IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 4 Ver. I (Jul. - Aug.2016), PP 63-69
www.iosrjournals.org

Divergent Effects of *Nigella Sativa* Linn and Omebrazole on the Healing Of Gastric Ulcer in Adult Female Albino Rats

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Abstract: *Gastric ulcer is one of the most common gastrointestinal tract diseases, and has affected on humans for centuries. Helicobacter pylori infection, use of drugs such as Nonsteroidal anti-inflammatory drugs (NSAIDs), and stress are some of the known factors that can cause gastric ulcers. NS (Nigella sativa) has been used in traditional medicine. It used for the treatment of a variety of diseases including gastrointestinal disorders. The aim of this study to evaluate the balanced between defensive and aggressive factors in the stomach and investigated the treated effects of NS ethanolic extract against Indomethacin induced gastric ulcer in rats. 48 Rats were divided at random into 6 groups 8 for each, 1st G negative control group treated with CMC 0.5 ml /kg BWt, 2nd NSG treated with NS 150mg/kg BWt, 3rd OG treated with Omebrazole 3 mg/ kg BWt, 4th IG Indomethacin group treated with 30mg / kg BWt, 5th INSG Indomethacin and NS, and 6th IOG Indomethacin and Omebrazole with the same dose. These studies revealed the successful effectiveness of the alcoholic extract of N-sativa seeds in doses of 150 mg/kg daily for two weeks in inducing significant (P< 0.05) treatment against gastric ulcer compared with IG. INSG and IOG showed a significant increase of RBCs x10^6/mm^3, Neutrophils %, Prostaglandin E_2 (PGE_2 ng/l), NO_3 µmol/ml and Reduced Glutathione GSH. and significant decrease of WBCs, HB, Lymphocytes %, PH, Total Acidity, Free Acidity , NO_2 and MDA. Our results suggest that NS extract possesses significant antiulcerogenic actions in gastric ulcer induced by using indomethacin (as NSAIDs) that might be related to its anti-inflammatory and antioxidant activity.*

Keywords: *Rats Gastric Ulcer, Nigella sativa, Omebrazole, Indomethacin.*

I. Introduction

Gastric ulcers are a serious problem in many parts of the World. The aetiology of gastric ulcers is influenced by various factors Ulcers are worsened by inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), stress, hereditary predisposition and infection by *Helicobacter pylori* **(Repetto, 2002).** Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion), prostaglandins, innate resistance of the mucosal cells factors. (**Tripathi, 1999).**Gastric ulceration in man is associated with reduced gastric mucosal prostaglandin E2 (PGE_2) synthesis, this finding which persists despite healing by proton pump inhibitors **(Pugh *et al*., 1989).** The mechanism of ulcerogenesis in the different model has similarities to human model gastric ulcer with increased secretion of gastric acid **(Kirkegaard *et al*., I980),** reduced acid neutralizing capacity **(Leung *et al.,* I985)**, inhibition of gastric alkaline mucosal secretion in response to the increased luminal acid **(Briden *et al*., I985),** impaired gastric microcirculation **(Leung *et al.,* I985; Ikeda and Kitajima I985)** and a decrease in mucus formation **(Shiina *et al.,* I985).** Other changes may be of pathophysiological importance: a dose-related increase in tissue histamine and histidine decarboxylase levels **(Boesby *et al.,* I983)**. Gastric ulcers associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) remain a major clinical problem. NSAIDs inhibit cyclooxygenase by reducing the intrinsic ability of the mucosa to resist injury induced by endogenous and exogenous aggressors. Indomethacin (INDO), a representative of NSAIDs family, causes gastric ulcers through various processes, including generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis **(Bech *et al.,* 2000).** Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which in turn, aggravate the ulcer **(Miller, 1983).**

