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RESEARCH ARTICLE

ACUTE ORAL TOXICITY STUDY AND LD_{50} DETERMINATION OF 5-FLUOROURACIL IN WISTER ALBINO RATS

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ABSTRACT

The present study was designed according to the Operation for Economic Co- operation and Development (OECD) Guideline 423 to determine acute oral toxicity of 5-Flurouracil. The Wister albino rats were divided into five groups. Group I served as normal control. Group II to V gavaged with 5-FU At dose rate of 5mg/kg, 50mg/kg, 300 mg/kg and 2000mg/kg. Body weight was measured and blood samples were collected on day 0, 7 and 14 and analysed for various biochemical parameters. Necropsied rats were subjected for histopathology. LD_{50} cut off value of 5-Flurouracil was found to be around 200 mg/kg. There was significant ($P< 0.001$) decrease in the body weight of group III and IV compared to the normal control and significant ($P< 0.001$) increase in the ALT, AST, BUN and serum creatinine value of group III and IV compared to the normal control. Histopathological examination of intestine showed villus atropy and inflammatory cell infiltration. Liver and kidney showed degenerative and hemorrhagic changes.

KEY WORDS: Acute toxicity, 5-Fluorouracil, LD_{50}

INTRODUCTION

5-Fluorouracil (5-FU), a pyrimidine analogue that interferes with thymidylate synthesis, has a broad spectrum of activity against solid tumors, which is employed most extensively in clinical chemotherapy for the treatment of carcinomas of the colon or rectum, skin and breast cancer [1].

5-FU is metabolized to 5-fluorouridine 5'-triphosphate (FUTP), 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP), and 5-fluoro-2'-deoxy 5'-triphosphate (FdUMP). Incorporation of FUTP into RNA is accompanied by profound effects on the synthesis, stability, processing, and methylation of RNA. Incorporation of FdUTP leads to inhibition of DNA elongation and DNA fragmentation. FdUNP inhibits thymidylate synthase, a crucial enzyme for the de novo synthesis of thymidylate, necessary for the synthesis of DNA [2].

Metastatic colon cancer is the third leading cause of cancer death in the United States [3]. 5-FU is widely used for treatment of colorectal cancers, other gastrointestinal cancer [4].

5 FU and its precursors commonly used as chemotherapeutic drug for several intestinal and non intestinal cancer, but there is less data available on its acute toxicity on different oragans. So this study is done according to the standard guidelines.

Materials and Methods

Experimental animals

Female Wistar albino rats weighing 150-200 g were obtained from Central Animal Facility, Indian Institute of Science, Bangalore. They were maintained under standard laboratory conditions. The study was carried out with a prior approval by the Institutional Animal Ethical Committee, Veterinary College, Bangalore.

Toxicity testing

Fifteen Female albino rats of Wistar strain were weighed and randomly distributed into five groups of three rats each. Group I served as normal control, gavaged with normal distilled water. Group II, III, IV and V were gavaged with 5-FU in normal distilled water at the dose rate of 5mg/kg, 50 mg/kg, 300mg/kg and 2000mg/kg according to the OECD guidelines 423.

Observations

As per the Paragraph 24 and 25 of OECD Guidelines 423, Wellness parameters of animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

Serum biochemistry

About 2 ml of blood from each animal of all groups was collected on day 0, 7 and 14 and serum was separated and subjected for biochemical analysis like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN) and Creatinine (Cr). The biochemical constituents were estimated from serum samples to investigate the toxic effect on organs and tissues using semi automatic biochemical analyzer (Merck, Germany) with ready to use kits (Ecoline®, Merck, India).

Histopathology

At the end of the study the overnight fasted rats were sacrificed under ether anesthesia. The organs were examined for gross pathological changes and the representative samples from liver, kidney, and intestine were collected in Neutral Buffered Formalin (NBF) for histopathology by cutting sections of 4-5µ thicknesses and stained with haematoxylin and eosin.

Statistical analysis

The data obtained from the present study were subjected to statistical analysis. The data were analyzed by using two-way ANOVA. $P<0.05$ was considered as significant. The analysis was carried out by Bonferroni's multiple comparison test. Mean values and standard error of mean was calculated and all the values are expressed as mean ± SEM [5]

RESULTS AND DISCUSSION

Clinical signs and Body weight

The present study conducted as per the OECD guidelines 423 revealed that 5-Fluorouracil have been found toxic at 300 mg/kg body weight (Group IV) of experimental animals as in the first 4 hours of observation 2/3 morbidity was observed and in the next 24 hours of observation mortality in the ratio 2/3 were found. All Group V animals found dead first 30 min of observation.

