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(RESEARCH ARTICLE)



Dihydroquercetin (taxifolin) attenuates dexamethasone activity on prostaglandin E-2 but potentiates thromboxane-A2 action in gastric acid secretion in wistar rats

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Abstract

The activity of dexamethasone and taxifolin {(2R, 3R)-2-(3, 4-Dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one} supplementation on prostaglandin E2 and thromboxane A2 in gastric acid secretion and anti-ulcer was studied. Twenty male Wistar rats (180g-200g body weight) were used. The rats were randomly selected into four groups containing 5 rats each. Group 1 was the control group fed on normal rat feed. Group 2 received 3mg/kg of Dexamethasone (intraperitoneally) at one day interval. Group 3 received 3mg/kg of Dex. intraperitoneally and 1mg/kg body weight of taxifolin orally while group 4 received 1mg/kg body weight of taxifolin. At the end of 6 weeks, basal and peak gastric acid output was measured by continuous perfusion of rats stomach under anaesthesia with normal saline at the rate of 1ml/min. Gastric acid, mucus secretion, ulcer index, PGE-2 and thromboxane A2 activity were determined according to standard procedures. Results showed a significantly ($p < .05$) decreased prostaglandin and mucus secretion level and a raised thromboxane concentrations and gastric acid output in dexamethasone administration. Taxifolin significantly ($p < .05$) lowered thromboxane A2 concentration in Dex treatment while increasing the prostaglandin E2 level. We conclude that Taxifolin decreases dexamethasone-induced gastric acid secretion, increases prostaglandin activity but reduces thromboxane concentration.

Keywords: Prostaglandin E2; Thromboxane A2; Taxifolin; Dexamethasone; Gastric acid

1. Introduction

Dexamethasone is a synthetic glucocorticoid that appears to have a double face effect. Its activity in man and experimental animals has generated a long standing debate on whether treatment with glucocorticoids leads to peptic ulcer or not [1,2] Available reports have shown that the use of glucocorticoids (dexamethasone) could be gastroprotective under physiologic conditions [3] on one hand, and on the other hand, could be causal to many disease states such as hypertension associated with increased oxidative stress and excess production of reactive oxygen species (ROS) often generated by nicotinamide adenine dinucleotide phosphate-oxidase (NAPDH) [4] and contributing to tissue damage, nitric oxide (NO) deficiency and endothelial dysfunction ([5], hormonal changes in the reproductive system and ulceration in the gastric mucosa. For example, various works have demonstrated that the administration of glucocorticoids to animals can attenuate gastric mucosal erosion formation [6,7, 8]. Similarly, It has also been shown that pretreatment with dexamethasone [6, 7] may protect the stomach from ethanol or non-steroidal anti-inflammatory drugs (NSAID)-induced injury, respectively thereby maintaining the gastric mucosal integrity [9, 10].

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Prostanoids including prostaglandins (PGE₂) and prostaglandin-like substance such as thromboxane -A₂ (THX-A₂), with potent biological activity, either exogenous or endogenous have also been reported to be pivotal in maintaining the integrity of the mucosal wall [11]. They are found in high concentration in the gastric mucosa and gastric juice. Endogenous prostaglandins inhibit acid secretion, stimulate mucus and bicarbonate secretion, alter mucosal blood flow, and protect against acute mucosal damage [12]. Our concern in this work, is to find out the possible physiologic actions of dihydroquercetin (taxifolin) on dexamethasone induced gastric acid secretion and denudation of the mucosal epithelium.

2. Material and methods

2.1. Experimental Design

Twenty male Wistar rats (180g-200g body weight) were used. The rats were randomly selected into four groups containing 5 rats each. Group 1 was the control group fed on normal rat feed. Group 2 received 3mg/kg of Dexamethasone (intraperitoneally) at one day interval. Group 3 received 3mg/kg of Dex. intraperitoneally and 1mg/kg body weight of taxifolin orally while group 4 received 1mg/kg body weight of taxifolin. At the end of 6 weeks, basal and peak gastric acid output was measured by continuous perfusion of rat's stomach under anaesthesia with normal saline at the rate of 1ml/min. Basal gastric acid secretion and maximal (Histamine -induced), prostaglandin E₂ and thromboxane A₂ activity were determined according to standard procedures. Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria. The research work involved the use of experimental animals-Wistar rats (approval number FBMS/CRUTECH/18/020).

