

Preparation of Potassium Dicitro Bismuthate Complex as Antigastric Ulcer

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ABSTRACT

The present study was designed to synthesis potassium dicitro bismuthate (III) complex. The complex was characterized by infrared spectra, conductivity and elemental analysis of Bi. The effect of gastro protection was investigated for the prepared complex against HCl- ethanol induced ulcer in rats. The complex was administered to rats at 50 and 100 mg/kg doses orally before one hour of receiving 0.2ml of HCl- ethanol mixture to induce gastric ulcer. Sucralfate (100mg/kg, orally) was used as standard drug. The severity of gastro mucosal damage induced by HCl-ethanol was analyzed in term of ulcer index value. Administration of potassium dicitro bismuthate (III) one hour before HCl-ethanol had significantly decreased ulcer index value when compared to saline treated group. Sucralfate (100mg/kg, orally) also produced a significant decrease in ulcer index when compared with the saline treated group. There was no significant difference between potassium dicitro bismuthate (III) and Sucralfate in degree of protection against ulcer. Thus it can be concluded that potassium dicitro bismuthate (III) has antiulcer activity in rats.

Keywords: citric acid, bismuth complex, antigastric ulcer, Sucralfate

(, / 100 , 50) —

—

ulcer index

— (, / 100) Sucralfate

INTRODUCTION

The importance of citric acid (CA) in abiotic and biological systems has been well established over the years (Ali *et al.*, 2008, Carla *et al.*, 2008, Tselesh, 2008, Maria *et al.*, 2008). The nature of the metal-citrate complex formed depends upon the nature of the metal, the pH of the solution, type of coordination bond and the stoichiometric ratio of metal to citric acid.

Citrate is an important biological ligand for metal ions. It is present at high concentration (*ca.* 200 mM) in blood plasma and forms strong complexes not only with natural metal ions such as Ca^{II} , Mg^{II} and Fe^{III} , but also with ions of toxic (*e.g.* Al^{III}), (Petrova *et al.*, 2008) therapeutic (*e.g.* Bi^{III}) and diagnostic (*e.g.* $^{67}\text{Ga}^{\text{III}}$) importance.

Peptic ulcer, one of the most widespread disease, is believed to be due to imbalance between aggressive and protective factors (Alkofahi and Atta, 1999) in the stomach such as acid-pepsin secretion, mucosal barrier, mucous secretion, blood flow, cellular regeneration, prostaglandin, epidermal growth factor (Govindarajan *et al.*, 2006, Lima *et al.*, 2006) bacterial products (*Helicobacter pylori*) and drugs (Peskar and Maricic, 1998). The current medical treatment of peptic ulcer is generally based on the inhibition of gastric acid secretion by H_2 as Omeprazol and antimuscarinics, as well as, the acid in depended therapy provided by Sucralfate and bismuth (Bighett *et al.*, 2005). Potassium dicitro bismuthate (III) has a topical action in the upper gastrointestinal tract (Coghill and Shepherd, 1988) and it is commonly used to treat a variety gastrointestinal disorder (Lourence and Keith, 2008). Potassium dicitro bismuthate (III) precipitate (an active metabolite) when it reaches the acidic environment of the stomach (Wagstaff *et al.*, 1988).

The aim of the present study is to prepare potassium dicitro bismuthate (III) complex and characterize by molar conductivity, infrared spectra, and elemental analysis and its effect as antiulcer activity in experimentally induced ulcer in rats.

MATERIALS AND METHODS

All the materials (BDH) were used without any further purification.

Preparation of Bismuth complex

The complex was prepared by adding bismuth nitrate (1mmole) in 25 ml of deionized water to an aqueous solution of citric acid (2 mmole) with continued stirring until a white precipitate was formed. The precipitate was washed with a small amount of deionized water. Bismuth citrate was suspended in (25ml) of deionized water, sodium carbonate powder was added with stirring until the solution become neutral. Absolute alcohol was added to the solution with stirring until a white precipitate was formed; the product was washed with 10 ml of dry ether and dried (yield 80%). Melting point more than 350°C , molar conductivity ($345, 125 \text{ ohm}^{-1} \text{ cm}^2 \text{ mole}^{-1}$) in water and ethanol, respectively.

