutrophilendothelial cell adhesion. *Circ. Research.* **86**: 205-213.

Konturek, P. C., Kania, J., Gessner, U., Konturek, S.J., Hahn, E.J., and Konturek, J.W. (2004). Effect of ascorbic acid-releasing acetyl salicylic acid on gastric mucosal damage before and after *H. Pylori* eradication therapy. *European Journal of Pharmacology.* **506**:69-77.

Lillie, R.D., (1954). Histopathological technigues and practical histochemistry. Mc Graw-Hill. U.S.A.

Matzke, G.R., (1996). Nonrenal toxicities of acetaminophen, aspirin and non-steroidal anti-inflammatory agents. *American Journal of Kidney Disease*. **28**: 63-70.

Michaud, D.S., Spiegelman, D., Clinton, S.K., Rimm, E.B., Willett, W.C., and Giovannucci, E.L., (1999). Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *Journal of Nutritional Cancer*. **91:** 605-613.

Odashima, M., Otaka, M., Ohba, R., Jin, M., Wada, I., Horikawa, Y., Matsuhashi, T., Hatakeyama, N., Oyake, J., and Watanabe, S., (2007). Attenuation of gastric mucosal inflammation induced by aspirin through inhibition of selective type III phospshodiesterase in rats. *Dig. Dis. Sci.*, **52**: 1355-1359.

Polat, A. and Emre, M.H. (2006). Effects of melatonin or acetylsalicylic acid on gastric oxidative stress after bile duct ligation in rats. *Journal of Gastroenterology*. **41**:433-439.

Sanchez-Fidalgo, S., Martin-Lacave, I., Illanes, M., Motilva, V. (2004). Angiogenesis, cell proliferation and apoptosis in gastric ulcer healing. Effect of a selective COX-2 inhibitor. *European Journal of Pharmacology*. **505**: 187-194.

Sathish, R., Sahu, A., and Natarajan, K., (2011). Antiulcer and antioxidant activity of ethanolic extract of *Passiflora foetida* L. *Indian Journal of Pharmacology*. **43:** 336-339.

Scheon, R.T., and Vende,r RJ. (1998). Mechanisms of NSAID-induced gastric damage. *American Journal of Medicine*. **86** : 449-58.

Souza, M.H.L.P., Lima, O.M., Zamuner, S.R., Fiorucci, S., and Wallace, J.L., (2003). Gastritis increases resistance to aspirin-induced mucosal injury via COX-2-mediated lipoxin synthesis. *American Journal of Physiological Gastrointestinal and Liver Physiology*. **285**: 54-61.

Tan, D.X., Chen, L.D., Poeggeler, B., Manchester, L.C., and Reiter, R.J., (1993). Melatonin: A potent endogenous hydroxyl radical scavenger. *Journal of Applied Pharmaceutical Science*. **1**: 57-60.

Tour, N.S., and Talele, G.S., (2011). Gastric antiulcer and anti-inflammatory activities of *Calotropis procera* stem bark. *Brazilian Journal of Pharmacology*.**21:** 1118-1126.

Tsai, L.Y., Lee, K.T., and Liu, T.Z.,(1998). Evidence for accelerated generation of hydroxyl radicals in experimental obstructive jaundice of rats. *Free Radical of Biological Medicine*. **24:**732–737.

Verhoeven, D.T.H., Goldbohm, R.A., van Poppel, G., Verhagen, H., van den Brandt, P.A. (1996). Epidemiological studies on brassica vegetables and cancer risk. Cancer *Epidemiol Biomark Prev*. **5**:733-48.

Wang, M.L., Shih, C.K., Chang, H.P. and Chen, Y.H. (2012): Antiangiogenic activity of indole-3-carbinol in endothelial cells stimulated with activated macrophages. *Journal of Food Chemistry*. **2**: 185-188.

Whelton, A. and Watson, A. J. (1998) Non steroidal anti-inflammatory drugs: Effects on kidney interstitial nephritis. *African Journal of Biomedical Research*. **9:** 203-216.