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Histopathology and Anticolon Cancer Effects of Turmeric Ethanolic Extracts in Wistar Rats

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Abstract

Colon cancer is cancer that begins in the large intestine. It usually begins as small, non cancerous clumps that form inside the colon. It typically affects older adults and can occur at any age. The aim of this study is to investigate the histopathological changes of the colon of rats experimentally induced with cadmium chloride as well as the anticancer effect of the ethanolic extract of *Curcuma longa*. Thirty six healthy albino rats of both sexes were classified into six groups with each group comprising of five rats as follows ; group I: normal control rats, group 2: Cadmium Chloride induced rats, group 3: Aduracil-5 fluorouracil treated rats, Group 4: dimethyl sulfuroxide treated rats, groups 5 and 6 rats were treated with 875mg/kg and 437.5mg/kg of *Curcuma* ethanolic extracts respectively after initial induction of cancer with cadmium chloride. Comparative observation of the cadmium induced colon showed histopathological damage as evidenced by empty goblet cells,

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lacerated and suppressed mucosa, destruction of the surface epithelium, features which were lacking in the control and *Curcuma longa* treated colon. *Curcuma* treated colon exhibited marked improvement of the histomorphology whilst cadmium treated colon clearly showed tumor cells, cancer cells or invasive inflammatory cells. Adruracil-5fluorouracil{an established anti colon cancer drugs} treated colon showed mild improvement in the histomorphology of the colon while the dimethyl sulfuroxide treated colon revealed an insignificant impact. The ameliorative effect on rat colon occasioned by administration of *Curcuma* ethanolic extract suggests that the plant product may have therapeutic activity against colon inflammation in albino rats.

Keywords: Curcumin, Histopathology, Cadmium Chloride, Wistar Rats, Anti-colon Cancer, Carcinogen

Introduction

Colon cancer, a disease of the large intestine is now of great public health concern all over the world. (Chan *et al.,* 1983) Dietary factors are considered to play a major role in cancer etiology. Cancer is a serious clinical problem that posses significant social and economic challenges to the healthcare system (DeVincenzi *et al.,* 1991). Despite improved imaging and molecular diagnostic techniques, colon cancer continue to affect millions of people globally (Bardin *et al.,* (2014). Colorectal cancer is the third most common and leading cause of cancer related mortality (Ableman, 1993) cadmium is a toxic, hazardous and carcinogenic non essential heavy metal found in the air, water, soil and food. It is known to produce toxic effects in humans as well as in rats. The agency for toxic substance and disease registry (ATSDR) in Atlanta Georgia has listed cadmium as number 7 in its top 20 last of hazardous substances. Brouck *et al.,* (1975) interestingly, in contrast to the toxic activity of cadmium chloride. Inducing wistar rats with cadmium chloride initiated a drastic histopathological change in the architectural of the colon. Alteration of the mucosa epithelial glands were destroyed. So turmeric was used to treat this condition for a period of 14 to 56 days, It was reported that high dose of turmeric extract exhibited considerable protective efficacy in the rats than the low dose turmeric ethanolic extract treated rats. (Huang *et al*., 1987). Thus with these properties, this study intend to examine the histopathology changes in the colon associated with cadmium chloride necrosis, inflammation and ulceration. Hence, the aim ascertain the efficacy *curcumin* in rendering protection against cadmium chloride induced colonic toxicity at the cellular level.

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Methods

Collection of samples

Rhizomes of turmeric plants were bought from oil mill Market, in Port Harcourt, Rivers State. Properly cleaned of soil and dust by washing with tap water.

Plant extraction

After collection, the rhizomes were allowed at room temperature (25^{o}C – 30^{o}C) to constant room weight over a period of seven 30days. The rhizomes were taken to market, and pulverized by an industrial blending mechanize. A fine golden yellow earthy' smelling powder was obtained *Curcumin* powder. It is a warm bitter and pepper like flavor earthy mustard like-aroma. The powder was dissolved in ethanol, allowed for 72hrs for properly maceration and subsequently evaporated in a desiccation and obtained a fine extract. After the extract was taken and filtered by using a 0.45 millipore filter paper. With a rotary evaporator at 40^{o}C and 200rpm and subsequently, on a steam bath at 40^{o}C. The semi-solid extract obtained was bottles and labeled accordingly. The fine dark brown extracts were stored in desiccators at room temperature until when the need arises.

Experimental design

Thirty-six (36) animals were divided into 6 groups, 6 rats in each group.
Group 1 (normal) with six animals
Group 2 (negative control) had six animal
Group 3 positive control) had six animal
Group 4 (TEE – group low dose)
Group 5 (TEE – group High dose)
Group 6 (Dimethyl Sulphuroxide)
Through out the experiment, group 1 animal were not treated but were given free access to normal animal feed and water *adlabium*.
Group 2 animals were induced with Cadmium Chloride
Group 3 animals were induced with cadmium chloride latter were treated with standard Adrucil – 5- fluorouracil anticolon cancer drug.
Group 4 and 5 were treated with turmeric ethanolic extract 437. 5mg/kg low dose,while 875mg/kg represent high dose respectively. While group 6 was treated with DMSO. On day 14, 28, 42, 56 from each group rats were humanely sacrificed using diethyl ether as anesthesia, colon harvested for assessment and examination.