Physical measurements: The FT-IR were measured as KBr disc by using FT-IR Spectrometer (Fourier transform infrared spectroscopy) (Bruker 27, TENSOR, Germany).

Molar conductance was obtained approximately $10^{-3}M$ in a Jenway conductivity meter model 4070. Content of Bismuth was determined by the following method (Malairajan *et al.*, 2008). Moistened about 0.5g, accurately weighed with sulphuric acid, ignite at a temperature not exceeding $500^{\circ}C$, and dissolve the product in 2ml of nitric acid and 4ml of water, add 50ml of glycerol and 0.2g of sulphuric acid, allow to stand for one minute, and add 200ml of water and 0.3ml of catechol violet solution; if a violet color is produced add dilute ammonia solution, drop wise, until a blue color is produced; titrate with M/20 sodium edentate until a yellow color is produced; each ml of M/20 sodium edentate is equivalent to 0.01045g of Bi.

Pharmaceutical study

Healthy rats of either sex weighing 200-250 gm were selected for this study. The animals were housed and fed with standard diet and water. Animals were deprived of food at least 24 hrs. before starting of the experiment but were allowed free access to water. The rats were divided into 4 groups, each group consists of five animals. Group 1 served as a saline control and was given normal saline 0.9% (1 ml/kg, orally), group 2 received (100 mg /Kg, orally) sucralfate (Alma company, Syria) as standard control. Group 3 and 4 received (50, 100 mg /Kg, orally) potassium dicitro bismuthate (III), respectively. After one hour all the animals were treated with 0.5 ml of HCl –ethanol mixture, orally (0.3 M hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were sacrificed by cervical dislocation, one hour after administration of HCl –ethanol mixture (Malairajan *et al.*, 2008). The stomachs were removed, opened along the greater curvature and examined for lesion severity, lesion severity was determined by measuring ulcer index, by measuring each lesion in mm along its greater length (Singh *et al.*, 2008, Abay and Abdulwahib, 2008). The ulcer lesions observed were scored according to the severity according to previous study (Nwafor *et al.*, 1996).

Histological evaluation

Sections of tissue from stomach were examined histologically to study ulcerogenic and or antiulcerogenic activity of tripotassium dicitro bismuthate (III). The slides were examined microscopically (Olympic Company) for pathomorphological changes such as congestion, hemorrhage, edema and erosion using an arbitrary scale for the assessment of severity of these changes (Al-Howiriny *et al.*, 2005).

Histopathological studies

The stomach tissues were removed from the rats and fixed in 10% normal saline for at least 48 hrs. These were then processed routinely and the tissues were embedded in paraffin wax. Histological sections were cut at 5 - 6 μm and stained with routine haematoxylin and eosin (HE). These were then examined by a histopathologist. The lesions observed were assessed for the following, mucosal atrophy, and the presence of inflammatory cells in the wall, eosinophils, lymphocytes and plasma cells. These were graded according to mild (+), moderate (++) or severe (+++). Photomicrographs of representative lesions were taken at various magnifications.

Statistical analysis

The data were expressed as mean \pm SD, difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at $p < 0.05$.

RESULTS AND DISCUSSION

A- Structure assignment

Fourier transform-infrared spectroscopy (FTIR)

The following factors affect metal-ligand stretching vibrations: i) oxidation state of the metal atom, ii) coordination number and symmetry, iii) coordination bond strength, iv) the base strength of the ligand, and, v) bridging ligands.

Citric acid

The structure consists of two methylenic carbons (CH_2), three carboxylic acid (COOH) groups, and a tertiary alcohol group ($\text{C}_3\text{C-OH}$). The sharp peak at 3500 cm^{-1} is due to α -hydroxyl O-H stretching, reflecting intermolecular hydrogen bonding. A broad group of bands between 3300 and 2500 cm^{-1} is due to O-H stretching vibrations resulting from hydrogen bonding indicating a dimeric structure. The C-O stretching vibrations for the alcoholic group occur at 1140 , 1175 , 1210 , and 1240 cm^{-1} . Carboxylic acid dimer peaks are indicated at 1350 to 1290 cm^{-1} and at 940 cm^{-1} due to C-O stretching vibrations. The primary C=O stretching vibration for COOH groups is seen as a doublet peak at 1740 cm^{-1} and 1710 cm^{-1} . The split structure signifies the non-equivalence between the two terminal carboxylates and the central carboxylate group. The sharp peak at 1430 cm^{-1} manifests the tertiary alcohol in-plane O-H stretching vibrations, while the moderately broad peak at 935 cm^{-1} is due to the dimeric carboxylic acid O-H out-of plane stretching vibration (Dimitr *et al.*, 2002).