2.2. Preparation of animals for collection of gastric acid

Prior to the collection of gastric acid, animals were fasted for 12-18 hours in order to get rid of all fecal matter in the stomach. When animals were ready, they were anaesthetized intra-peritoneally using 25% urethane at a dose of 6ml per Kg body weight of the animal. The animals were laid in a dissecting board, and the trachea cannulated for proper aeration. Incision was also made along the *linea alba* to expose the stomach. A thread was passed at the pyloric end under the pyloric sphincter. Also, an incision on the duodenum was made near the pyloric sphincter, cannulated and firmly tied.

An orogastric infusion tube was carefully passed through the oesophagus to the stomach and ligated just behind the tracheal cannula to prevent reflux. The exposed end of the orogastric tube was passed through a water bath to maintain the perfusate at 37°C and to pre warm the experimental solution. The other end of the orogastric tube was attached to a 50ml syringe mounted on a perfusor pump. The animal was warmed with a table lamp to maintain the body temperature at 37 ° C and temperature monitored.

2.3. Measurement of gastric acid output

The method of [13] modified by [14] was used. The rats were first perfused with 0.9% normal saline at the rate of 0.1ml/min. The aliquots were collected after 10 minutes of infusion and were titrated with 0.01 N NaOH using phenolphthalein as an indicator. Change in colour from colourless to pink indicated the end point of the titration and the volume of the base used was recorded. The first 10-20 minutes effluent collected was not used because of possible increase in acid secretion due to trauma. When a stable acid secretion was obtained, cimetidine (11.40mg/kg body weight,) was administered, followed by histamine (100mg/kg body weight,) and acid output determined every 10 minutes using the method described above.

2.4. Cytoprotection study

The cytoprotection experiment was done using the method of [15] 2ml of acid-alcohol (prepared from equal volumes of 0.1N HCl and 70% methanol) was introduced into the stomach of the rat through a duodenal incision with the help of a syringe and the pyloric-duodenal junction was ligated once again and left for 2 hours. The orogastric tap was reopened to flush, to get rid of any food materials. The ulcer score was then carried out after the method described by [15]. A magnifying lens and a vernier caliper were used to measure the extent of ulceration.

2.4.1. Determination of Ulcer Score

- The ulcer scores were done as follows:
- Grade
- 0=No lesions
- 1= Haemorrhagic erosions (less than 5mm).
- 2= Haemorrhagic erosions (greater than 5mm or small linear ulcers).
- 3= Many small linear ulcers (greater than 2mm or small linear ulcer).
- 4= Multiple linear ulcers of marked size.

2.5. Drug Treatment

Drugs used in this study were cimetidine (Sigma, UK), and histamine (Sigma, UK),

2.6. Determination of Thromboxane A2 and PGE-2

Thromboxane A2 and PGE2 were determined using the ELISA KIT/ manufacturers instruction.

2.7. Statistical analysis

All data obtained in this study were expressed as mean \pm standard error of mean. Collected data was analyzed using ANOVA (analysis of variance) using *Graph Pad Prism version 5.0 for Windows (Graph Pad Software, San Diego, CA, USA)*. Bonferroni multiple comparison post hoc test was used to compare the level of significance between control and experimental groups. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Effects of dexamethasone and taxifolin on mucus secretion

Mucus secretion was significantly ($p < 0.05$) reduced in dexamethasone (3mg/kg body weight) group when compared to control. There was also a significant ($p < 0.05$) increase in gastric mucous secretion in the treatment groups compared to control and the Dex group (figure 6).