Several pharmaceutical products have been employed for the treatment of gastroduodenal ulcers and peptic diseases, resulting in decreased mortality and morbidity rates. However they are not completely effective and they produce many adverse effects **(Rates, 2001).** Omeprazole is a proton pump inhibitor which has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion for about 15 years **(Ekstrom *et al.,* 2004).** In recent years, there is growing interest in alternative therapies and the use of natural products, especially those derived from plants **(Rates, 2001; Schmeda-Hirschmann and Yesilada, 2005).** Plant extracts are some of the most attractive sources of new drugs and have been shown to produce

promising results for the treatment of gastric ulcer **(Alkofahi and Atta, 1999; Schmeda-Hirschmann and Yesilada, 2005).** *Nigella sativa Linn* (N. sativa) commonly known as black seed or black cumin, is an annual herb from the botanical family of Ranunculaceae. The seeds of the plant have been used in the Southeast Asia, Middle and Far East as a natural remedy to treat many diseases, including asthma, hypertension, diabetes, hypercholesterolemia, inflammation, arthritis, tumor, gastrointestinal disturbances and gynecological disorders for over 2000 years **(Ali and Blunden, 2003; El-Din *et al.*, 2006 and Ramadan, 2007).** The aims of the present study to determine the efficacy of an extract of *N. sativa* in gastric ulcer induced with indomethacin.

II. Materials And Methods

Drugs and chemicals

Indomethacine (INDO) is powder suspended in 0.5% Carboxy Methyl Cellulose (CMC) for oral administration (30 mg/kg) according to **Halter et al. (2001).** It was purchased from Pfizer company, *Nigella sativa* (N.S): ethanolic extract of N.S, purchased from local market, where needs N.S and crushed as powder for extraction with ethanol alcohol (95v/v)**.,** Omebrazole (OME): is proton pump inhibitors have widely used as acid inhibitors agent for treatment a disorder related to gastric acid secretion. It is powder purchased from Cidco company dissolved in 0.5% CMC for oral administration (3mg/kg) and act as reference drugs according to **Li *et al.* (2004).**

Preparation of Plant

Seeds of *N. sativa* Linn were purchased from the local market and their identification was confirmed. The seeds were dried and crushed into coarse powder which was used for extraction with ethanol alcohol (95% v/v) using Soxhlet apparatus. The extract was evaporated under vacuum. The extractive value (%w/w) of the alcoholic dry extract was 4.25%. N.S constituents were analyzed by gas chromatography (GLC) for glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, tannins, phenolic acids, gums and mucilage using standard procedures of analysis **(Evans, 2002 and Harborne, 2007).**

Experimental Animals

In performing the present work, adult female albino rats Sprague Dawley weighing 150 ±20g were used. Rats were brought from laboratory animal breeding of National Organization of Drug Control and Research (NODCAR) rats were housed under control condition for adaptation one week and fed with standard basal diet formulated in accordance with composition authorized by Association of **Official Analytical chemists (1988),** which consists of about 78.5 % carbohydrate (inclusive of 50 % crude cellulose fiber), 15.2 % protein, 3.2 % lipids, 2.1 % salt mixture and 1 % Multivitamins.

The rats were allowed free access to feeding and drinking *ad libitum*. They were placed in cages of adequate size each comprising 8 animals allowing free spontaneous motility. They were kept through the period of the experiment under properly controlled environmental conditions in the animal house with respect to ambient temperature (22-25 0C) and 12 hours dark and 12 hours light period **(Adeniyi *et al.*,2006 and Bahuguna Yogendra *et al.*, 2008).**

III. Experimental Design

The aim of the present study was to determine the role of N.S seed extract in comparing with OME as reference drug in treatment of gastric ulcer in female adult rats after two weeks. Forty eight rats were fasted over night prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment then food withheld 2h for releasing gastric enzymes, then Pyloric ligation was carried out in each animal before indomethacin administration and ulcer induction to collect gastric juice under light ether anesthesia. Rats were injected orally with a single oral dose of 30mg/kg of indomethacin, and After 6 hr later, rats were divided to 6 groups for treatment of induced ulcer as follow: 1st G negative control group treated with CMC 0.5 ml /kg BWt, 2nd NSG treated with NS 150mg/kg BWt, 3rd OG treated with Omebrazole 3 mg/ kg BWt, 4th IG Indomethacin group treated with 30mg / kg BWt, 5th INSG Indomethacin and NS, and 6th IOG Indomethacin and Omebrazole with the same dose. Rats were treated with the same drugs and doses for two weeks, after that rats were killed and stomach tissues were removed. The stomach was incised along the greater curvature and observed for ulcers **(Bhatnagar et al., 2005; Muralidhran and Srikanth. *et al.*, 2011).**