potassiumdicitro bismuthate (III) complex

The association of a hydroxide group in metal bonding is indicated by the absorption peak at 3610 (O-H) and the $\text{C}\alpha$ -OH stretch is reflected in the peaks at 910 , 850 , and 830 cm^{-1} . Complexation of the α -hydroxyl group of citric acid is indicated by the absence of a peak at 3500 cm^{-1} compared to citric acid. Additional evidence for the association of the α -hydroxyl group is indicated by the shift in the C-O bending vibrations of the hydroxyl group to higher energies (1190 , 1230 , 1260 , 1300 cm^{-1}) compared to those in citric acid. Lattice water is indicated by the shoulder peak at 3380 cm^{-1} . Involvement of the citric acid carboxylate groups with the metals is suggested by the asymmetric C=O stretching peaks at 1640 and 1570 cm^{-1} . The position of these peaks is strong indication of unidentate as well as bidentate coordination to the metal. The shift in the symmetric C=O stretch (medium) to 1440 cm^{-1} signifies bidentate or bridging carboxylate coordination, while unidentate coordination is confirmed by the peak at 1360 cm^{-1} . The presence of a sharp peak at 1790 cm^{-1} is due to the C=O stretch of the free carboxylate group (Dimitr *et al.*, 2002).

The molar conductivity measurement: A high value of molar conductivity for the reflects complex has a 1:3 ratio of metal to ligand in both solvents. These results suggested a structure of complex Fig.1, the suggested structure was further confirmed by metal content analysis of bismuth (calculated; 29.665, found 30.01%)

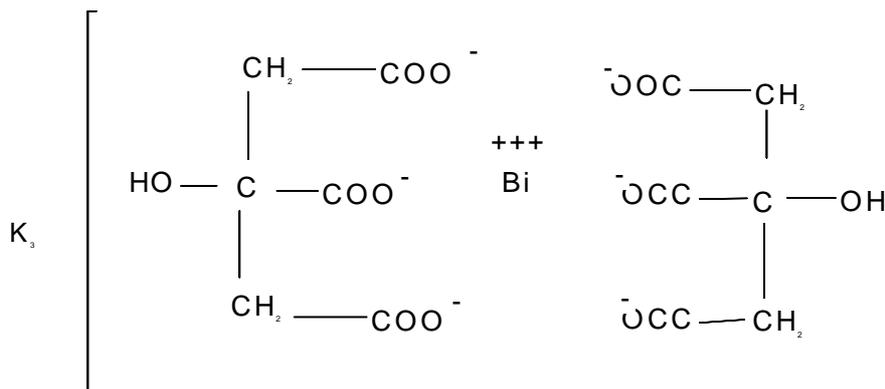


Fig. 1: The suggested structure for tripotassium dicitro bismuthate (III) complex.

B- Antiulcer activity of the complex

In the present study, the potassiumdicitro bismuthate (III) was evaluated for its antiulcer activity against HCl –ethanol induced gastric ulcer in rat. Oral administration of HCl – ethanol produces sever ulceration, potassium dicitro bismuthate (III) at (50,100 mg/Kg, orally) as well as standard drug Sucralfate at (100 mg /Kg , orally) significantly reduce lesion score of ulcer in comparison to saline treated group (Fig. 2,3,4).