LD_{50} Value: As per observations and calculations from Acute Oral Toxicity (OECD Guidelines 423), the LD_{50} value of 5-Flurouracil was found to be more than 50 mg/kg body weight but less than 300 mg/kg body weight. According to the guidelines LD_{50} cut off value lies between 200 to 300mg/kg.

Table 1 indicates the wellness parameters used for evaluation of acute toxicity observed before and after the administration of the test substance for Group III and Group IV. At lower limit dose of 5 mg/kg body weight (Group I), no significant changes were observed in body weight and other wellness parameters. But a significant ($P<0.001$) decrease in body weight of groupIII rats as compare to normal control group on day 7 and 14 (Table 2 and Fig.1) and diarrhoea was most common in all rats of Group III and Group IV, which was comparable to the result of the effect 5-FU at dosage of 15mg/kg and 18 mg/kg on male rats, in which it observed that significant decrease in body weight and signs like diarrhea, lethargy [6]. One of the dose-limiting toxicities (DLT) of oral 5-Fluorouracil was diarrhea in breast cancer patients [7].

Shindoh *et al* also observed diarrhea and decease in bodyweight in in cynomolgus monkeys at 0.1 and 0.5 mmol/kg dose of capecitabine, which is prodrug for 5 FU [8]. The probable reason for diarrhea and decrease body weight could be due to either decrease in the feed intake [6] or inhibition of RNA and DNA synthesis and induction of apoptosis by 5FU in the highly proliferative organ like intestine [9,10].

Biochemical parameters

The mean values of ALT, AST, BUN and serum creatinine in group II rats on day 7 and 14 were significantly higher ($P<0.001$) compared to normal control group (Table 3 & 4). These findings are supported by the earlier studies, in which AST and ALT were significantly higher ($p < 0.001$) with 5-FU treated groups of mice than the control group [11].

Bano *et al.*, also find similar results in which they used 400mg/m^2 of 5 FU in humans, there is significant increase in post treatment ALT,AST and Creatinine when compared to pretreatment [12].

Histopathology

Photomicrographs (Fig. A & B) of the group III and IV showed duodenal villus epithelia cell degeneration and desquamation which results in the shortening of the villi. And also it showed hemorrhages and congestion. Mucosa and sub mucosa infiltrated with the inflammatory cells. Liver and kidney showed slight hemorrhagic and degenerative changes (Fig C & D).

These findings are in accordance with earlier study, in which they used 5 FU at 0.5 mmol/kg and 1 mmol/kg in rats and mice [8]. The probable reason for intestinal damage due to

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Table.1.Observation for the Test at 50 mg/kg(G_1) and 300 mg/kg(G_2) Body weight of an animal.

Observatio	30 Min.			4 Hrs.			24 Hrs.			48 Hrs.			1 Week			2 Weeks		
	C	G_1	G_2	C	G_1	G_2	C	G_1	G_2	C	G_1	G_2	C	G_1	G_2	C	G_1	G_2
Skin and	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Eyes	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Mucous	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Salivation	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Lethargy	N	N	All	N	1/3	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Sleep	N	N	2/3	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Coma	N	N	2/3	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Convulsion	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Tremors	N	N	N	N	N	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Diarrhea	N	N	All	N	1/3	2x1	N	1/3	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Morbidity	N	N	2/3	N	1/3	2x1	N	N	2x1	N	N	2x1	N	N	2x	N	N	2x1n
Mortality	N	Nil	Nil	N	Nil	2x1	N	Nil	2x1	N	Nil	2x1	N	Nil	2x	N	Nil	2x1n

C= Control, E= Extract, N= Normal, 2x1n= 2 Expired and 1 Normal, 2/3= observation ratio

Table 2: The mean animal body weight (g) values of control, 5mg/kg and 50mg/kg groups at different intervals of time.

Days	NC	5mg/kg	50mg/kg
0	188.33±4.40	187.45±4.70	185.00±2.88
7	191.66±8.81	190.78±4.91	165.00± 1.78***
14	197.33± 6.56	192.66±2.84	163.33±1.66**

Values are expressed as Mean ± SE, n=3. *P < 0.05, **P < 0.01, ***P < 0.001 compared to normal control group

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Table 3: The mean serum ALT(IU/L) and AST (IU/L) values of control, 5mg/kg and 50mg/kg groups at different intervals of time.