IV. Analysis Of Gastric Juice

The gastric contents was drained and centrifuged at 3000-4.500 rpm for 10 min. The gastric volume and pH were measured by pH meter **(Moore, 1968).** Free and total acidity were calculated by multiplying gastric juice volume by the measured free and total acid concentrations, respectively **(Hara *et al.*, 1991 and Feldman, 1998).**

Biochemical analysis

Serum Malondialdehyde (MDA) level was measured by the method of **Karatep (2004)** by HPLC. (1978). Nitric oxide (NO) content was determined by HPLC method of **Papadoyannis *et al.*, (1999),** The thiols of reduced glutathione were detected by HPLC using the method of **Jayatillke and Shaw (1993**). Prostaglandin E2 (PGE2) assay was performed with PGE2 enzyme immunoassay kit. The supernatant was used for determination of prostaglandin E2 by using an EIA kit according to supplier's instructions **(**Sigma-Aldrich). At the end of experimental periods, rats were scarified. Blood was collected and hebarinized blood was used for hematological analysis experiment and measurement different hematological parameter as Hb%, RBCs and WBCs total count and differential count **Schalm *et al.*, (1975).**

V. Statistical Analysis

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1989). Significant differences among means were evaluated using Duncan's Multiple Range Test of SAS (1989).

The following linear model was applied: $Yijk = \mu + \alpha i + \xi ij$

Yij = Observation measured

M = Over all mean

αi = Effect of treatment .

ξijk = Experimental error assumed to be randomly distributed ($\sigma^2 = 0$).

VI. RESULTS AND DISCUSSION

Table (1): Antiulcerative Effects of *Nigella sativa* (mg / kg B.W) and other antiulcer drugs Omeprazole (mg/kg B.W) on blood WBCs x 10^3/ml, RBCs x 10^6/mm^3, Neutrophils%, Lymphocytes% and Hb g/dl against indomethacine induced gastric ulceration in Rats .

	Parameters					
Interval	**Groups**	**WBCs x 10^3/ml**	**RBCs x 10^6/mm^3**	**Neutrophils %**	**Lymphocytes %**	**Hb g/dl**
After 2 weeks of Treatment	**CMC**	**5.56 ± 0.24**	**5.26 ± 0.21**	**35.21 ± 1.76**	**52.43 ± 1.95**	**12.38 ± 0.46**
	OME	**4.52 ± 0.16**	**5.12 ± 0.22**	**37.66 ± 2.11**	**49.89 ± 2.11**	**12.50 ±0.46**
	NS	**4.91 ± 0.19**	**4.99 ± 0.22**	**35.40 ± 2.51**	**52.34 ± 2.61**	**12.63 ±0.50**
	INDO	**6.92± 0.13^a**	**4.49 ± 0.22^a**	**29.54 ± 1.91^a**	**58.15 ± 1.91^a**	**10.29±0.45^a**
	INDO+OME	**5.68 ± 0.34^b**	**5.36 ± 0.23^b**	**35.89 ± 1.36^b**	**51.79 ± 1.28^{ab}**	**12.37±0.54^b**
	INDO+NS	**6.21 ± 0.35^{ab}**	**5.23 ± 0.29^b**	**34.18 ± 1.47^b**	**51.90 ± 1.57^{ab}**	**13.11±0.58^b**

- Data represents the means ± SEM.
- a significantly different from control CMC group at $P < 0.05$
- b significantly different from INDO group against (INDO+OME and INDO+NS) at $P < 0.05$.

Data presented in Table (1) recorded the effect of NS and Omeprazole on serum WBCs, RBCs, Neutrophils%, Lymphocytes% and Hb g/dl against indomethacine induced gastric ulceration in Rats.

INDO produce gastric ulcer with significantly increase in total serum WBCs, Lymphocytes%, however decrease in RBCs, Neutrophils%, and Hb g/dl ($P < 0.05$) as compared with control and there are no difference between two +ve control groups (NS and OME) against--ve control (CMC) . On the other hand after treatment for two weeks with OME and NS as compared with INDO showed a significant increase in RBCS, neutrophil and Hb but they induced a significant decrease in total leukocytes ,lymphocyte.

