Original Research Article

Gastro protective effects of Tadalafil on ethanol- induced and reserpine –induced gastric ulcer in rats

Abstract

Background: Drug repurposing is a system whereby drugs already in use are redirected for another therapeutic use. Tadalafil is PDE5 inhibitors. This research study investigated the potential of tadalafil which is used in the management of erectile dysfunction on ethanol-induced and reserpine –induced gastric ulcer in rats. **Method:** Cialis® which contain 20mg of tadalafil was used. Male rats weighing 180 -252g were orally administered 95% Ethanol at 1ml/200g of rats to induce gastric ulcer at 1h post tadalafil administration. In another group, 5mg/ml of Reserpine in 0.5% acetic acid was administered to rats to induce gastric ulcer, also at 1h post tadalafil administration. Omeprazole was used as standard drugs. After 4h, stomach tissues were removed and assessed for Ulcer Index and Protective ratio. **Result:** 50mg/kg of tadalafil exhibited a significant reduction in Ulcer Index in reserpine-induced and ethanol-induced models when compared to standard. **Conclusion:** Tadalafil has gastro protective potential at lower concentration.

Keywords: Gastric ulcer, Tadalafil, Ethanol, Wistar rats

Introduction

Drug repositioning is a system whereby drugs already in use is redirected and channeled for another therapeutic use. It is the system of redirecting the use of drugs already in existence for another clinical indication. Drug repurposing involves the utilization of a drug for a totally different therapeutic application. It is also known as drug rechanneling, re-profiling, or re-routing (1). Drug repurposing is necessitated by the fact that traditional drug discovery and development process has become quite exorbitant/expensive taking an average of US$1.8billion and a long duration of time with an average of 13 -15 years. The long development process, high cost, drug resistance, toxicity and a very low success rate have revealed the unavoidable need for drug repurposing of old conventional drugs for a new therapeutic application (2). Alternatively, it is also known as the evaluation of currently used drugs for a new therapeutic purpose. A few numbers of drugs were discovered through drug repurposing such as penicillin (while investigating on influenza), chlordiazepoxide (while discovering synthetic dyes), imipramine; an anti-depressant was identified while investigating for a chlorpromazine-like substance for the management of schizophrenia. Iproniazid was a drug initially utilized for the management of tuberculosis but later re-channeled as an anti-depressant leading to the discovery of the group, monoamine oxidase inhibitors (MAO-I) (3). Peptic ulcers Diseases (PUDs) are commonly characterized depending on anatomical sites as (gastric or duodenal). The former occurred down

the stomach‘s lesser curvature while the later are frequently found in duodenal bulb, the gastric acid most exposed region, (4). Helicobacter pylori (H. pylori) had been suspected as the key etiological factor for (90%) duodenal and (80%) gastric ulcer cases (5). It has been discovered that Peptic ulcer can be resistant to conventional anti- ulcer therapy. European Medicines Agency and the Ministry of Health in Nigeria has banned all Histamine$_2$- Receptor Antagonist base on the report issued by the US Food and Drug Administration after discovering that the popular heartburn medication Zantac (Ranitidine) contains low levels of the nitrosamine impurity (N-nitrosodimethlamine) (NMDA) (6). Tadalafil is thereby being investigated for its gastro protective potential in ethanol- induced and reserpine –induced rats.

Materials and Methods

Experimental Animals

Albino wistar rats (180-200g) were purchased from the Department of Pharmacology Animal House, University of Port Harcourt, and Rivers State. The animals were classified in five in standard cages at room temperature 25°C with 12-hour dark/ 12-hour light cycles and they were provided with feed and water *ad libitum*. Prior to the studies, the rats were conveyed to the laboratory and permitted to take only water at will. All animals used for this research studies were kept in clean cages and in accordance the directives of the Federal Government Legislation on animal cares. Ethical approval was obtained from the Ethical Committee for Animal Care of the University of Port Harcourt, Rivers State.

