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Ameliorating effect of coconut water on the epithelium and gastric goblet cells of albino wistar rats induced with castor oil

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Abstract

Determination of ameliorating effect of coconut water on the epithelium and gastric goblet cells was evaluated using castor oil induced-diarrhoea in adult Wistar rats. 30 adult male Wistar rats weighing 150 – 250 g were used in this study. The animals were randormly divided into 5 groups of 6 animals each and were fed with Growers feed and clean drinking water ad libitum. Group A was the control and received 5 ml of distilled water, group B received 2ml of castor oil orally, group C received 2ml of castor oil + 30ml of coconut water/kg bd. wt, group D received 2 ml of castor oil + 40 ml of coconut water/kg bd. wt. while group E received 2 ml of castor oil + 4 mg of loperamide/kg bd. wt orally for 5days. The animals were sacrificed for histological studies and determination of serum levels of Na^+, Cl^-, K^+ and HCO_3^-. Alcian blue stain was used. Data were analysed with window SPSS (version 15.0) using analysis of variance (ANOVA). Result reveals that coconut water given orally to rats in all test groups significantly reduced copious diarrhoea, elevate electrolyte levels in blood and protects the gastric mucosa and other components of the stomach from the irritating stimuli produced by castor oil induced-diarrhoea. It is concluded that coconut water may be useful in the treatment of diarrhoea especially in areas where conventional anti-diarrhoeal drugs like loperamide and others are not readily accessible. Coconut water may be used as an emergency anti-diarrhoel medium.

Keywords: Diarrhoea, coconut water, electrolyte, goblet cells, epithelium

1. Introduction

1.1 Background

Coconut (*cocos nucifera*, L) is the most important and extensively grown palm tree worldwide. Every part of the plant is useful and in many cases human lives would be impossible in its absence [1]. The leave and trunk provide building material and the fruit is used as medicine [2]. The fruit is the most marketable part; the envelope (mesocarp) called the husk is processed into ropes, carpets, geotextiles and growing media. The hard brown shell (endocarp) can be processed into very high activated charcoal. The inner part of the nut (endosperm) is divided into two edible parts: a white kernel and a clear liquid called coconut water [3]. For more than a century, the coconut pulp or kernel has been considered as a cash crop because of its high fat content; however, nowadays, coconut is more than just an oil seed. The dried kernel was a very important international commodity in the first part of the 20th century. Food and chemical industries processed the lauric oil extracted from the dried kernel into margarine or detergent. However in the past 20 years, the volume of world trade in dried kernel has decreased by 75% percent while the export of fresh coconut has increased by three hundred percent, the market for canned coconut milk, coconut cream and coconut juice/water is increasingly considerably [4, 5, 6]

Coconut is no longer an international oil commodity but is becoming a valuable fresh fruit. Coconut water differs from coconut milk which is the oil liquid extracted from the grated fresh kernel. In most cases, coconut water comes small and scarce as coconut tree plantations are more related to garden. As a consequence the coconut water remains a traditional and underused resource which could thus be considered as an exotic beverage by most people living far from the coconut production area [7]. An increasing international demand for this product could be a highly positive issue for thousands of Africans and small Asian farmers. Coconut water is not only a tropical beverage but also a traditional medicine [8], a microbiological growth medium[9] and a ceremonial gift [10] and can be processed into vinegar [11, 10]. In the Indian ayurvedic medicine it is believed to increase semen, promote digestion and to clear the urinary path [8]. There are many references to medicinal uses of coconut in Sri Lanka, a country where coconut is consumed on a daily basis [2]. Coconut water is traditionally prescribed for burning pain during urination, dysuria, burning pain of the eye, indigestion, hiccups or even expelling of retained placenta. In case of emergency in remote regions of the world and during World War II, coconut water was used as a short term intravenous hydration and resuscitation fluid [11, 12]. In the early 1960s, coconut water was already known to favour microbial growth especially "Nata de coco'' bacterium [13]. Total world coconut cultivation area in 1996 was estimated at 11 million hectares, and around 93% is found in Asian and Pacific regions [14]. The two biggest producers, Indonesia and the Philippines, have about 3.7 million hectares and 3.1 million hectares, respectively. India is the third largest producer. In the South Pacific countries, Papua, New Guinea is the leading producer. In Africa, Tanzania is the largest producer while in Latin America Brazil accounts for more than one half of the total coconut area for that region [15].