Challenging Apparently healthy Animal with Cadmium Chloride

Thirty (30) animal (groups 2- 6) were challenged with an induction dose of 20mg/kg of cadmium chloride. After induction, we observed signs like

weakness, anorexia, non-productive cough, watering stool, standing of the hairs or furs. Sluggishness, non-agility.the animals were prepared for treatment.

Preparation of the extract concentration and anticancer drug

The brown colour paste of turmeric extract were prepared by dissolving 5mg in 1ml of dimethyl sulfuroxide (DMSO) which was given to the rats by gavage. 0.02ml of Adrucil -5flourouracil (500mg/10ml) was administered intraperitonaeally to rats respectively.

Organ collection and assessment

After the animals were anaesthetized with diethyl ether in a desiccators, the colon was removed aseptically and was weighed and was kept in 10% buffered formaline for histological analysis.

Histopathology studies

Formalin fixed colon were removed from fixative and various histological procedures were conducted on the colon noting the length and its colour.

Grossing; received a colon tissue with smooth external surface measuring 11 x 1.0cm with grayish to tan in colour. It was cut to a size of 2mm to 4mm thickness. This was done to allow the fixative to readily penetrate the tissue.

Ascending grades of alcohol; the tissue was exposed to different concentrations of alcohol for processing by standard method described by Baker (1945). The various stages includes

Dehydrate; using ascending grade of alcohol concentration 2hour in each

Clearing; using two changes of xylene to remove alcohol.

Impregnation in two changes molten paraffinic wax. This step will take 2 hrs in each solution

Embedding in mould to give it a solid and firm support.

Microtomy; cutting tissue blocks into sections. colon tissues are sectioned using the precision device called the microtomy.

Staining colon sections with hematoxyhn and Eosin (H xE) and finally mounting it with a DPX mountant, cover slip and view under a light microscope.

Results

The effects of cadmium chloride in wistar rats resulted in a decreased activity rate in the rats. The animals at certain point were losing the furs on them, they look emaciated and became small physically. Some of the animals

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died. Cadmium chloride was administered intrarectally to all the animals. A slight decrease in their feeding as well as in sleeping habit was observed. The rats in normal control group were given only food and water all through the treatment period. There was no death recorded. The histopathology report reveals that the colon mucosa epithelial remained normal. No sign of toxicity seen. The rats in Adrucil-5-flourouracil group were treated with 0.5ml of 40mg/kg of Cdcl for cancer induction. Then after 28 days were treated with 0.2ml of 500mg/10ml adrucil 5-flourouracil from day 28 to day 56, 2 rats were recorded dead, the histology reveals that there was mild mucosal disruption.

The rats in Cadmium control group were treated with only cadmium chloride from day 0 to 28 days and was observed for the rest of the experimental period. Three (3) rats were recorded dead, the histology reveals severe laceration of the colon mucosal. The rats in the low and high dose Curcumin extract group were treated with 0.25ml (437.5mg/kg) and 0.5ml (875mg/kg) daily orally from day 28 to day 56 of the treatment period, no death was however recorded. Histology reveals no healing and eodenatous mucosa. The rats in the DMSO group were treated with 0.2ml of dimethyl surlfuroxide orally from day 28 to day 56 of the treatment period. DMSO was used to dissolve *Curcumin* extract. It was reported to have anti-cancer property hence we decided to make a group to ascertain that claim. Three (3) rats died in this group. Histology report reveals severe mucosal ulceration and inflammation.

The weight of the colon after administration of Cadmium Chloride ($CdCl_2$) as shown in table 4 revealed that there was no weight gain in the colon of the experimental rat in the Adrucil control, but at day 42 and 56 in the Cadmium group as a result of edematous and inflammatory action of Cadmium on the mucosa walls of the colon ensured weight gain.

During Cadmium Chloride induction, there was increased stool consistency scores, Cadmium decreased weekly weight gain this finding is consistent with previous reports by (Chiarenza *et al.*, 1989).

Table 1: Grossing Report of Rat Colon in control, Cadmium, Adrucil and DMSO Groups

Normal Control Group	The proximal and distal portions of the colon was the target for investigation. Normal colons shows smooth external surface with no obvious abnormally or defect present. The physical appearance is grayish white measuring 6.0 x 1.0cm.
Cadmium Group	Colon showed undulating surface measuring 6.0x2.5cm showing a tan to dark colour
Adrucil Group	Colon showed smooth external surface measuring 6.0x2.0cm showing grayish to tan colour appearance.
Dimethyl Sulfur Oxide Group	Colon tissue showed undulating and irregular shape measuring 6.0x2.0cm, showing soft to firm constituency appearing grayish white.

