



**ANTICOLON CANCER EFFECTS OF TURMERIC ETHANOLIC EXTRACTS IN
WISTAR RATS EXPERIMENTALLY INDUCED WITH CADMIUM CHLORIDE**

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ABSTRACT

Colon cancer also known as colorectal cancer is caused by a number of factors ranging from hereditary to dietary needs. Colon cancer is mostly diagnosed in the aged people but screening can be done in much earlier age. This study is geared towards investigating 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, 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. Aduracil-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 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 poses 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 nonessential heavy metal, cadmium is found in the air, water, soil and food. It is known to produce server 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, *Curcumin* derived from *Curcumin longa* (Turmeric) is attributed with many beneficial properties and it contains healing properties. Inducing wistar rats with cadmium chloride initiated a

drastic histopathological change in the architectural of the colon. Alteration of the mucosa epithelial, glands preporia was destroyed. So turmeric was used to treat this condition for a period of 14 to 56 days, the positive influence on the colon was excellent.

Turmeric countered the cadmium induced necrosis. 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.

MATERIALS AND 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 to remove sand and dust from the plant.

Plant extraction

After collection, the rhizomes were allowed at room temperature (25°C – 30°C) to constant room weight over a period of 7 to 30 days. 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 proper 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°C and 200rpm and subsequently, on a steam bath at 40°C. The semi-solid extract obtained with bottles were 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 sulfuroxide)

Throughout the experiment, group 1 animal were not treated but were given free access to normal animal feed and water *adlibium*.

Group 2 animals were induced with cadmium chloride

Group 3 animals were induced with cadmium chloride latter were treated with standard Aducril – 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 anti cancer 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 Aducril -5flourouracil (500mg/10ml) was administered intraperitoneally to rats respectively.

Organ collection and assessment

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

Histopathological 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 concentration 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

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 -5-flourouracil 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.

Keywords

- Normal: (Animals not expose to any form of treatment but were fed *ad libitum*)
- Adrucil -5-flourouracil group (Animals treated with anti-colon drug)
- Dimethyl Surfur Oxide group (Animal treated with Dimethyl Surfur Oxide)

Table 2: Grossing Report of Animal Colon Treated with Curcumin 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 6.7x2.0cm
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

Table 4: Histopathological Scoring of Rat Colon after Cadmium Induction and Treatment with Plant Extract.

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...

DISCUSSION

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 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 28days 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 recorded. Histology reveals no healing and eodenatous mucosa.

The rats in the DMSO group were treated with 0.2ml of dimethylsulfuroxide 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 were dead in this group. Histology report reveals severe mucosal ulceration and inflammation. The weight of the colon after administration of Cadmium Chloride (CdCl₂) as shown in table 4 revealed that there was no weight gain in the colon of the experimental rat in the Adrucil-5-flourouracil 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 there was weight gain.

During cadmium chloride induction, there was increase in stool consistency scores, cadmium decreased weekly weight gain, this finding is consistent with previous reports by (Chiarenza *et al.*, 1989). Histological

evaluation showed that cadmium caused erosion of colonic epithelium, increased severity of colonic injury. The majority of 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 cadmium 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 humans do not have effective pathway for cadmium elimination, hence cadmium 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 experiment, 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 hostile 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 photomicrograph view are similar to the results of various studies where cadmium has be 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 has given validity to our work where similar cadmium induced aberrations is observed. *Curcumin* in this experiment demonstrated a protective property or agent which clearly initiated decrease congestion and inflammation as 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). *Curcumin* also reduced ulcerative colitis (Ammon and Wahl, 1991). *Curcumin* has been shown to protectively coat the intestinal mucus membrane and reduce acid secretion. However, we have information available clearly delineates the protective effects of *curcumin* against cadmium chloride induced colonic toxicity. Photomicrograph of colonic tissue of rats induced with cadmium and then treated with turmeric ethanolic extract high doses for 28, 42, 56 days 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. By this finding, it is clear that *Curcumin* exhibited total protective effect on the mucosal epithelium of induced colon.

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