Coconut water has a specific taste and flavour different from the well-known fragrance of the coconut kernel. This beverage has an aroma which has never been fully characterised. Like organic acid composed of malic, succinic, citric, acetic and tartaric acid [16, 17] which contribute to the aroma of the fresh liquid. The most significant change during the ripening process is the volume of nut water [18]. As the nut matures, there is an increase in the nut holding water capacity until the kernel begins to form a jelly inside the cavity of the fruit. Then, the water volume decreases as it is gradually used by the fruit to form the kernel. Compared with other components of coconut water, sugars varied the most throughout the ripening process [19, 20, 21].

Castor oil is a stimulant laxative used in the treatment of constipation, Castor oil is a colourless to very pale yellow liquid with a distinct taste and odour once first ingested. Its Boiling Point is 313 °C (595 °F) and its density is 961 kg/m [22]. It is a triglyceride in which approximately 90 percent of fatty acid chains are ricinoleate. Oleate and linoleates are the other significant components. Castor oil and its derivatives are used in the manufacturing of soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceuticals and perfumes [23].

The oil obtained from the seeds of castor oil plant *Ricinus comuinis* is one of the oldest drug known to man. The first mention of it as a laxative can be found in 3500 year old ancient Egyptian papyrus scrolls, it was also used for medicinal purposes in Greek and Roman times and for many centuries, it has also been used to induce labour. Castor oil produces permeability changes in the intestinal mucosal membranes to water and electrolytes resulting in fluid and watery luminal content that flows rapidly through the small and large intestines [24]. This is brought about by the irritant effect of ricinoleic acid liberated by pancreatic lipases which hydrolyse the oil derived from the seeds of *Ricinus communis*. Clinically, diarrhoea may result from disturbed bowel function, in which case there is impaired intestinal absorption, excessive intestinal secretion of water and electrolyte and a rapid bowel transit [25].

2. Method

Coconut water was obtained from fresh Coconut from a local village in Uruan local government area of Akwa Ibom State. The water was extracted and preserved in the refrigerator. Phytochemical analysis of coconut water was done according to the methods of [26] and [27]. Test for electrolytes, carbohydrates and other sugars, dietary fibre and vitamins were carried out. Thirty adult albino rats (weighing 150-250g) of wistar strain were used for this research. The animals were housed in standardized environmental conditions, maintained at 12hr light and dark cycles and kept at room temperature of 27-30 ºC in clean cages in a well-ventilated room at the animal house of the Faculty of Basic Medical Sciences, University of Uyo. They were fed with Growers feed and clean drinking water ad libitum. All rats were handled according to standard guidelines for the care and use of laboratory animals (Association for Assessment and Accreditation of Laboratory Animal Care, 2009) and the research was approved by the ethical committee of the Faculty of Basic Medical Sciences, University of Uyo. The animals were divided into 5 test groups of 6 animals each. Group A was the control and received 5ml of distilled water, group B received 2 ml of castor oil orally, group C received 2ml of castor + 30ml/kg bd.w of coconut water orally, group D received 2ml of castor oil + 40ml/kg/bd.w of coconut water orally while group E received 2ml of castor oil +4mg/kg bd.w of loperamide orally. 4hrs after the last dose schedule the animals were anasthesised using chloroform and sacrificed where the stomachs were taken out and preserved in 10% buffered formalin and blood sample taken to determine the serum levels of the electrolytes. The tissues were processed histologically.

2.1 Staining method

Alcian blue staining method was used

2.2 Data analysis

Data were analysed using analysis of variance (ANOVA). All data were expressed as means ± standard error of mean. Data were analysed using window SPSS (version 15.0).

3. Result

3.1 Phytochemistry of coconut water

Phytochemical analysis of coconut water indicated the presence of the following constituents; potassium, sodium, magnesium, manganese, vitamins (B_1, B_2, B_3, B_5 and B_6), protein, dietary fibre, fat, calcium, iron, magnesium, folate, sugars, carbohydrates, phosphorus, potassium, zinc and water.