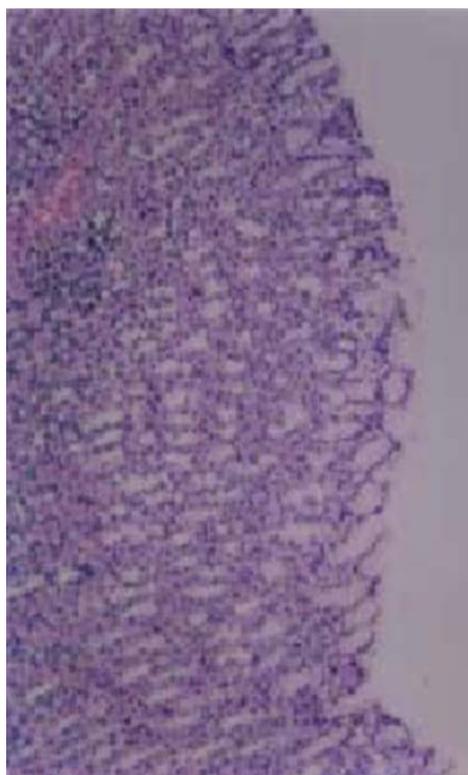


Fig. 2 : Showing normal appearance for section of gastric mucosa (H&E. x100).

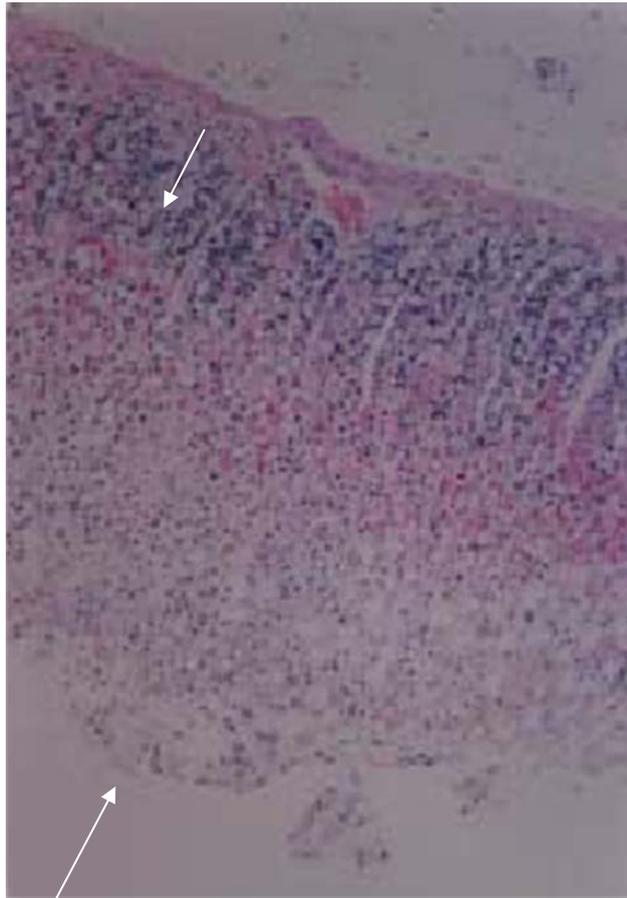


Fig. 3 : Section of gastric mucosa after treated with ethanol-HCl, showing inflammation, hemorrhage and mucosal ulceration. (H&E.x100) .

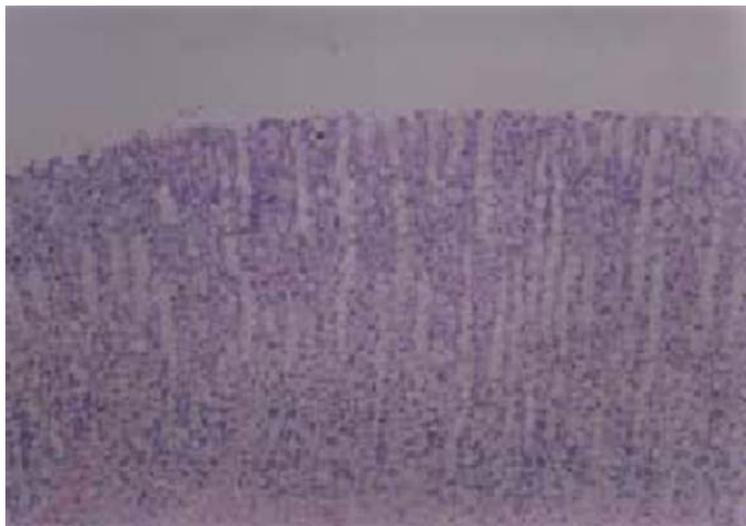


Fig. 4 : Gastric mucosa after treated with potassiumdicitro bismuthate (III) complex (H&E. x100). - value are mean \pm SE 5 rat /group . Significantly different from the control group. $P <$