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Keywords:
Normal: (Animals not expose to any form of treatment but were fed *ad libitum)*
Adrucil group (Animals treated with anti-colon drug)
Dimethyl SuIphuroxide group (Animal treated with Dimethyl Surfur Oxide)

Table 2: Grossing Report of Animal Colon Treated with Ethanolic Extract

Day 7 (TEE,LD)	Received colon showed smooth external surface and patchy area of dilated areas measuring 7. 1 x 1.5cm
Day 7 (TEE,HD)	Received colon showed Undulating external surface, firm and distended areas with tan colour measuring 6.7x 2.0cm
Day 14 (TEE,LD)	Received partly tan to grayish white colon with firm portions having undulating surface length measuring 7.0 x 2.0cm
Day 14 (TEE,HD)	Received colon showed firm tumor measuring 3x1.5cm with soft areas at distal regions of the colon measuring 6.0 x 4.0cm
Day 28 (TEE,LD)	Received colon showed soft grayish undulating external surface measuring 6.5 x 1.5cm
Day 28 (TEE,HD)	Received colon showed undulating smooth external surface with grayish white colour measuring 6.5 x 1.5cm
Day 42 (TEE,LD)	Received colon showed smooth external surface with partly grayish colour measuring 6.0 x 1.0cm
Day 42 (TEE,HD)	Received colon showed smooth external surface partly grayish colour measuring 6.0 x 1.0cm
Day 56 (TEE,LD)	Received colon showed smooth grayish colour and pasty tan measuring 6.0 x 1.0cm
Day 56 (TEE,HD)	Received colon showed smooth grayish colour appearance measuring 6.5x1.0cm

Keywords:
TEE, LD: (Rats treated with low dose turmeric ethanolic extract)
TEE,HD: (Rats treated with high dose turmeric ethanolic extract)

Table 3: Colon Weight Gain after Cadmium Administration in the Control Groups

Colon weight of rat in various groups.	Days 14	28	42	56
	Colon weight in grams(g)			
Normal control	0.01	0.01	0.01	0.01
Adrucil -5-flourouracil group	0.01	0.01	0.01	0.01
Cadmium group	0.02	0.02	0.03	0.03

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Table 4: Histopathological Scoring of Rat Colon after Cadmium Induction and ric

Groups	**No of Rats**	**$Cdcl_2$ 40mg/kg (Day 0)**	**Histopathology Report After Induction**	**Adrucil 500mg/10mls (Day 7)**	***Curcumin* Extract 437.5/875mg/kg (Day 14)**	**DMSO 0.2mls (Day 28)**	**No of Death (Day 42)**	**Histopathology Report After Treatment (Day 56)**
Normal Control	9	-	No Ulceration or inflammation.	-	-	-	Nil	Normal colon
Cadmium Control	9	0.5	Severe necrosis	-	-	-	3	Lacerated colon
Adrucil Control	9	0.2	Severe Inflammation	0.2	-	-	2	Mild Mucosal disruption
***Curcumin* Low Dose**	9	0.5	Severe laceration	-	0.5	-	Nil	Complete reversal/repair
***Curcumin* High Dose**	9	0.25	Severe ulceration	-	0.25	-	Nil	Mucosal epithelia intact
DimethylSulfur Oxide	9	0.2	Severe ulceration	-	-	0.2	3	Moderate ulceration…

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Histological Plates of the Colon
Photomicrograph of the Rat Colon

The histopathology profile of the animal colon in Normal control group (Plate 1) reveals no aberrations, laceration, and the goblet cells present were filled, the mucosal *epithelial remain intact. The Photomicrograph of colon showing histopathological profile of* the animal colon treated with Andrucil -5 flourouracil standard in positive control (Plate 2, 7 and 12) showed localized area of glands within the mucosa, forming vague lymphoid folicules, enlarged goblets cells and lacerated muscular layer, reveals mucosal damage. The photomicrograph of rat colon in damage control treated with Cadmium Chloride ($Cdcl_2$) (plate 3, 8 and 13) showed blunt, suppressed and obviously delineating histopathological damage and total damage to the goblets cells were seen. Severe necrosis, inflammation and ulceration was seen and no smooth muscles. The photomicrograph of colon treated with (DMSO) (plate 4, 9 and 14) revealed severe inflammation as well as marked necrosis. The photomicrograph of colon treated with low dose turmeric ethanolic extract (plate 5, 10 and 15) revealed mild ulceration of the mucosal epithelial cell while the high dose turmeric ethanolic extract (plate 6, 11 and 16) displayed mild inflammation of the goblets cells with improved histomorphology suggesting that turmeric extracts exhibited anti-inflammatory responses to the histopathological damaged by cadmium.