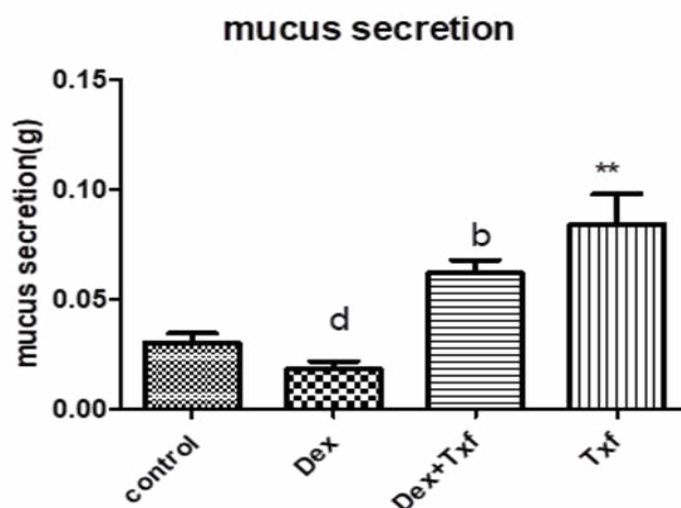


Figure 1. Showing effect dexamethasone and taxifolin treatment on mucus secretion in rats. Values are expressed in mean \pm SEM n=5, **= $p < 0.01$ vs control;

3.2. Effects of dexamethasone and taxifolin on gastric acid secretion

Basal concentration of gastric acid output were significantly ($p < 0.05$) lower in the treated groups. Gastric acid output following Histamine induced stimulation was significantly ($p < 0.01$) lower in Dex group compared to control and other treatment groups (figure 2a). Similarly, the administration of cimetidine significantly ($p < 0.05$) blocked gastric acid secretion in the treated group when compared to control (figure 2b)

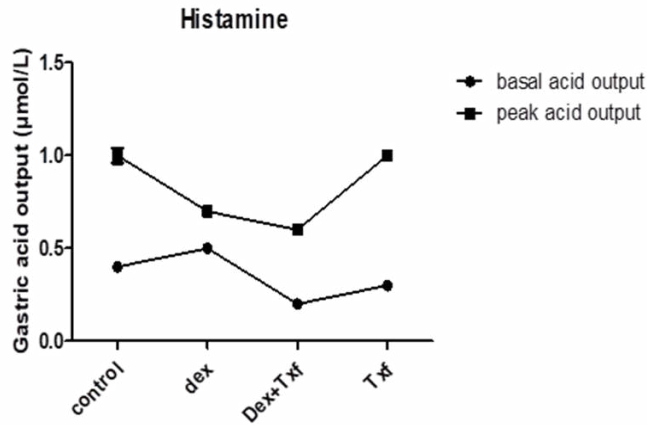


Figure 2a: showing effect of histamine introduction on gastric acid output in dexamethasone treated Wistar rats and taxifolin supplementation. Values are expressed in Mean \pm SEM

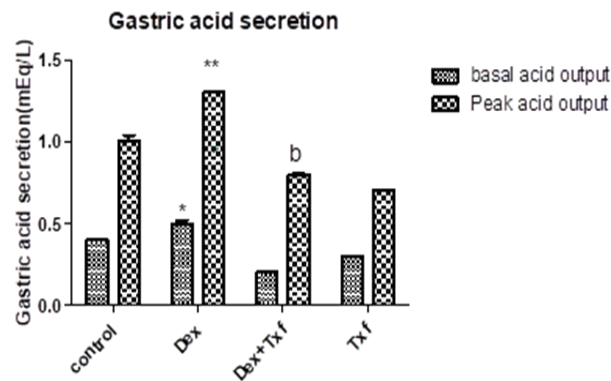


Figure 2b Showing effect of dexamethasone treatment and taxifolin (dihydroquercetin- 1 mg/kg) supplementation on gastric acid secretion. Values are expressed in Mean \pm SEM; n=5.

3.3. Effects of dexamethasone and taxifolin on prostaglandin E2 activity

The serum concentration of prostaglandin E2 was significantly ($p < 0.01$) reduced in dexamethasone treated rats (figure 5). Treatment with taxifolin significantly ($p < 0.01$) increased PGE-2 concentration when compared to the Dexamethasone group.