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers and may produce loss of blood causing hemorrhagic anemia from gastrointestinal tract **(Pratt, 1992).** This precipitated low Hb concentration and RBC count which causing low capacity to carry oxygen with the resultant anoxia cyanosis and respiratory acidosis which in turn caused the death of the rats within a short period of time **(Makinde, 1991).** Also there is increase in the total WBC following hemorrhage this according to (**Bush 1991)**. This increase in total WBCs count may be due to the increased hemopoitic activity as a result of the hemolysis of RBCs So This result is compatible with **Gilman et al (1985**) where indomethacin inhibits the motility of polymorphonuclear leukocytes.

As far the neutrophils there was a relative decrease in this parameter which might have been due to stress leading to demargination in circulation **(Bush 1991).** There was a relative decrease of neutrophil in blood stream and increase of neutrophil infiltration into ulcerated gastric tissue. **Fujita et al. (1998)** observed that an increase in neutrophil infiltration into ulcerated gastric tissue delayed the healing of gastric ulcers in rats this according to **Shimizu et al. (2000).** INDO would extensively damage the gastric mucosa leading to increased neutrophil infiltration into the gastric mucosa. Oxygen-free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory effect on gastric ulcers healing in rats **(Suzuki *et al.*, 1998 and Su *et al.*, 2002)**.Also neutrophils mediate lipid peroxidation through the production of superoxide anions. In addition

neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants **(Zimmerman *et al.*, 1997).**

It also need to be stressed that there was increasing in lymphocytes following administration of INDO to the rats showed lymphocytosis and monocytosis as a result of severe stress that the animals were subjected. This may be due to the production of specific or non specific antibodies against different antigens, since lymphocytes are responsible for achieving the defense mechanism in the body **(Cheng and Koo, 2000).**

NS might improve and ameliorate some disturbed hematological parameters of ulcerative rats **(Al-Jishi *et al.*, 2000). Aqel and Shaheen (1996**) they found that low dose of N.S extract caused significant increase in Hb, PCV and RBCs, also iron is essential for bacterial invasion to the body in which, iron is associated with iron binding protein transferring, lactoferrin, heptaglobin and feritin, hence N.S. eliminate iron from the body fluid to be conjugated with proteins. In addition **Hedaya (1995)** showed that NS made relaxation of circular muscles may protect the gastric mucosa and prevent hemolytic anemia and increase RBCs count and HB% also promote WBC and neutrophils to control level which are compatible with our result. In addition NS prevent lymphocytes apoptosis **(Mandor *et al.*, 1998).** In addition NS has antibiotic action and acts as immunoenhancer. This indicates that the treatment with NS affect the defense mechanism and immune response to inhibits the inflammation resulting from INDO treatment **(Pal *et al.*, 2001 and Zauoi *et al.*, 2002).**

Table (2): Effect of *Nigilla sativa* (mg / kg B.W) in comparing with anti ulcer drugs Omeprazole (mg/kg B.W) on gastric mucosa PH, Free acidity and Total acidity, against peptic ulcer by Indomethacin.

	Parameters			
Intervals	**Groups**	**PH**	**Free acidity**	**Total acidity**
After 2 weeks of Treatment	**CMC**	**2.80 ± 0.13**	**12.46 ± 0.40d**	**22.14 ± 1.15**
	OME	**3.11 ± 0.21**	**11.82 ± 0.44**	**19.98 ± 0.99**
	NS	**3.18 ± 0.21**	**12.31 ± 0.54**	**22.27 ± 1.08**
	INDO	**2.59 ± 0.15[a]**	**50.01 ± 1.03[a]**	**23.17 ± 0.88[a]**
	INDO+OME	**2.73 ± 0.12[a]**	**29.38 ± 0.86[ab]**	**24.43 ± 1.05[a]**
	INDO+NS	**3.15 ± 0.17[b]**	**27.13 ± 0.95[ab]**	**47.69 ± 0.99[ab]**

- Data represents the means ± SEM.
- a significantly different from control CMC group at $P < 0.05$
- b significantly different from INDO group against (INDO+OME and INDO+NS) at $P < 0.05$.