Mucosa Epithelia
Lamina Propria
Epithelium
Intestinal Glands
Muscularis mucosa

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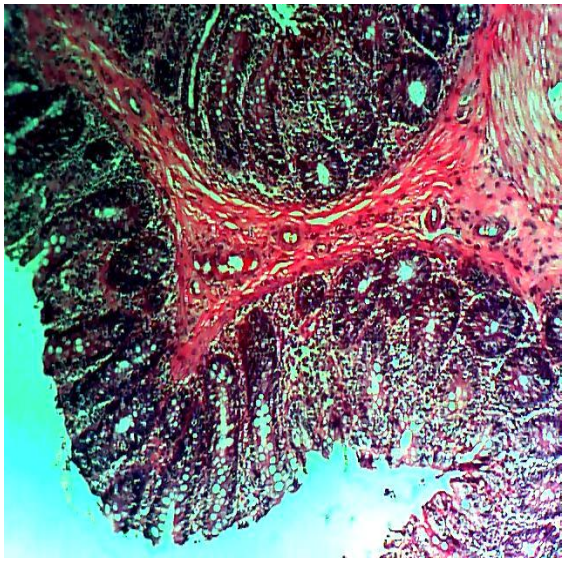

Plate 1: Photomicrograph of Normal colon
(Hematoxylin and eosin stain). Magnification X10. day 28

Normal group;
Photomicrograph of colon tissue of normal rats showing normal histo-architecture with the epithelium, muscularis mucosa, intestinal glands, lamina propria and mucosa epithelia were seen intact no laceration or ulceration, no aberration in the colonic mucosa.

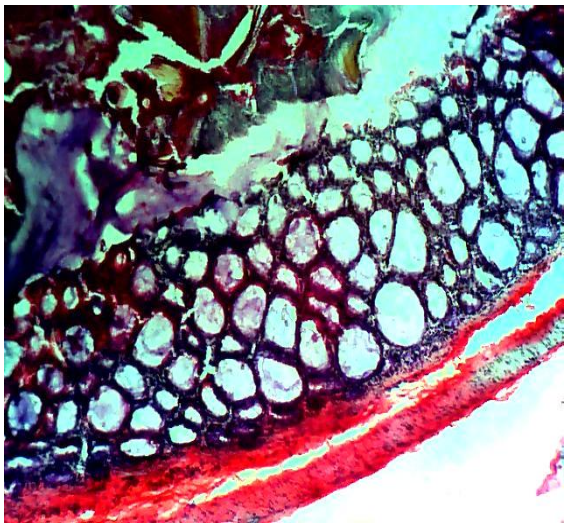

Plate 2: Photomicrograph of the colon of Adrucil-5-flourouracil treated Colon.
(Hematoxyline and eosine stain .MagnificationX10) day 28

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Photomicrograph of colon tissue showing localized area of glands within the mucosa, forming vague lymphoid folicules, enlarged goblets cells and lacerated muscular layer.

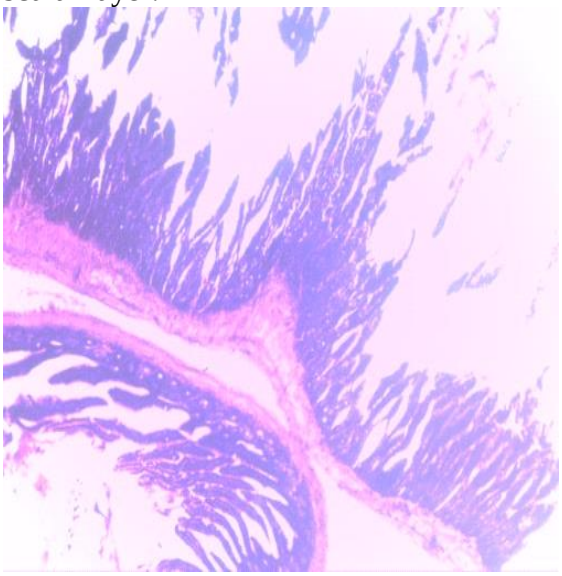

Plate 3: Photomicrograph of Cadmium treated colon
(Hematoxyline and eosine stain; Magnification X10)day 28

Photomicrograph of colon tissue showing area of necrosis and sever inflammation, no smooth muscles and cancer cells within the muscular area.