Effect of tadalafil on ethanol-induced gastric ulceration in rats

According to Adinortey et al (7), ethanol-induced Model wistar rats of male sex (weight 180 – 252 g) were divided into 4 Groups (n= 6). Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Ulcer lesion was established with 0.5 ml of 95% ethanol. Group 1- 3 received Tadalafil (50, 100 &200mg/kg) + Ethanol (1ml /200g); Group 4 standard Omeprazole (30mg/kg). Ethanol was
given 1 hour after the drugs were administered. All drugs were administered intragastrically via the aid of an orogastric cannula . 4 h later, the animals were killed by cervical dislocation. The stomach tissues were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions. The ulcer score, ulcer index, and Preventive ratio of drugs were calculated using the procedure of Kulkarni (8).

Effect of tadalafil on reserpine in acetic acid induced ulceration in rats

As described by Adinortey et al. (2013), male adult albino rats grouped into 4 (n=6) were used for this experiment. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1-3 received tadalafil (50, 100 and 200mg/kg) respectively, while Group 4 received Omeprazole (30mg/kg), 1 h prior to administration of 0.25 g/kg reserpine. All drugs were administered intragastrically via the aid of an orogastric cannula. 4 h later, the animals were killed by cervical dislocation. The stomach tissues were removed and opened along the greater curvature. The tissues were fixed with 10%

Formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions. The Ulcer index and Preventive ratio of drugs were
Calculated using the procedure of Kulkarni (8).

Ulcer Index: According to Kulkarni (8) method, ulcer indicator can be gauge by application of the following scores including the figure and ulcer severity;

0.0 = usual color of
1.5 = hemorrhagic lines,
2.0 = ulcers having (>3 but =5mm2) area
3.0= ulcers > 5mm2,

Ulcer index (UI) = UN + US + UP × 10

Where UI = ulcer index, UN = average number of ulcers per animal, US = average of severity score, and UP = fraction of rats that developed ulcer. The percentage –protective- ratio and percentage- curative- ratio are correspondingly, obtained from the equation below;

Percentage protective ratio = $\frac{100 - [\text{UI pre-treated}]\ [\text{UI control}] \times 100,}{\text{UI control X } 100}$

Statistical Analysis

Results were expressed as the mean ± S.E. Data were analyzed by one-way ANOVA followed by Dunnet's post hoc test, and $p < 0.05$ was considered to be statistically significant.

Results

Table.1: Effects of Tadalafil on Ethanol-induced gastric Ulceration in Rat

Treatment	Ulcer Index	Protective ratio (%)
TAD 50mg/kg	1.18± 0.12**	35.58
TAD 100mg/kg	2.25± 0.35	64.70**
TAD 200mg/kg	2.45±0.37	42.72
Omeprazole 30mg/kg	3.87± 1.25	79.50

** Significant relative to control: *p<0.05. Values represent mean ± S.E.M (n= 5), TAD = Tadalafil, control = omeprazole**.**

Table.2: Effects of Tadalafil on Reserpine in 0.5% acetic acid-induced gastric ulceration in rats.

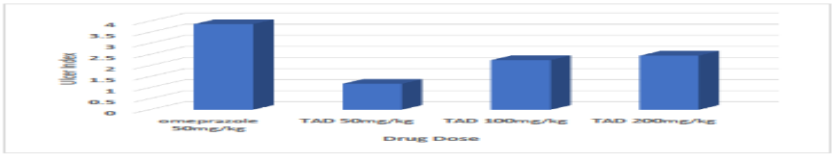
Treatment	Ulcer Index	Protective ratio (%)
TAD 50mg/kg	5.2 ± 1.34	56.80
TAD 100mg/kg	3.7 ± 2.18	48.20
TAD 200mg/kg	1.5 ± 0.13**	22.17**
Omeprazole 30mg/kg	2.8 ± 1.16	35.60

** Significant relative to control: *p<0.05. Values represent mean ± S.E.M (n= 5), TAD = Tadalafil, control = omeprazole**.**