3.2 Electrolyte Analysis

Analysis of K^+, Cl^-, Na^+ and HCO_3^- ions indicated a relative reduction in the haematological levels of the ions in group administered with castor oil alone. Pretreatment with coconut water elicited significant ($p<0.05$) increase in the haematological levels of the ions in groups administered with 30ml/kg bd.wt of coconut water with 2ml/kg bd. wt of castor oil, 40ml/kg bd.wt of coconut water with 2ml/kg bd. wt of castor oil and 4mg/kg bd.wt of loperamide respectively. The activity was similar with that of the standard anti-dairrhoeal drug, Loperamide (Table I**).**

Table I: Serum electrolyte analysis in rats

Group	Treatment	K^+ ($mmolL^{-1}$)	Na^+ ($mmolL^{-1}$)	Cl^- ($mmolL^{-1}$)	HCO_3^- ($mmolL^{-1}$)
Control	Water	5.00 ± 0.00	140.20 ± 0.20	94.10 ± 0.50	21.50 ±1.60
B	Castor oil	2.10 ± 0.20^{i}	125.00 ± 0.40^{i}	79.00 ± 2.80^{i}	18.50 ±1.20^{i}
C	Cw (30ml/kg)	4.30 ± 0.00$^{i, ii}$	138.50 ± 0.20$^{i, ii}$	90.60 ± 0.20$^{i, ii}$	20.30 ±0.20$^{i, ii}$
D	Cw (40ml/kg)	4.80 ± 0.10^{ii}	139.20 ± 0.10^{ii}	92.20 ± 0.20^{ii}	20.80 ± 0.20^{ii}
E	Lp (4mg/kg)	5.00 ± 0.10	140.10 ± 0.20	93.60 ± 0.40	21.40 ± 1.60

Cw- coconut water, Cw - coconut water, LP - loperamide, n = 5, $^{i, ii}$ $p<0.05$ compared to control

3.3 Microscopic Observations

The Alcian Blue sections of the stomach of control rats given water showed the presence of well-defined/distinct mucosa with epithelial lining (EL), numerous Goblet cells (GCs) picking the Alcian Blue stain within the mucosa. muscularis mucosa (MM**)** and submucosa (SM) with component blood vessel (BV) at magnifications of X100 and X400. Alcian Blue sections from the stomach of rats administered with 2ml of castor oil alone showed severe surface epithelial erosion (SEE), degeneration of connective tissue components (DCT) in the submucosa and Goblet cell metaplasia at magnifications of X100 and X400. Alcian Blue Sections from the stomach of rats administered with 30ml/kg b.w of coconut water and 2ml of castor oil showed mild epithelial erosion (MEE) and mild Goblet cell distortions (MGCD) at magnifications of X100 and X400 while the Alcian Blue sections of the stomach of rats administered with 40ml/kg b.w of coconut water and 2ml of castor oil showed improvement in the histological appearance at magnifications of X100 and X400 when compared to the positive control administered with 4mg/kg b.w of loperamide, the standard anti-diarrhoeal drug with 2ml of castor oil at magnifications of X100 and X400. In both groups the Goblet cells appear to be so distinct with pale staining basophilic cytoplasm.

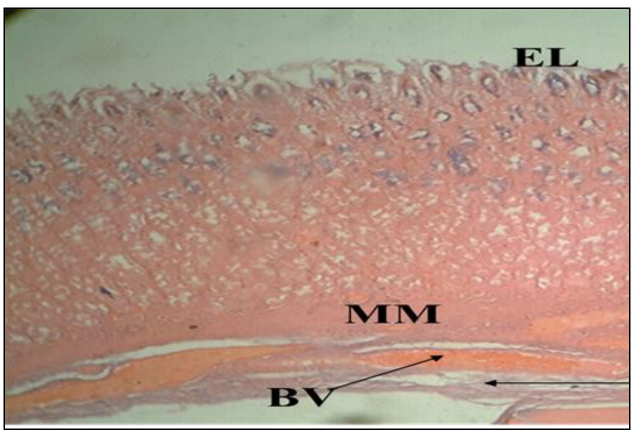

Fig 1: Photomicrograph of the stomach from rats in control group (A) showing well defined/distinct mucosa with epithelial lining (EL), Goblet cells (GCs), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100).

Fig 2: Photomicrograph of the stomach from rats in group A (2ml/kg bd. wt of castor oil showing severe epithelial erosion (**SEE**) (Alcian Blue X100).