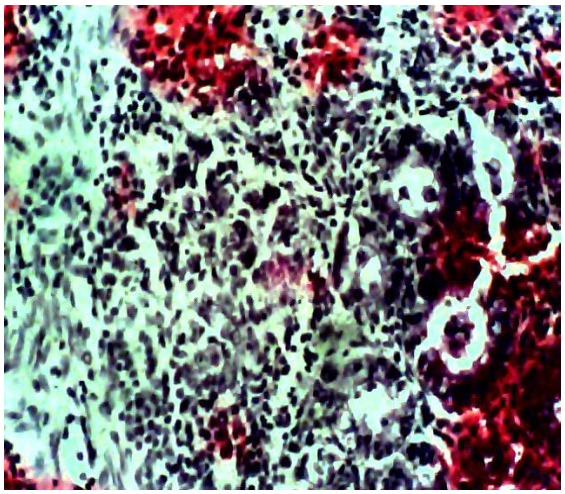

Plate 4: Photomicrograph Dimethylsulfuoxide treated column.
(Hematoxylin and eosin stain; Magnificationx10) day 28

Photomicrograph of colon tissue showing goblet cells lacerated and damaged, muscularies with glandular hyperplasia.

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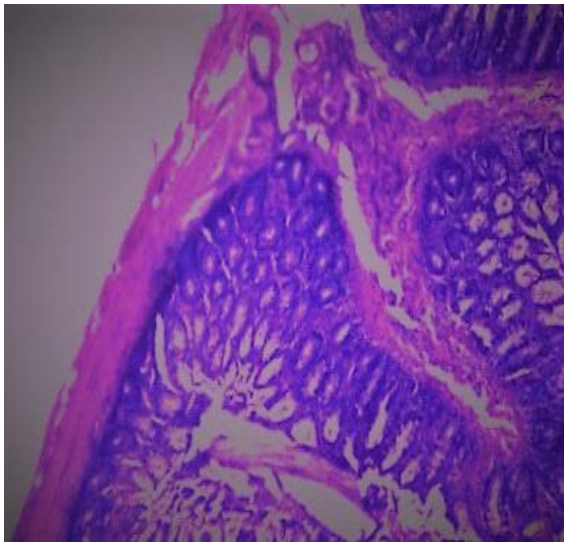

Plate 5: Photomicrograph Low dose *Curcumin* treated colon.
(Hematoxylin and eosin stain; magnificationx10) day 28

Photomicrograph of colon tissue showing goblet cells lacerated and damaged, muscularies with glandular hyperplasia.

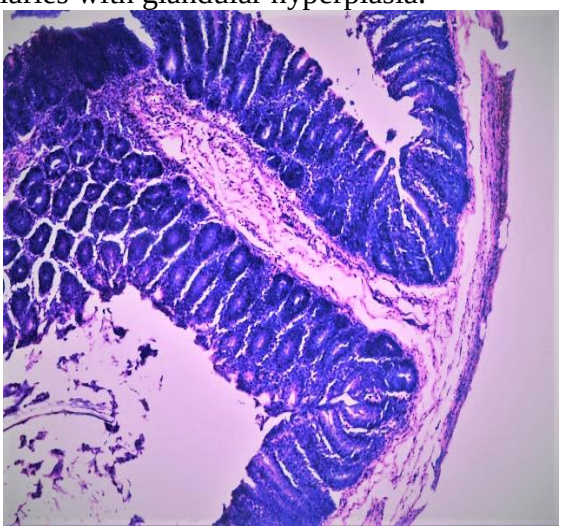

Plate 6: Photomicrograph of High dose *Curcumin* treated colon
(Heamatoxyline and eosine stain; magnificationx10)day 28

Photomicrograph of colon tissue showing areas of glands within the muscular area and goblet cells, few cancer cells with inflammatory cells.

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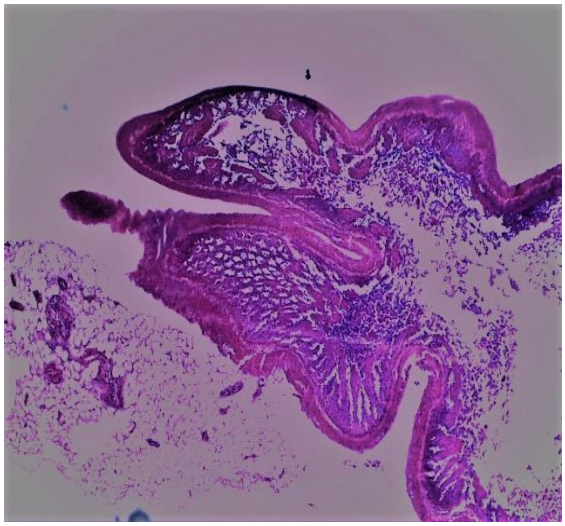

Plate 7: Photomicrograph of Adrucil-5-flourouracil treated colon.
(Hematoxylin and eosin stain; MagnificationX10) Day 42

Photomicrograph of colon tissue showing area of necrosis and inflammatory cells within the mucosa.