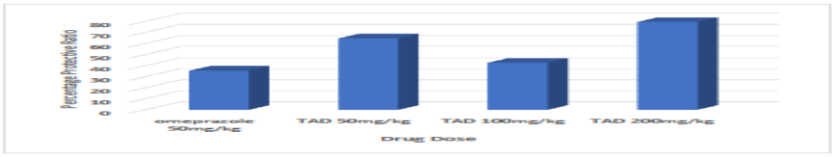
From the above table, in the ethanol-model, there was a significant reduction in Ulcer Index of group treated with Tadalafil (50mg/kg, 100mg/kg and 200mg/kg) in comparison with omeprazole ($p< 0.01$). Hence, pretreatment with tadalafil revealed a significant inhibitory effect against alcohol-induced gastric ulceration as seen in table 1above. The Preventive Ulcer ratios are 64.70, 35.58, 42.72 and 79.50 for the treated group and standard respectively. Tadalafil 50mg/kg and 200mg/kg produced more significant gastric protection when compared to control.For the 25 mg/kg reserpin in 10 ml/kg 0.5% acetic acid induced model, it was observed that group treated with Tadalafil (50, 100 and 200mg/kg) and omeprazole (30mg/kg) showed a significant reduction in Ulcer Index in comparison with control group . The Percentage protective ulcer ratios are 48.20, 22.17, 35.60 and 56.80 respectively. The group treated with Tadalafil 100 and 200mg/kg displayed more significant gastro protection in this model

Results

Bar chart representing Ulcer index and Protective ratio against Drug dose in Reserpine-induced Model

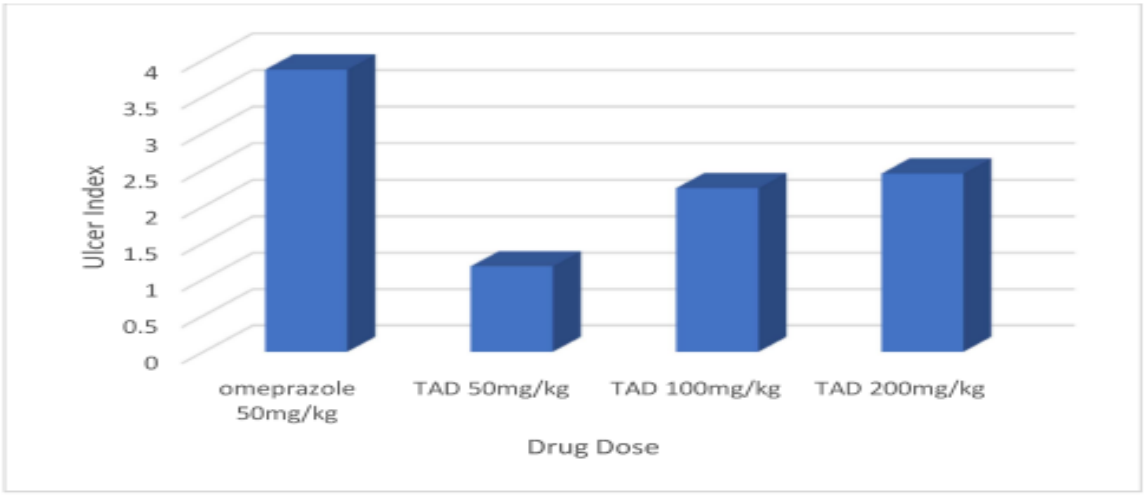


A bar chart representing ulcer index against drug dose



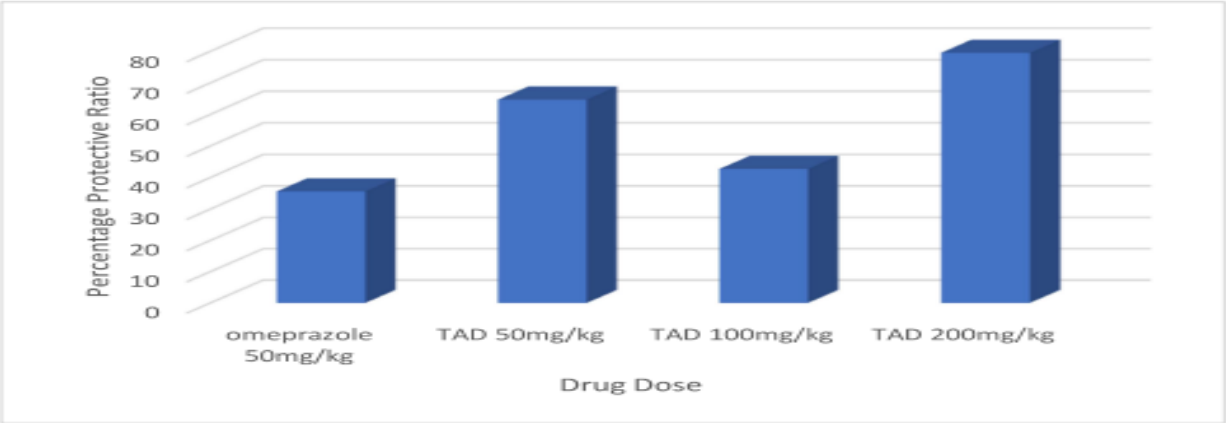
A bar chart representing percentage protective ratio against drug dose