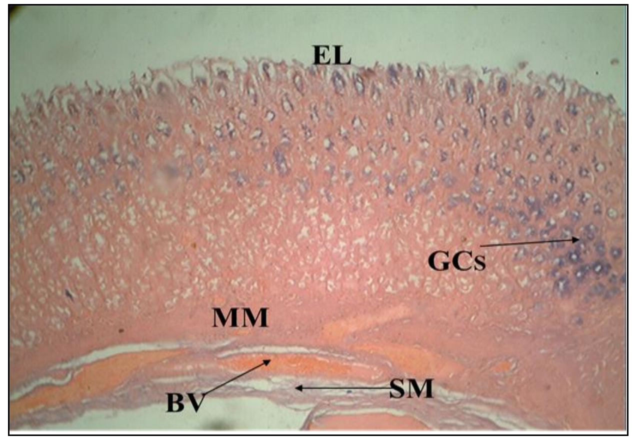

Fig 3: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL), Goblet cells (GCs), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100).

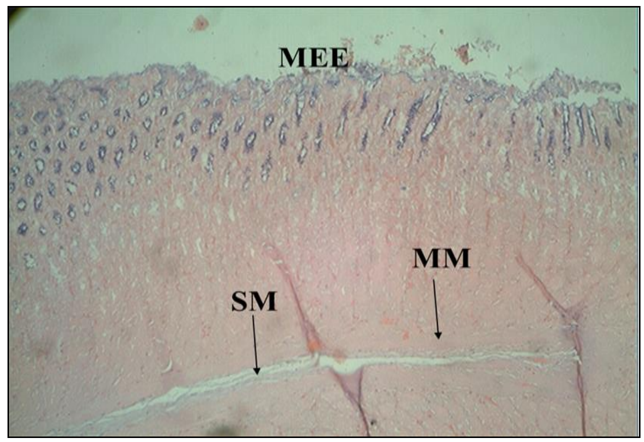

Fig 4: Photomicrograph of the stomach from rats in group A_3 (30 ml/kg bd. wt of coconut water + 2ml/kg bd. wt of castor oil) showing mild epithelial erosion (MEE), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100).

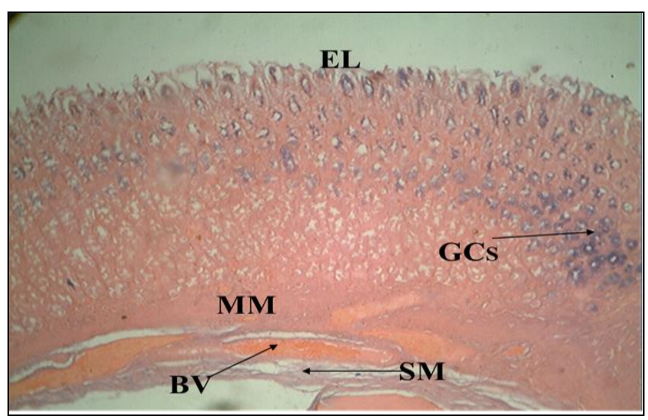

Fig 5: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL), Goblet cells (GCs), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100).

Fig 6: Photomicrograph of the stomach from rats in group A_4 (40ml/kg bd. wt of coconut water +2ml/kg bd. wt of castor oil) showing improvement in the mucosa with epithelial lining (EL), muscularis mucosa (MM) and submucosa (SM) with component blood vessel (BV) (Alcian Blue X100)

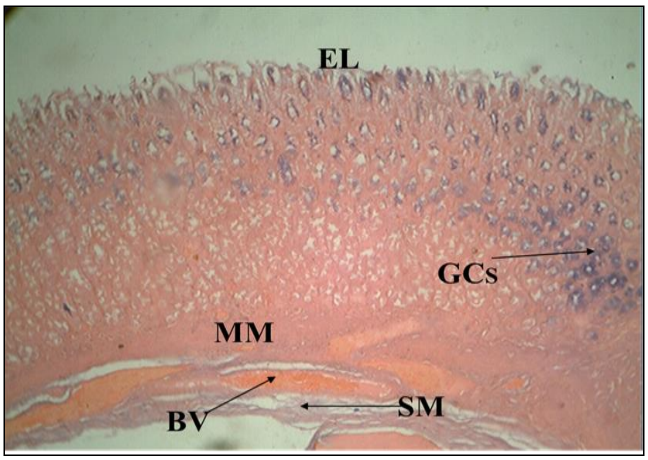

Fig 7: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL), Goblet cells (GCs), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100).