Sever heamorrhagic lesions were found in the stomachs exposed to HCl –ethanol, the mean lesions area in saline treated group (7.44mm^2) was significantly reduced in potassiumdicitro bismuthate (III) treated groups (50 , 100 mg /Kg) and Sucralfate (100 mg /Kg) to (1.39 , 0.45) mm^2 and (0.45) mm^2 ,respectively (Fig. 5) .

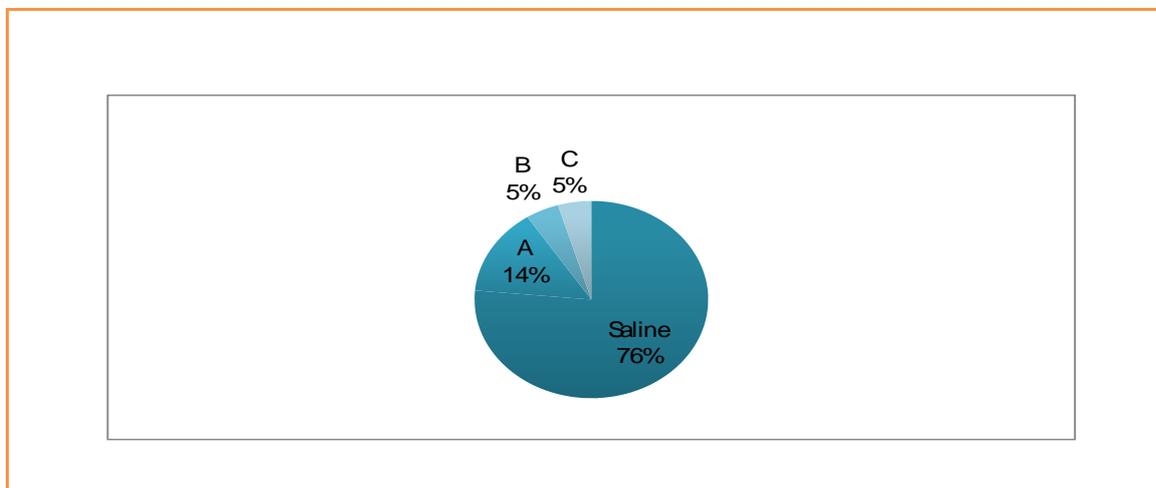


Fig. 5 : Effect of tripotassiumdicitro bismuthate (III) (50, 100 mg/kg, orally) and Sucralfate (100 mg /Kg ,orally) on lesion score on HCL-Ethanol induced gastric ulcer in rat - Value are mean \pm SE 5 rat /group.
(A) potassiumdicitro bismuthate (III) 50mg/Kg, (B) Sucralfate 100mg/Kg, (C) potassium dicitro bismuthate (III)100mg/Kg .

Potassiumdicitro bismuthate (III) treated groups have shown significant percentage protection (74.67 % , 91.2 %) at ($P < 0.05$) with the dose of (50, 100 mg /Kg ,orally) respectively in comparison to saline treated group (0.0%), the high dose of tripotassiumdicitro bismuthate (III), is almost same as that of standard Sucralfate group (93.2 %) (Table1).

Table 1: Effect of tripotassiumdicitro bismuthate (III) complex and Sucralfate drug on ethanol induced gastric ulcer in rats.

Treatment	Dose mg\ kg	No of rats	Ulcer index	% Protection
Control (Saline)		5	28.72 ± 7.9	0.0
Sucralfate	100	5	$1.57 \pm 1.3^*$	93.2
tripotassiumdicitro bismuthate (III)complex	50	5	$6.34 \pm 3.9^*$	74.67
tripotassiumdicitro bismuthate (III) complex	100	5	$2.52 \pm 2^*$	91.2

- Value is mean \pm SE 5 rats /group.

*Significantly different from the control group. $p < 0.05$.