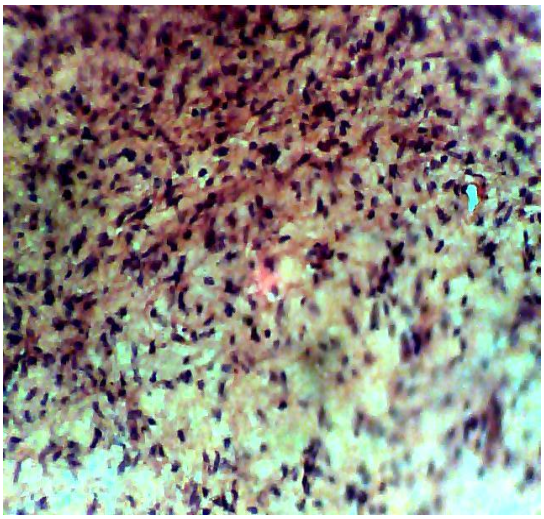

Plate 8: Photomicrograph of cadmium treated colon
(Hematoxylin and eosin stain; MagnificationX10) Day 42

Photomicrograph of colon tissue showing necrosis and sever inflammation, no smooth muscles and few cancer cells with inflammatory cells.

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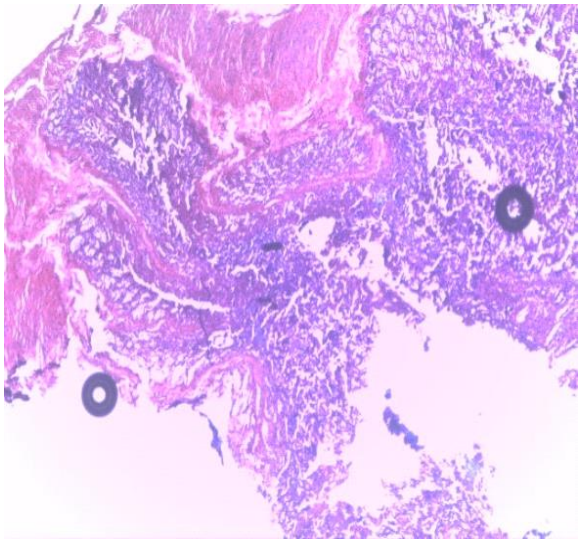

Fig 9: Photomicrograph of Dimethylsulphuroxide treated colon.
(Hematoxylin and eosin stain; MagnificationX10) Day 42

Photomicrograph of colon tissue showing goblet cells appear constricted with inflammatory cells.

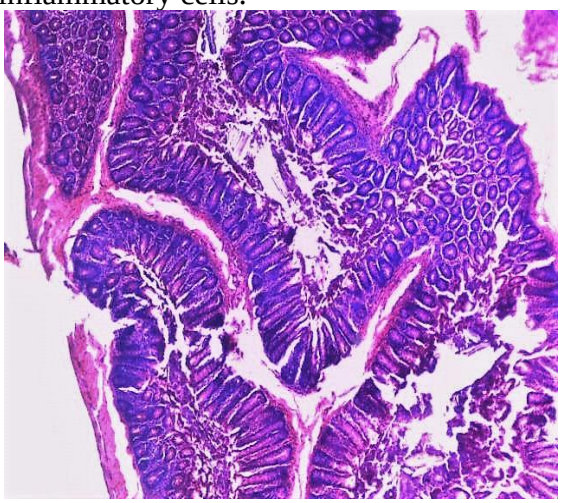

Plate 10: Photomicrograph of Low dose *Curcumin* treated colon.
(Hematoxylin and eosin stain;MagnificationX10) Day 42

Photomicrograph of colon tissue showing mild inflammation and blunt mucosa, goblet cells enlarged. Inflammation within the muscularis, goblets cells are reduced and area of necrosis within the adipose tissue.

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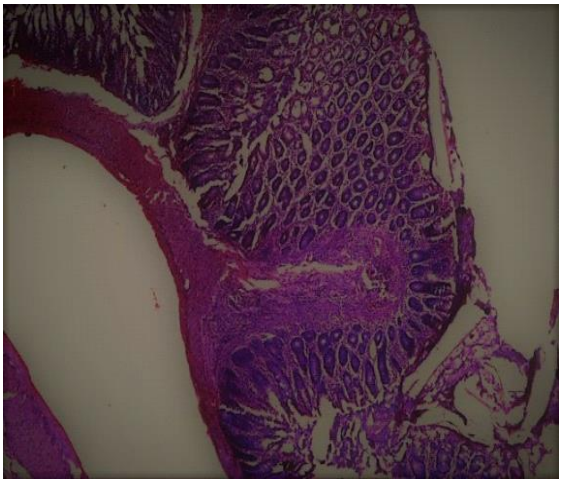

Plate 11: Photomicrograph of High dose *Curcumin* treated colon Hematoxylin and eosin stain; MagnificationX10) Day 42

Photomicrograph of colon tissue showing moderate inflammation and no cancer cells.