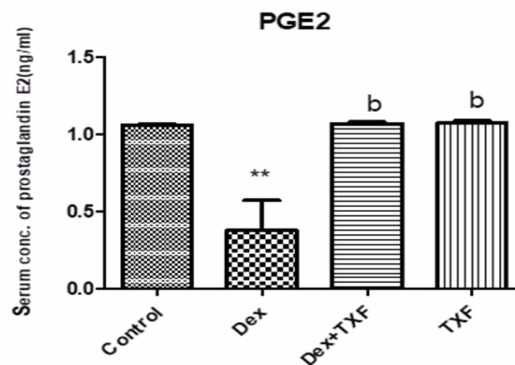


Figure 3. Showing effect of Taxifolin on prostaglandin E2 in dexamethasone treated rats. Values are expressed in Mean \pm SEM n=5 **= $p < 0.001$ vs control b= $p < 0.01$ vs Dex; c= $p < 0.05$ vs TXF

3.4. Effects of dexamethasone and taxifolin on thromboxane A2 concentration

The serum concentration of thromboxane A2 was significantly ($p < 0.01$) increased in dexamethasone treated rats. Treatment with taxifolin significantly ($p < 0.01$) reduced Thromboxane A2 concentration when compared to the Dex.group (figure 4).

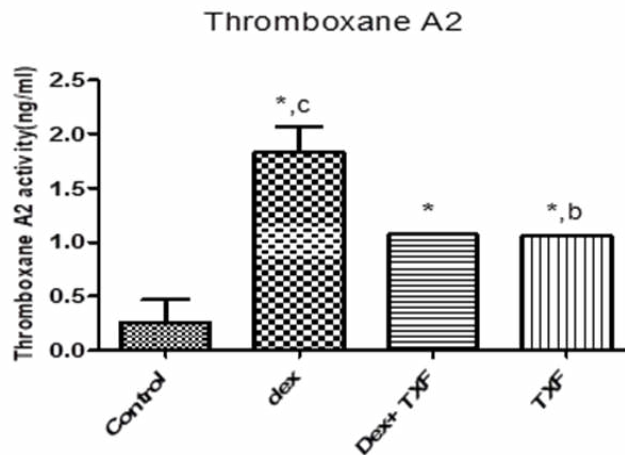


Figure 4 Showing effect of Taxifolin on Thromboxane A2 activity in dexamethasone treated rats. (Values are expressed in Mean \pm SEM n=5 *= $p < 0.05$ vs control; b= $p < 0.05$ vs Dex; c= $p < 0.05$ vs TXF)

3.5. Effects of dexamethasone and taxifolin on gastric ulceration

Ulcer score was significantly ($p < 0.05$) higher in Dex group than control. Treatment with taxifolin significantly ($p < 0.05$) reduced ulcer score. Dexamethasone alone lowered gastic ulceration as shown in figure 5

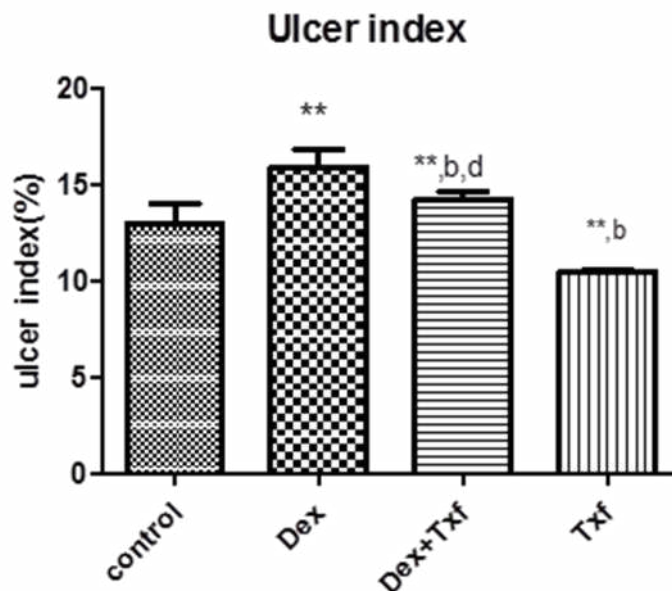


Figure 5 Showing effect of dexamethasone (3 mg/kg) treatment and taxifolin (Dihydroquercetin – 1mg/kg) supplementation on gastric acid secretion. Values are expressed in Mean \pm SEM; n=5. **= $p < 0.05$ vs control; b= $p < 0.05$ vs Dex; c= $p < 0.05$ vs TXF

