**Tocotrienol - Rich Fraction and its Effects on Parameters Affecting Gastric Mucosal Integrity after a Single Exposure to Indomethacin**

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**Abstract:** The effect of treatment with a tocotrienol-rich fraction (TTRF) on acute single exposure to indomethacin was investigated. Forty-eight male rats of the Sprague-Dawley (200-250g) species were randomly assigned into two groups (N and T). The N group was fed with a commercially prepared normal rat diet and the T group was fed with an identical diet enriched with TTRF 150mg/kg diet. Each group was further subdivided into two subgroups that was either challenged (NI and TI) or not challenged with indomethacin (NX and TX). After eight weeks of treatment the NX and TX rats were killed and the stomachs isolated whereas the NI and TI rats were challenged with a single dose of indomethacin (80mg/kg body weight) orally and after six hours the rats were killed. Measurements for malondialdehyde (MDA), glutathione content, PGE₂, gastric acid concentration and gastric adherent mucous (GAM) were done. Gastric PGE₂ content and acid concentration were comparable in the NI and TI groups compared to its corresponding group that was not challenged. The gastric MDA content and GAM concentration were increased in the NI and TI compared to its corresponding group that was not challenged. This indicated that indomethacin increased MDA and treatment with TTRF could not inhibit the rise of MDA whereas TTRF has no effect on GAM concentration. The glutathione ratio was however, only elevated in the TI group compared to the TX, which indicates that in acute mucosal injury by indomethacin, TTRF is able to preserve the ratio of the endogenous antioxidant. We conclude that TTRF has beneficial effects on gastric parameters.

**Keywords:** TTRF, indomethacin, MDA, glutathione, PGE₂, gastric acid, gastric adherent mucous

**Introduction**
The non-steroidal anti-inflammatory drugs (NSAIDs) have been among the most widely used drugs and although the non-selective NSAIDs have been notorious in causing gastrointestinal lesions, the mechanism of how these lesions are generated have remained obscure. The emergence of the selective NSAIDs is indeed a breakthrough as it ameliorates the gastrointestinal side effects. The use of the non-selective NSAIDs is still popular due to its relatively cheap cost. Hence, studies exploring the possibilities of preserving gastric mucosal integrity during treatment with non-selective NSAIDs are still being conducted (Sonata et al., 1994; Taha et al., 1996; Havely et al., 1998).

The therapeutic effects and major toxic side effects of NSAIDs have been attributed to the ability of these drugs to inhibit the synthesis of prostaglandin (PG), through a direct action on prostaglandin H synthetase, which serves both as a cyclooxygenase (COX) and as a peroxidase (Davies and Wallace, 1997). PGs increase both the synthesis and the release of gastric mucous while NSAIDs has the opposite effects. Free radicals production by NSAIDs is among the more probable mechanism suggested that disrupt the gastric mucosal integrity (Ali et al., 1996; Granger et al., 1986). The body has endogenous antioxidant, which under normal conditions is adequate to protect the organs. In situations that differ from normal such as exposure to noxious stimuli, vulnerable organs such as the lung, liver and stomach need a high level of nonprotein sulphhydrals (mainly reduced glutathione) to maintain integrity (Kosover and Kosover, 1978; Boyd et al., 1979). In such situations, exogenous antioxidant may prove to be beneficial.

Alpha tocopherol (vitamin E) is a naturally occurring antioxidant in the biological systems and is present in the cell membrane of various tissues including intestine and stomach (Granger et al., 1986). The biological activity of vitamin E is generally believed to be due to its antioxidant action rendering it capable to inhibit lipid peroxidation in biological membranes by scavenging the chain propagating peroxyl radicals, thus blocking the free radical chain reaction. Tocotrienol, the vitamin E that may be obtained from palm oil has been shown to be a better antioxidant (Serbinova and Packer, 1994; Afaf and Applequist, 1986). In our efforts to search for avenues to minimise disturbances in the gastric environment due to NSAIDs, this study is carried out to determine the effects of TTRF on important gastric parameters after exposure to indomethacin. This study also investigated the effects of a single exposure of indomethacin on the same parameters.