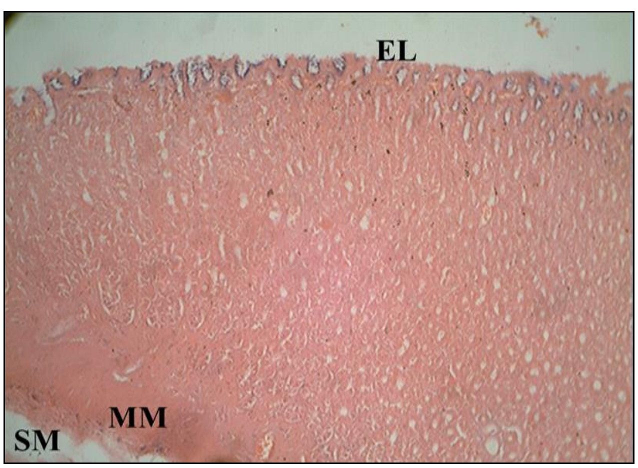

Fig 8: Photomicrograph of the stomach from rats in group A_5 (4mg/kg bd. wt of loperamide + 2ml/kg bd. wt of castor oil) showing improvement in the mucosa with epithelial lining (EL), muscularis mucosa (MM) and submucosa (SM) (Alcian Blue X100)

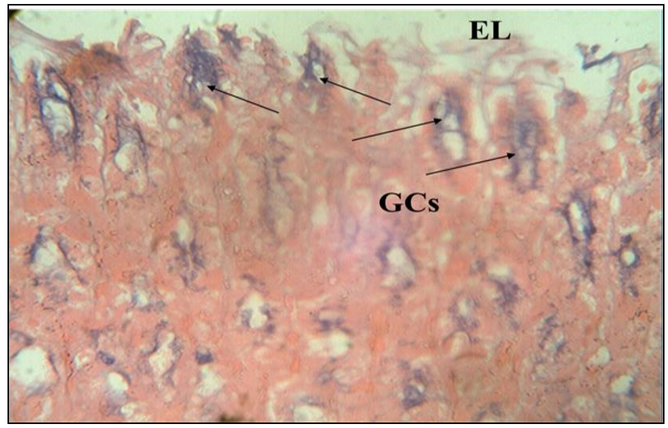

Fig 9: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

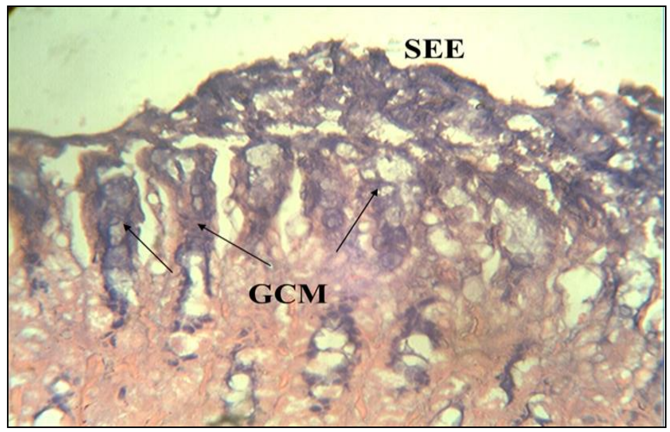

Fig 10: Photomicrograph of the stomach from rats in group A_2 (2ml/kg bd. wt of castor oil) showing severe epithelial erosion (**SEE**) and goblet cell metaplasia (**GCM**) (Alcian Blue X400).