4. Discussion

The use and benefit of dexamethasone as a proinflammatory and as an anti-inflammatory drug has over the time been controversial. To some, it serves a gastroprotective function and has the ability to maintain blood flow, mucus secretion and gastric mucosal integrity [16] while to others it is said to be detrimental to both the cardiovascular system and the gastrointestinal tract. To this end, we examined the effect of taxifolin (dihydroquercetin) supplementation on prostaglandin and thromboxane – A₂ (THX-A₂) on gastric acid secretion in dexamethasone treated rats. Our results showed that dexamethasone, a synthetic glucocorticoid, potentiates gastric acid secretion and gastric mucosal excoriation. Previous experimental reports have demonstrated that dexamethasone treatment may stimulate gastric acid secretion [17,18,19] by inhibition of prostaglandin synthase and peroxidase enzyme, thereby inhibiting the biosynthesis of prostaglandin [20]; by blunting the H⁺ + K⁺ ATPase inhibitor and suppressing epithelial cell proliferation via inhibition of cyclooxygenase -2 (Cox - 2) and reduced mucosal wall regeneration [21]. One mode of action of glucocorticoid is its regulation of gene expression through glucocorticoid receptors that are mostly found in the parietal cells [22]. Interestingly, our result showed that increase in gastric acid secretion caused by dexamethasone may not be histaminergic.

We also demonstrated that treatment with dexamethasone caused a significant decrease in prostaglandin E₂ concentration and a raised level of thromboxane A₂. Clinical trials have proven that endogenous prostaglandins may modulate gastric acid secretion via inhibition of histamine – stimulated cAMP formation and improved mucus and bicarbonate secretion [12].

The high level of thromboxane A₂ (THX –A₂) derived probably from Cox-1 in a series of metabolic reaction involving thromboxane A₂ synthase, observed in this study, may have been responsible for the gastric ulceration, prevention of mucosal epithelial proliferation and delayed healing [23]. THX- A₂ affects vasoconstriction and endothelial adhesion molecule expression [24, 25]. There was however no pathology identified in all groups.

Supplementation of dexamethasone with taxifolin yielded a better gastroprotection and reduced both gastric acid secretion and thromboxane A₂ level but upregulated PGE₂ and mucus secretion.

Taxifolin is an effective polyphenolic compound and a food and nutritional supplement with gastroprotective ability against a variety of ulcerogenic agents [26]. This compound scavenges free radicals because of its conjugation structures and protects the gastrointestinal mucosa lesions produced by various experimental ulcer models and against different necrotic agent [27]. Being a flavanoid, it shows an outstanding propensity to exhibit efficacy and anti-ulcerogenic activity via precipitation of microproteins on ulcerated areas thereby forming a barrier to easy penetration on the surface of the mucosal lining thus inhibiting easy uptake of substances that may be damaging and also preventing degradation by proteolytic enzymes [28,29,30]. It also inhibited basal histamine induced secretion as well as augmented cimetidine (H₂-receptor blocker) inhibitory activity. These results indicate that with its flavonoid property, taxifolin may be acting via inhibition of the H₂-receptors.

Other earlier possible mechanisms proposed have been scavenging of free radicals, inhibition of inward flow of calcium ions, modulation of mucosal membrane permeability and promoting mucus secretion. Mucus in the gastric mucosa offers protection against excoriation of the mucosal lining [31] by the corrosive activity of the acid available in the stomach. In this study, taxifolin elicited increased mucus secretion which forms a gel layer that serves as a primary barrier against acid induced excoriation.

5. Conclusion

Taxifolin decreases dexamethasone-induced gastric acid secretion, increases prostaglandin activity but reduces serum thromboxane A₂ concentration.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declare that there is no conflict of interests regarding the publication of this paper.

Statement of ethical approval

Ethical approval for the study using experimental animals was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria (approval number FBMS/CRUTECH/18/020)..

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