**Materials and Methods**
In this study, forty-eight male rats of the Sprague-Dawley (200-250g) species were randomly assigned into two groups (N and T). The N group was fed with a commercially prepared normal rat diet and the T group was fed with an identical diet enriched with TTRF 150mg/kg diet. The TTRF enriched diet was prepared by dissolving 150mg of palm oil in a sufficient amount ofacetone, pouring it over 1 kg of rat pellet and allowing the acetone to evaporate. The normal rat pellets were treated with acetone only. Each group was further subdivided into two subgroups that was either challenged (NI and TI, each n = 12) or not challenged with indomethacin (NX and TX, each n = 12). After an eight-week study period the NX and TX rats were killed and the stomachs isolated. Whilst the NI and TI were first challenged with a single dose of indomethacin (80mg/kg body weight) orally and were killed only after six hours post challenged. Of the twelve rats in each group, measurements for MDA, glutathione content, PGE₂, and gastric acid concentration were done in six rats while the remaining stomachs (n = 6) were used for the analysis of gastric adherent mucous.

The lower end oesophagus and pylorus were clamped and the stomach was removed. Gastric tissue MDA content was measured using a modified method described by Ledw czyw et al., 1988. The gastric tissue was homogenised in distilled water, centrifuged and the diluted supernatant was added with trichloroacetic acid. After 15 minutes at room temperature, thioarbituric acid was added and the samples were incubated in 100°C water bath for 30 minutes. After cooling, n-butanol was added and the absorbancy of the upper phase was read. Gastric glutathione content was measured using a well-established method (Griffith, 1980). The gastric tissue was homogenised in 4 volumes of 5% TCA/0.01N HCl and centrifuged at 17000 X g for 15mins at 2°C. The supernatant was separated for GSH and GSSG assay. The ratio for reduced glutathione to oxidised glutathione
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Fig. 1: Effects of TTRF and indomethacin on gastric MDA content. There was an increase in the gastric MDA content after challenged with indomethacin for both groups (NI and TI) compared to their respective controls (NX and TX) (P = 0.002).

Fig. 2: Effects of TTRF and indomethacin on glutathione ratio. There was an increase in the glutathione ratio in the treated group that was challenged with indomethacin (TI) (P = 0.015) compared to the corresponding control (TX). The glutathione ratio in the normal challenged group (NI) did not differ from the normal unchallenged group (NX).

Results

Effect on gastric malondialdehyde (MDA) content: The effects of TTRF and indomethacin on gastric MDA content are shown in Fig. 1. There was an increase in the gastric MDA content after challenged with indomethacin for both groups (NI and TI) compared to their respective controls (P = 0.002). There was a 3.5 fold increment in MDA content for the NI group and 3 fold increment for the TI group compared to the treated, unchallenged group. On a percent basis, the increment in MDA is smaller in the treated group (TI).

Effect on glutathione ratio: The effects of TTRF and indomethacin on glutathione ratio are shown in Fig. 2. There was an increase in the glutathione ratio in the treated group that was challenged with indomethacin (TI) compared to the corresponding control (TX). The glutathione ratio in the normal challenged group (NI) did not differ from the normal unchallenged group (NX).

Effect on gastric tissue content of PGE: The effects of TTRF and indomethacin on gastric tissue content of PGE are shown in Fig. 3. The gastric tissue content of PGE in the NI group did not differ from the unchallenged group (NX). There was also no difference in the gastric tissue content of PGE in the treated group whether or not the rats were challenged with indomethacin. This also demonstrated that indomethacin did not have an effect on gastric tissue content of PGE, as the content remain unchanged in the untreated group (NI), compared to whether or not challenged (NI vs NX).

Effect on gastric acid concentration: The effects of TTRF and indomethacin on gastric acid concentration are shown in Fig. 4. The gastric acid concentration in the NI group did not differ from the unchallenged group (NX). Similar observations were made in the groups treated with TTRF that is there was no difference in the gastric acid concentration in the TTRF group whether or not the rats were challenged with indomethacin. This also demonstrated that indomethacin did not have an effect on gastric acid concentration as the concentration remain unchanged in the untreated group (NI), compared to whether or not challenged (NI vs NX).
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Fig. 4: Effects of TTRF and indomethacin on gastric acid concentration. There were no differences in the gastric acid concentration in the normal diet and TTRF group whether or not the rats were challenged with indomethacin.

Fig. 5: Effects of TTRF and indomethacin on gastric adherent mucous quantity. There was an increase in gastric mucous quantity after challenged with indomethacin for both normal (NI) and the TTRF groups (TI) (P = 0.041 and P = 0.002 respectively) compared to their corresponding controls.