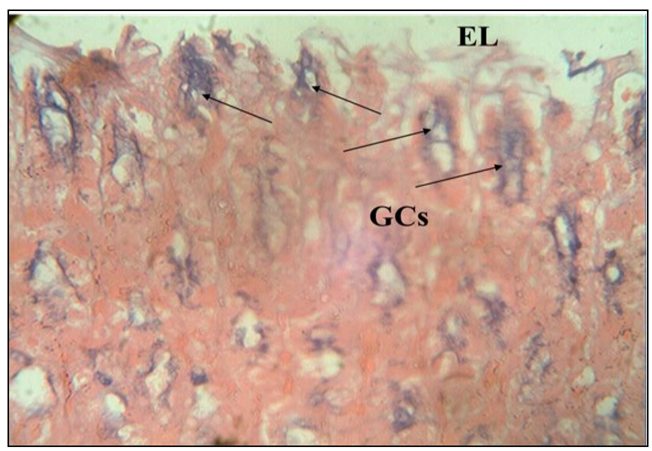

Fig 9: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

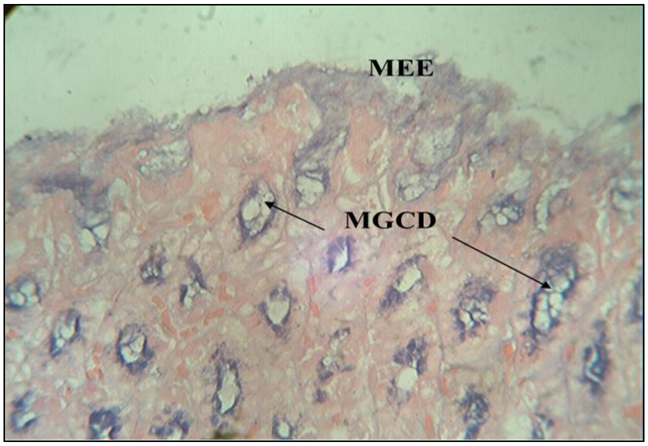

Fig 12: Photomicrograph of the stomach from rats in group A_3 (30ml/kg bd. wt of coconut water + 2ml/kg bd. wt of castor oil) showing mild epithelial erosion (MEE) and mild Goblet cell distortion (MGCD) (Alcian Blue X400).

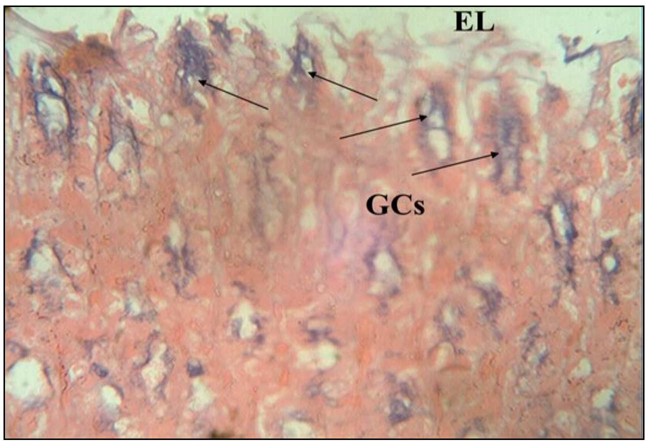

Fig 13: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

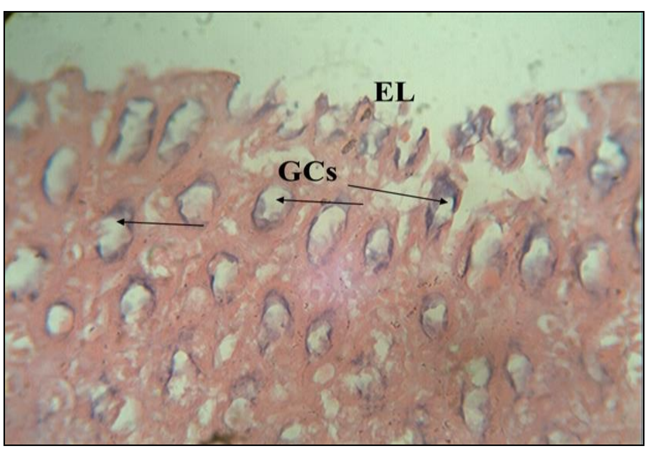

Fig 14: Photomicrograph of the stomach from rats in group A_4 (40ml/kg bd. wt of coconut water + 2ml/kg bd. wt of castor oil) showing improvement in the epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

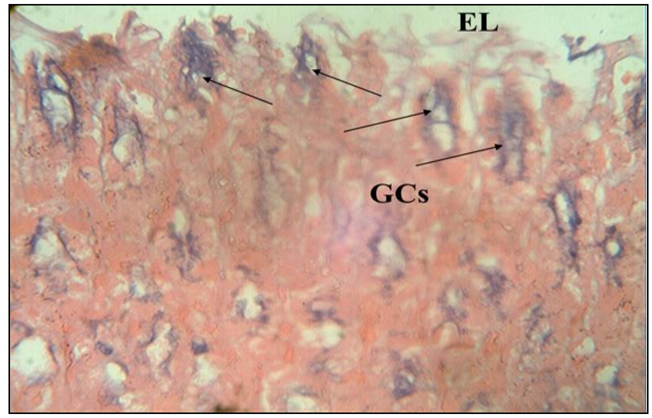

Figure 15: Photomicrograph of the stomach from rats in control group (A_1) showing well defined/distinct mucosa with epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