Data presented in Table (2) recorded the treatment effect of NS and Omeprazole on pH, free and total acidity in gastric mucosal of female albino rats.INDO produce gastric ulcer with significantly increase in pH, free and total acidity ($P < 0.05$) as compared with control in gastric mucosal of female albino rats. On the other hand the treatment with OME and NS as compared with INDO showed a significant decrease in this parameter.

The major aggressive factor responsible for ulcers is the content of acid present in gastric juice **(Grossman, 1978).** When the concentration of hydrogen ions in gastric juice decreases, that reflects of high pH and a decrease in mucous and bicarbonate secretion **(Tennkton *et al.*, 1991).** The genesis of ulcer and gastric damage is facilitated by hydrogen ions hence increase in free and total acidity **(Lüllmann *et al.*, 2000).** OME is highly selective for the proton pump inhibitor and undergo catalyzed conversion into active form within the acid forming space. The active inhibitors react with SH (thiol) group of the proton pump, resulting in inhibition of acid formation (**Nagaya, 1991; Wallace and Granger, 1996**). Also OME is substituted benzimidazoles and it inhibits acid secretion by acting on the hydrogen-potassium exchanger (H+: K+-ATPase) for the apical plasma membrane of the gastric mucosa **(Satoh, 1989 and Li *et al.*, 2004).**

From these results, NS showed the antiulcer action that exerted by their purified fraction thymoqunion inhibition of gastric aggressive factors i.e. acid and pepsin because ability to interfere with the indomethacin–induced inflammatory effects which is in accordance with **Alghamdi (2001)**

Table (3): Effect of *NS* (mg / kg B.W) in comparing with anti ulcer drugs Omeprazole (mg/kg B.W) on serum MDA nmol/ml, GSH nmol/ml, NO_2µmol/l, NO_3 µmol/l and PGE2 ng/l against peptic ulcer by Indomethacine

	Parameters					
Intervals	**Groups**	**MDA nmol/ml**	**GSH nmol/ml**	**NO_2µmol/l**	**NO_3µmol/l**	**PGE2ng/l**
After 2 weeks of Treatment	**CMC**	**0.99 ± 0.05**	**85.35 ± 4.91**	**27.87 ± 1.31**	**16.66 ± 0.90**	**276.0 ± 5.14**
	OME	**1.12 ± 0.06**	**91.74 ± 5.31**	**25.91 ± 1.81**	**16.29 ± 0.77**	**275.6 ± 5.02**
	NS	**0.99 ± 0.06**	**89.33 ± 4.10**	**28.05 ± 1.72**	**17.04 ± 0.71**	**265.7 ± 4.28**
	INDO	**2.79 ± 0.05[a]**	**69.48 ± 3.71[a]**	**28.05 ± 1.29[a]**	**18.38 ± 0.73**	**209.9 ± 5.43**
	INDO+OME	**1.87 ± 0.08[ab]**	**86.58 ± 4.51[b]**	**32.28 ± 1.49[ab]**	**17.75 ± 0.79**	**263.5 ± 5.69[b]**
	INDO+NS	**1.93 ± 0.07[ab]**	**90.16 ± 4.99[b]**	**30.35 ± 1.62[ab]**	**19.18 ± 0.82**	**244.2 ± 4.28[ab]**

- Data represents the means ± SEM.
- a significantly different from control CMC group at $P < 0.05$

- b significantly different from INDO group against (INDO+OME and INDO+NS) at $P < 0.05$.

Data presented in Table (3) recorded the treatment effect of NS and Omeprazole on PGE_2, and oxidative stress parameter (GSH, NO_2, NO_3, MDA) in serum rats

INDO produce gastric ulcer with significantly increase in lipid peroxidation (MDA), however decrease in GSH, PGE_2, NO_2, NO_3 as compared with control. On the other hand after treatment for two weeks with OME and NS as compared with INDO showed an ameliorate effects in this parameters.