Bismuth compound have been used to treat gastrointestinal disorder since 18th century (Scarpignato and Pelosini, 1999). The results of this study showed that the oral administration of tripotassiumdicitro bismuthate (III) prevent gastric mucosal injury caused by HCl –ethanol. The most commonly employed test in the evaluation of antiulcer and cytoprotective activities (Konturek *et al.*, 1998, Toma *et al.*, 2005), the ability of the gastric mucosa to resist injury by endogenous secretion (acid , pepsin and bile) and by ingested irritant (eg. Alcohol) can be attributed to a number of factors that have been generally referred as mucosal defense (Wallace, 2001).

The formation of gastric mucosal lesion by necrotizing agents such as ethanol caused ulcer due to perturbation of superficial epithelial cells, notably the mucosal mast cell leading to the release of the vasoactive mediators including histamine , thus causing damage gastric mucosa (Miller and Henahan, 1984). Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin (Hollander *et al.*, 1984), and generation of free radicals and the production of leukotriens (Toma *et al.*, 2005, Glavi and Szabo, 1992).

The result show that tripotassiumdicitro bismuthate (III) probably exhibits gastroprotective action against ulcerogenic HCl –ethanol because it has been found to stimulate mucosal generation and luminal release of PG it has been suggested that this protection mediated by PG , and the cytoprotective effect of tripotassiumdicitro bismuthate (III) observed in this study which could be attributed to the endogenous generation of PG , was responsible for maintaining the cellular integrity of the gastric epithelium, therefore, such endogenous release of PG would play a physiological role in protecting the gastric mucosa. (Robert *et al.*, 1984; Pugh and Lewin, 2008).

Bismuth compounds (Fraser, 2004) have antibacterial properties, and used successfully in the treatment of *Helicobacter pylori* induce ulcer (Larsen *et al.*, 2003). In addition to the bactericidal properties, bismuth has also shown to decrease mucin viscosity, prevent bacterial digestion of mucus, and reduce adherence of bacterial to gastric epithelium cells (Larsen *et al.*, 2003), as well as the bismuth drugs, speed the ulcer healing by affecting the binding of epidermal growth factor to its receptor sites in the gastric mucosa (Konturek *et al.*, 1988) and epidermal growth factor accumulated in the ulcer area also has been growth promoting action that accelerates ulcer healing similar to that of gastrin (Takeuchi and Johson, 1986). All these properties characterize that bismuth drugs used for treatment erosive ulcers in gastrointestinal tract (Maev *et al.*, 2008).

Histological study

Pretreatment with potassiumdicitro bismuthate (III) complex at (50, 100 mg /Kg, orally) completely protects the gastric mucosa against different histological changes (congestion, hemorrhage, edema, necrosis, inflammatory change, erosion and ulceration) caused in HCl –ethanol treated rats (Table 2).

These results were further supported by histopathological changes in the form of congestion, hemorrhage, edema, necrosis, inflammatory change, erosion and ulceration caused by the destructive stimuli in the gastric tissue, this confirms the cytoprotective ability of the tripotassiumdicitro bismuthate (III) complex which may be attributed to its selective binding to the ulcer base to form a protective barrier against acid – pepsin attack and to the enhancement of the re epithelialisation of the ulcerated mucosa (Wrkis *et al.*, 1982).

Table 2: The effect of potassiumdicitro bismuthate (III) complex potassium dicitro bismuthate (III)x on ethanol-HCl induced histopathological lesions in gastric mucosa

Treatment	Histopathological lesions					
	Ulceration	Edema	Necrosis	Congestion	Inflammatory change	Erosions
Control	-	-	-	-	-	-
(ethanol-HCl)	++	+	+	+++	+	++
Tripotassiumdicitro bismuthate complex 50mg/Kg orally	-	-	-	-	-	-

(-) Normal, (+) moderate effect,(++) sever effect,(+++) intensely sever effect.

CONCLUSION

Over all, The obtained results demonstrate that potassiumdicitro bismuthate (III) complex display a good anti-ulcer activity , this protective effect might be have been mediate by both cytoprotective mechanisms and the compound have ability to restore mucosal PG to normal levels, thereby mediating PG dependent mechanism might play some role in the healing of chronic peptic ulcer.

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