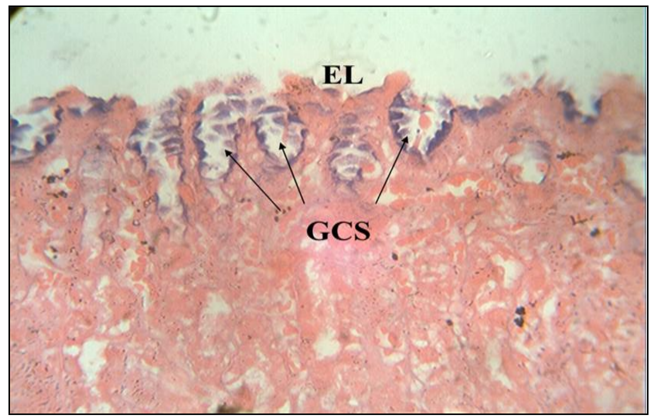

Fig 16: Photomicrograph of the stomach from rats in group A_5 (4mg/kg bd. Wt. of loperamide + 2ml/kg bd. wt of castor oil) showing improvement in the epithelial lining (EL) and Goblet cells (GCs) (Alcian Blue X400).

4. Discussion

Results of this finding show that rats with copious diarrhoea indicated decrease in the level of plasma electrolytes when compared to the control, there was reduction in the plasma levels of sodium, chloride, potassium and bicarbonate ions. This may be due to the widely reported loss of electrolytes during diarrhoea resulting in body weakness, anaemia and sometimes death [28]. Rats pretreated with coconut water and loperamide expressed a relatively normal plasma electrolyte levels almost similar to those in the control. This increase might be due to the presence of electrolytes in coconut water suggesting that coconut water may improve dehydration during diarrhoea. Oxidative stress plays an important role in the pathogenesis of various diseases including diarrhoea with antioxidants being reported to play a significant role in the protection of gastric mucosa and glands against necrotic agents [29]. Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing body's defensive system against degenerative diseases, example in the case of diarrhoea.

Coconut water produces a dose dependent and significant ($P<0.05$) protection against castor oil induced diarrhoea from mechanisms such as direct toxin action, reduction of the secretion of bicarbonate and depletion of gastric wall mucus [30]. Anti-diarrhoeal agents are thought to increase endogenous glutathione and prostaglandin levels and decreases the release of histamine, increases the influx of calcium ion and generation of free radical [31]. Thus, the action of coconut water in this model suggests cytoprotective action possibly mediated through enhancement of mucosal defensive factors such as protection from free radicals. Furthermore histological sections from the sections of stomach of rats administered with 2ml/kg bd. wt of castor alone when compared to the control showed Goblet cell metaplasia. Goblet cells are the most thoroughly studied mucus-secreting cells, these cells have numerous large, lightly staining granules containing strongly hydrophilic glycoproteins called mucins and secretion from Goblet cells is activated by the presence of external stimulus [32]. Mucus secreted by the Goblet cells is responsible for protecting the gastric mucosa against damage. The result from this study reveals that atrophy and metaplasia indicated cellular adaptation in response to toxic stimulus from chemicals present in castor oil.

Administration of 30ml/kg and 40ml/kg bd. Wt. of coconut dose dependently improved surface epithelial erosion experienced in castor oil treatment group when compared to the group administered with loperamide, the standard anti-diarrhoeal agent. This effect may be correlated to the anti-oxidants present in coconut water. Naturally occurring anti-oxidants found in fruits and other vegetables seem to be more protective than supplements [33]. This would suggest that fruits and vegetables contain substances other than the identified anti-oxidants that can reduce the risk of gastritis and other gastric problems. Anti-oxidants are involved in protecting the body from oxidation, a natural process but some could also be attributed to the development of chronic diseases and the changes associated with ageing[33]. This suggesst that some of the proteins found in coconut water has anti-oxidant properties, however more supporting evidence is needed.

5. Conclusion

In conclusion, coconut water may be used as an emergency antidiarrhoel medium especially in cases where conventional medications like loperamide are not readily accessible.

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