The ulceration induced by indomethacin is attributed mainly to various processes, including generation of oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis **(Bech *et al.*, 2000).** These free radicals also damage the cellular antioxidant enzymes such as glutathione peroxidase, SOD and others, acting as the first line of cellular defense against oxidative injury which might contribute to mucosal injury **(Chattopadhyay *et al.*, 2006**) and lead to aggravated tissue damage during stomach ulceration **(El-Missiry *et al.*, 2001).**

The results of this study are in line with these previous data. Also Indomethacin produces damage to key biomolecules such as lipids. This was apparent from the stimulated lipid oxidation leading to increased accumulation of MDA as well as reduction in the gastric activity of GSH (**Lanas *et al.*, 2000).**

Several studies have demonstrated the importance of endogenous NO in the protection of gastric mucosa (**Kim and Kim, 1998; Tanaka *et al.*, 2001 and Whittle *et al.*, 1990).** NO formed by endothelial NOS plays an important role in the modulation of gastric mucosal integrity by interacting with sensory neuropeptides and endogenous prostaglandins **(Whittle *et al.*, 1990).** A previous report has shown that NO protected against indomethacin-induced gastric ulceration through maintenance of mucosal blood flow and reduction of leukocyte–endothelial cell rolling and adherence **(Calatayud *et al.*, 1999**). In the present study, indomethacin significantly reduced gastric mucosal NO level compared to control group. These findings are in accordance with Trip and Tepperman who reported a decrease in NO biosynthesis, as a result of decreased NOS activity that was associated with an increase in the extent of damage (**Tripp and Tepperman, 1995).**

In addition decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which, in turn, aggravate the ulcer **(Miller, 1983). Hiruma-Lima et al. (2006)** demonstrated that prostaglandin, is a key molecule that stimulates the complex array of ulcer healing mechanism, gets synthesized in the mucosal cells by cyclooxygenase (COX) enzymes. Also stimulates the secretion of biocarbonate and mucus, maintains mucosal blood flow and regulates mucosal turn over and repair. Suppression of prostaglandins synthesis by indomethacin results in increase susceptibility of stomach to mucosal injury and gastric ulceration through the inhibition of the cyclooxygenase enzymes **(Rainsford, 1987).** particulary PGs increase mucosal blood flow ,promote mucous secreation ,and increase bicarbonate secreation. As shown in the present results, OM treatment significantly reverted the indomethacin-induced changes in MDA and GSH. This significant reduction in MDA levels along with significant increase in GSH level suggest decreased lipid peroxidation and antioxidant activity of OM.. Proton pump inhibitors promote healing mechanism of gastric mucosa via antisecretory mechanism of PGE_2 by exerting some effects on COX expression in not only non steroidal inflammatory drugs ulcer but also in common ulcers **(Bush, 1991).**

NS provided a marked suppression of oxidative damage due to its excellent radical scavenging capacity; it brought MDA level closer to normal levels **(Okabe *et al.*, 1997).** Thimoqunione(TQ) which the main constituents of nigella sativa and its metabolite dihydrothymoquinone have an important role in lipid peroxidation and scavenging free radicals (antioxidant properties) according to (**Mansour *et al.*, 2002).** TQ can react non enzymatically with GSH, NADH and NADPH to form glutathionyl-dihydrothymoquinone, after rapid reaction with GSH, and dihydrothymoquinone after slow reaction time with NADH and NADPH, offering thus, an evidence for powerful free radical scavengers, even more than TQ it self **(Khalife and Lupidi, 2007).**There fore, when these metabolites are formed they can remove superoxide anions, as well as other free radicals formed, an effect that can spare the endogenous antioxidant defense molecules, GSH and SOD, and prevent lipid peroxidation. **(Brzozowski *et al.*, 2000).**On the other hand, TQ by reducing gastric oxidative injury increases bioavailability of mucosal defense systems, including GSH, SOD and NO. All these mechanisms finally maintain normal gastricmucosal barrier integrity **(Zaman *et al.*, 2004).**

In conclusion, Indometacin which induces gastric ulceration can be protected with NS due to its antioxidative and anti-inflammatory enhancing properties also through inhibition of more gastric acid. The mechanism of its gastroprotective activity may be attributed to reduction in pH, free and total acidity, ,total leukocytes ,lymphocytes and lipid peroxidation (MDA), with elevate in RBCs, HB%, neutrophils, GSH, NO, and PGE2 on serum, blood and gastric mucosa. The presence of thymoqunione phytoconstituent in this medicinal plant particularly might be responsible for these pharmacological actions.

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