	ALT			AST		
Days	NC	5mg/kg	50mg/kg	NC	5mg/kg	50mg/kg
0	51.54±2.01	51.84±1.41	44.53±4.49	55.71±1.57	55.74±2.35	56.28±1.25
7	53.34±1.57	61.44±2.97	93.15±3.33***	56.07±0.68	62.21±3.49	101.47±2.02***
14	52.63±0.85	57.30±3.17	68.88±2.91**	56.72±1.78	59.60±5.31	74.26±3.68***

Values are expressed as Mean ± SE, n=3. *P < 0.05, **P < 0.01, ***P < 0.001 compared to normal control group

Table 4: The mean serum creatinine (mg/dL) and BUN (mg/dL) values of control, 5mg/kg and 50mg/kg groups at different intervals of time.

	Creatinine (mg/dL)			BUN (mg/dL)		
	NC	5mg/kg	50mg/kg	NC	5mg/kg	50mg/kg
	0.50±0.02	0.55±0.06	0.56±0.04	17.34±1.24	17.41±1.65	18.16±1.93
	0.48±0.02	0.56±0.06	2.52±0.54***	17.62±1.10	18.55±0.38	34.5±0.87***
	0.49±0.00	0.54±0.05	2.11±0.18**	16.59±0.46	18.33±0.67	29.38±0.63**

Values are expressed as Mean ± SE, n=3. *P < 0.05, **P < 0.01, ***P < 0.001 compared to normal control group

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Figure A: Duodenal section showing epithelial cell degeneration, Shortening of villi, congestion and hemorrhages in group III (H & E, 10X).

Figure B: Duodenal section showing infiltration of inflammatory cells and shortening of villi in group IV (H & E, 20X).

Figure C: Kidney showing the cystic dilation of tubules, congestion and hemorrhage in group III (H & E, 40X).

Figure D: Liver showing congestion of vessels and sinusoids in Group III (H &E, 20X).

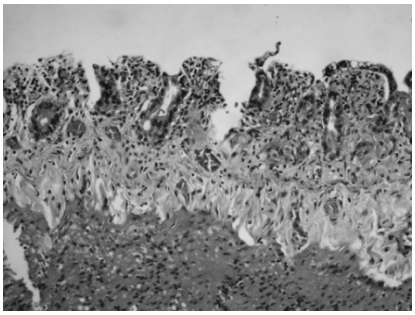

Figure A

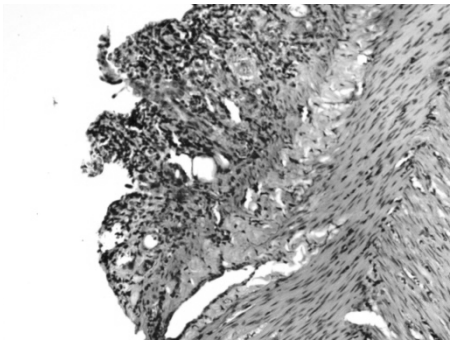

Figure B

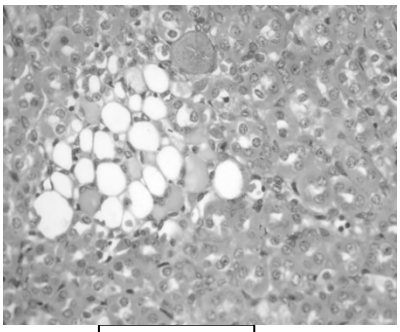

Figure C

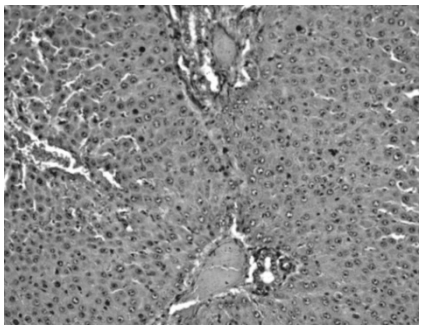

Figure D

Cytotoxic effects of 5-FU are caused by inhibition of mitosis due to inhibition of RNA and DNA synthesis. In addition, it has been reported that 5-FU induce apoptosis in tumor cells and also in the intestinal epithelium [13, 14]. Inomata *et al*., reported that 5-FU caused cell cycle arrest and apoptosis in intestinal epithelium cells in normal mice after both oral and intravenous infusion administrations [15].

CONCLUSION

5-FU is being cytotoxic drug, showed degenerative changes in the several vital organs, toxicity was highly intensive in the intestine mainly duodenum. Acute oral toxicity studies revealed that the LD50 cut off value between 200 to 300 mg/kg in rats.

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