Effect on gastric adherent mucous quantity: The effects of TTRF and indomethacin on gastric adherent mucous quantity are shown in Fig. 5. There was an increase in gastric mucous quantity after challenged with indomethacin for both normal (NI) and the TTRF groups (TI) (P = 0.041 and P = 0.002 respectively) compared to their corresponding controls.

Discussion
Just as many factors are involved in the maintenance of gastric mucosal integrity, factors causing the injury are also many and diverse. Some common identified aggressive factors include smoking, drugs such as NSAIDs and steroids. Helicobacter pylori, acid and pepsin. Although the gastric mucosa has protective factors such as adherent mucous layer, bicarbonate, phospholipids, prostaglandin and antioxidants, there are situations whereby these protections are breached. Given the widespread use of NSAIDs and their adverse effects on the gastric injury, studies such as the current one is indeed required.

Studies have shown that free radicals are involved in the development of mucosal damage by NSAIDs (Phan et al., 1997; Naiero et al., 1996). These excessive free radicals induce lipid peroxidations which is believed to be an important cause of destruction and damage to the gastric cellular membrane. In the current study, we found that MDA was increased after challenged with indomethacin. Elevated gastric MDA reflects an intensification of lipid peroxidation process. Antioxidants such as TTRF used in this study is expected to retard lipid peroxidation process but this was not the case in our study. Even though TTRF is unable to inhibit the rise of MDA, on a percent basis the increment in MDA is smaller in the treated group (TI) compared to the untreated group (NI). Amongst the factors causing the lack of antioxidant effects of TTRF is the dose of indomethacin used. If, in fact the dose of indomethacin used is high and indomethacin increases the production of free radical, it is highly possible that the amount of TTRF used is insufficient to scavenge the excessive free radical. Hence, it is possible to increase the dose of TTRF in future studies. In contrast to the findings on MDA, interestingly we found that another indicator of antioxidant status that is the glutathione ratio, increased in the TTRF group that was exposed to indomethacin. Similar changes were not seen in the TTRF group that was not challenged with indomethacin. These observations suggest that TTRF on its own does not increase glutathione synthesis or its production. The ratio is enhance, however only after exposure to indomethacin which indicates that TTRF is able to scavenge the free radical and this reduce the consumption of reduced glutathione (GSH).

Studies have shown that chronic exposure to indomethacin suppressed the gastric prostaglandin synthesis (Redfern et al., 1987; Srorock and Rees, 1992). In this current study, there was no difference in gastric PGE2 content. It is therefore evident that a single dose of indomethacin does not inhibit prostaglandin synthesis after a single exposure. Complete inhibition of COX leading to reduce in PGE2 content will consume a much longer duration and will probably not be seen in a single dose only after 8 hours. The PGE2 measured is most probably the pre-formed PGE2. Chronic treatment with indomethacin may lead to irreversible inhibition of PGE2, in which case the reduced in PGE2 coupled with lipid peroxidation may caused sustained gastric injury.

Acid an aggressive factor that will ultimately leads to gastrointestinal lesions. Current treatment of the GI lesions employs anti secretory agents that is the H2 receptor antagonist, proton pump inhibitor and antisecretaric agent. This study showed that there was no significant change in concentration after exposure to a single dose of indomethacin either in the normal or the TTRF treated group. Previous studies done (Feldman and Celebrin, 1984; Wagner et al., 1995) showed that long term exposure to indomethacin led to a significant increase in mean gastric acid concentration. Exogenous prostaglandin has been shown to inhibit basal and stimulated acid secretion in man and animals (Levine and Schvartzet, 1984). As mentioned above, our study showed no changes in the gastric PGE2 content. This might explained why there were also no changes in the acid secretion after exposure to indomethacin. After indomethacin exposure, there was increased in gastric adherent mucous content whether or not groups were treated with TTRF. A previous study done also showed that TTRF does not stimulate gastric mucous production (Nafeezza et al., 2000).

We found from this study that TTRF has no effect on PGE2 content, gastric acid and GAG concentration. TTRF was not able to inhibit the rise of MDA content by indomethacin but able to preserve the ratio of the endogenous antioxidant that is the glutathione. We conclude that TTRF has beneficial effects on gastric parameters.

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