Fig 1: Bar chart showing both Ulcer index and protective ratio against drug dose in reserpine –induced ulceration in rats.



A bar chart representing ulcer index against drug dose

Fig 2: Bar chart showing ulcer index against drug dose in ethanol –induced ulceration in rats.



A bar chart representing percentage protective ratio against drug dose

Fig 3: Bar chart showing percentage protective ratio against drug dose in ethanol –induced ulceration in rats.

From the above table, in the ethanol model, there was a significant reduction in ulcer Index of group treated with Tadalafil (50mg/kg, 100mg/kg and 200mg/kg) in comparison with control group/standard omeprazole ($p< 0.01$). The Preventive ulcer ratios are 64.70, 35.58, 42.72 and 79.50 for the treated group and standard respectively.Tadalafil 50mg/kg and 200mg/kg produced more significant gastric protection when compared to control. For the reserpine-induced model, it was observed that group treated with Tadalafil (50, 100 and 200mg/kg) and omeprazole (30mg/kg) showed a significant reduction in ulcer Index in comparison with control/standard.The Percentage Protective ulcer ratios are 48.20, 22.17, 35.60 and 56.80 respectively. The group treated with Tadalafil 100 and 200mg/kg displayed more significant gastro protection in this model.

Discussion

Currently, it has been discovered that Peptic ulcer can be resistant to conventional anti- ulcer therapy or reappear after initial treatment. Another study reported increase in the rate of resistance to Proton pump inhibitor (PPI).This research study investigated the potential of tadalafil which is used in the management of gastric ulcer in ethanol- induced and reserpine – induced gastric ulcer in rats. The development of ethanol-induced ulcers occur at the glandular section of the stomach and has been reported to trigger the production of leukotriene (LTC4) giving rise to the damage of rats gastric mucosa (9). Ethanol-induced lesions are associated with vascular changes; hence maintenance of the mucosal vasculature and normal blood flow is the crucial mechanism of cytoprotection (10). Reserpine in acetic acid induced gastric ulceration via paralyses of the cholinergic activity causing an elevation of adrenergic tone. Activation of the cholinergic afferent fibers produces gastro protective effects facilitated by the calcitonin gene related peptides (CGRP), and Nitric oxide (11). Stimulation of the adrenergic system brings about gastric acid production and pathogenesis of gastric mucosal ulceration. However, these receptors of cholinergic system facilitate the anti-secretary and gastric mucosal protective effects. Tadalafil is an active, reversible, competitive inhibitor of phosphodiesterase 5 (PDE5), an

enzyme that degrades cGMP. It interferes/block cGMP breakdown, leading to the buildup of cGMP which invariably bring about the dilation of smooth muscle of the blood vessels. Elevation of cGMP level enhances PDE5 actions (12).Tadalafil acts by increasing the blood flow to gastrointestinal tissues following increased cGMP level. As a phosphodiesterase inhibitor, tadalafil enhances the endogenous synthesis of NO (13) and these effects invariably produce anti-inflammatory effects via enhancing cGMP production. Tadalafil bring about production of more NO. No is widely known as a vasodilator via its capacity to maintenance mucosal vasculature and enhance more blood flow in GIT tissues thereby impeding tissue breakdown. Through promotion of nitric oxide, cholinergic activities are enhanced (11).

Conclusion

From the above result, it can be seen that there was a significant reduction in the ulcer index with tadalafil 50mg/kg in reserpine-induced model and increase in percentage protective ratio especially with 100mg/kg and 200mg/kg. This showed that tadalafil has gastro protective potential in ethanol-induced model and reserpine in acetic acid induced model.

Animal rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed.

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