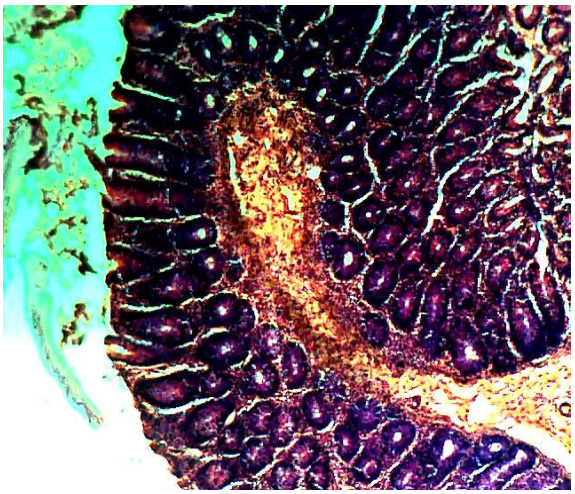

Plate 12: Photomicrograph of -5-Flourouracil treated colon (Hematoxylin and eosin stain; Magnification X10) Day 56

Photomicrograph of colon tissue showing mild inflammation and localized area in the muscularis filled with inflammatory cells.

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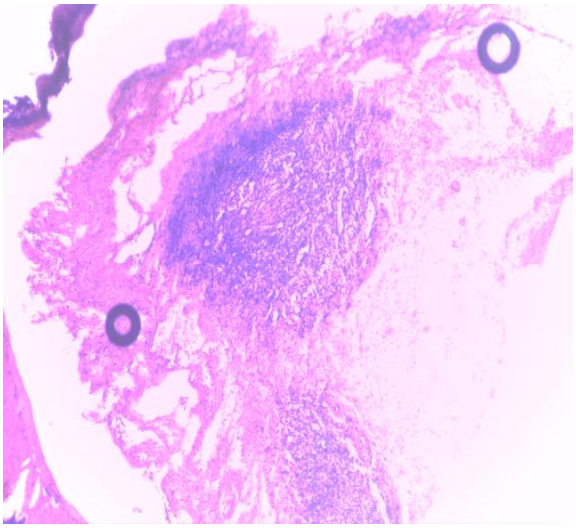

Plate 13: Photomicrograph of cadmium treated colon.
(Hematoxylin and eosin stain; Magnification X10) Day 56

Photomicrograph of colon tissue showing erosion of the mucosa epithelial, areas of necrosis and inflammatory cells and no cancer cells.

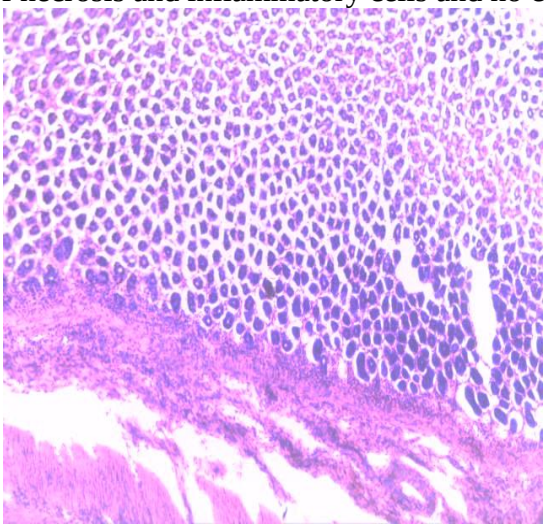

Plate 14: Photomicrograph of the colon of Dimethylsulfuroxide treated colon
(Hematoxylin and eosin stain; MagnificationX10.) Day 56

Photomicrograph of colon tissue showing ulcerated mucosal layer, complete damage of the goblet cells and mucosa epithelial.

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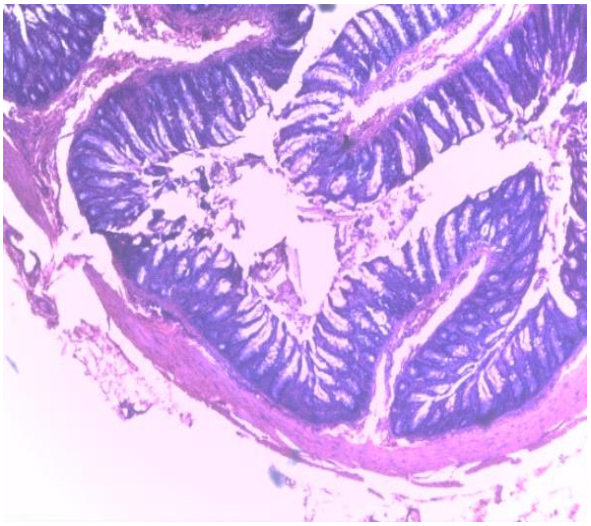

Plate 15: Photomicrograph of Low dose *Curcumin* treated colon
(Hematoxylin and eosin stain; MagnificationX10) Day 56

Photomicrograph of colon tissue showing mild area of inflammation in the muscular area, areas close to normal and no cancer cells seen.

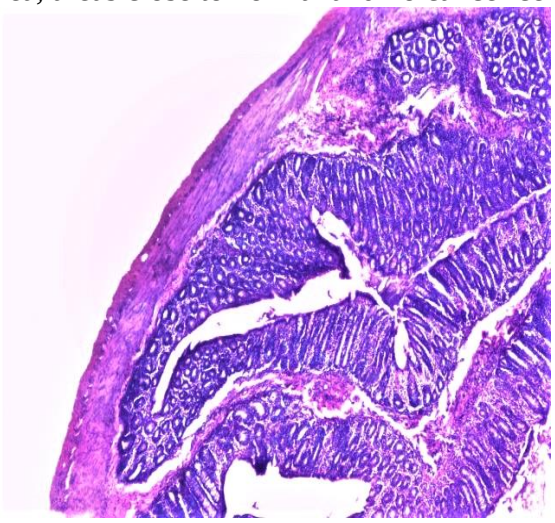

Plate 16: Photomicrograph of High dose Curcumin treated colon.
(Heamatoxylin and eosin stain; MagnificationX10.) Day 56

Photomicrograph of colon tissue showing mild eodema within the mucosa, reduced goblet cells and areas close to normal, muscularis with obvious vaocules and colon shows normal features of a colon.

Discussion

Histological evaluation showed that cadmium caused erosion of colonic epithelium, increased severity of colonic injury. The majority of

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cadmium exposure arise from ingestion of food substance due to uptake of cadmium by plant from fertilizer, sewage sludge, manure and atmospheric deposition (Ando *et al*., 1998), the two major ways humans can take up cadmium is by smoking and eating food especially in vegetarian. They feed mainly on fruit and vegetables, these food type are rich in cadmium and so can greatly increase concentration in the human body (Friberg *et al.,* 1985 Valiter *et al*., 1996). In human cadmium has a long half life which is declared to be of about 10 -30 years in kidney and 4.7 – 9.7 years in liver (Cheng and Wang 1990). Interestingly, about an average of 5% of the total orally ingested cadmium is absorbed in the intestine, but individual values ranges from less than 1% to more than 2%.

This reflects the fact that humans do not have effective pathway for cadmium elimination, hence shows the phenomenon of bioaccumulation. Once cadmium is absorbed into the human system, it remains resident for many years. Where cadmium toxicity is concerned, colon is of prime importance. About 90 –95% of cadmium that is excreted passes through the colon, hence colonic cells are exposed to cadmium in fecal matter as well to cadmium present in the circulation. In this present study, we assessed the toxic effects of cadmium chloride as well as to monitor the protective effects of *Curcumin* in colon of wistar rat on the basis of histopathological observations. The integrity of colon depends upon the balance between the hostle factors, one of them being cadmium which damages the mucosa, goblet cells etc and the protective factors such as certain internal secretions like mucin and certain external agents like *Curcumin* which render protection against cadmium toxicity.

The data obtained and photomicrograph view results are similar to the results of various studies where cadmium has been reported to cause variation on histo -architecture of colon. These reports revealed that intra rectal exposure as well as oral exposure to cadmium caused severe necrosis, hemorrhage and ulcers in the colonic epithelium as well induced decreased body weight and muscle atrophy (Farnsworth, 1992). (Adamsson, Piscator, and Nogawa, 1979). These various reports gave validity to our work where similar cadmium induced aberrations were observed. *Curcumin* in this study demonstrated a protective property or agent where it clearly initiated decrease congestion and inflammation in stagnant mucus membrane and no negative side effects have been associated with *Curcumin* supplementation (Mesa, *et al.*, 2003). *Curcumin* also reduced ulcerative colitis (Deodhar *et al.,* 1980) and (Ammon and Wahl, 1991). *Curcuim* has been shown to protectively coat the intestinal mucus membrane and reduce acid secretion. However, information available clearly delineates the protective effects of *curcuimin* against cadmium chloride induced colonic toxicity. Photomicrograph of colonic tissue

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of rats induced with cadmium and then treated with turmeric ethanolic extract high doses for 28, 42, 56 days have confirmed this claim.

Conclusion

Turmeric ethanolic extract (TEE) reversed the adverse pathological changes in the colon induced with cadmium chloride at 875mg/kg and 437.5mg. With the 875mg/kg dose being more effective at reversing colonic changes when compared with Adrucil -5- fluorouracil anticancer drug. This findings suggest that *curcuma longa* in turmeric has anti inflammatory potential as well as anti necrotic tendency. The treatment with high does tends to be more effective than the low dose. The rat colon treated with DMSO shows little or no significant effect in the colonic morphology. It is obvious that *Curcumin* exhibited total protective effect on the mucosal epithelium of induced colon.

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