

CHAPTER FOUR

4.1. RESULTS

4.1.1. Acute toxicity study

Acute toxicity study is a study in which the animals were treated with the *P. niruri* extract at a dose of 2 g/kg and 5 g/kg were kept under observation for 14 days. All the animals remained alive and did not manifest any significant visible of toxicity at these doses. Thus, clinical observations, serum biochemistry, and histopathology data did not show any significant differences between control and treated groups . We conclude that *P. niruri* ethanolic extract orally administered to rats was safe and that no drug-related toxicity was detected even at the highest dose investigated.

4.1.1.1. Behavioral Observation and Mortality

The fasted animals pre-treated with a dose of 2 g/kg and 5 g/kg of *P.niruri* were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible of toxicity at these doses. There were no abnormal signs, behavioral changes or body weight changes. There was no mortality in the above-mentioned doses at the end of 14 days of observation. Histology of liver and kidneys, hematology and serum biochemistry revealed no significant differences between groups. From these results it is concluded that the extract is quite safe even at these higher doses and has no acute toxicity (Table 4.1).

Table 4.1: The observation data for toxicology study of *P. niruri*

Dose	Occurrence of mortality				
	10 min	30 min	3 h	24 h	48 h
5 g/kg	0/6	0/6	0/6	0/6	0/6
2 g/kg	0/6	0/6	0/6	0/6	0/6
Vehicle 10% Tween 20	0/6	0/6	0/6	0/6	0/6

4.1.1.2. Analysis of Body Weight of Acute Toxicity

Throughout the 14 days, the changes of body weight of animals were observed Figure (4.1 and 4.2) tables (4.2 and 4.3) illustrated that there was no significant observed in any of rats groups pre-treated with *P. niruri* when compared to their respective vehicle group.

Table 4.2: Mean body weights of male rats pre-treated with *P. niruri* in acute toxicity test.

Dose	Body weight (g)	Body weight (g)
	Day 0	Day 14
5g/kg	171.25 ± 0.77	199.30 ± 0.58
2g/kg	174.87 ± 1.25	206.61 ± 0.63
Vehicle	170.50 ± 0.98	197.69 ± 0.85

All values expressed as mean ± SEM No significant body weight changes observed in all rates groups.

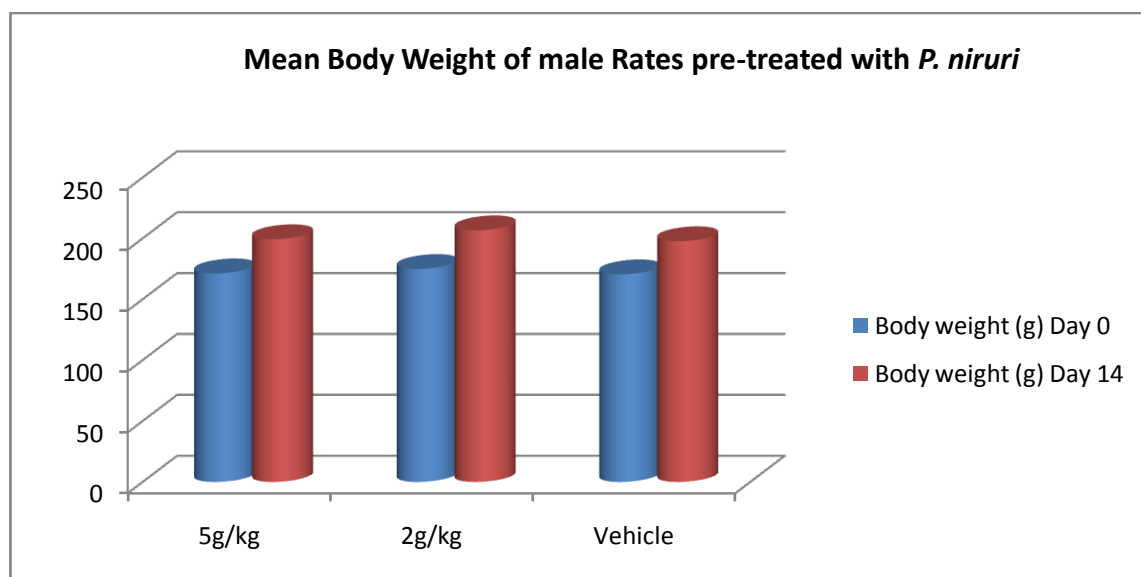


Figure 4.1: Mean body weights of male rats pre-treated with *P. niruri*. All values expressed as mean \pm SEM No significant body weight changes observed in all rates groups.

Table 4.3: Mean body weights of female rats pre-treated with *P. niruri* in acute toxicity test.

Dose	Body weight (g)	Body weight (g)
	Day 0	Day 14
5g/kg	162.05 \pm 0.98	179.10 \pm 0.78
2g/kg	164.67 \pm 1.33	177.97 \pm 0.99
Vehicle	165.36 \pm 0.94	180.75 \pm 1.68

All values expressed as mean \pm SEM No significant body weight changes observed in all rates groups.

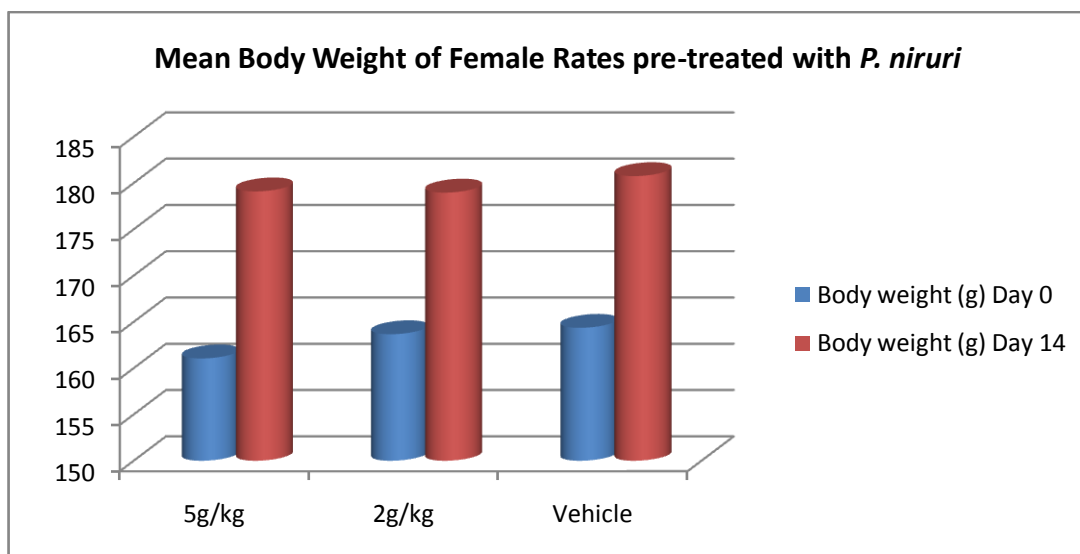


Figure 4.2: Mean body weights of female rats pre-treated with *P. niruri*.
All values expressed as mean \pm SEM
No significant body weight changes observed in all rates groups.

4.1.1.3 Hematology

The total white blood cell for all rats was counted. Table 4.4 and Figure 4.3 showed that there are no significant differences between all groups.

Table 4.4: Total white blood cell (WBC count ($10^9/L$))

Group	Vehicle	2 g/kg	5 g/kg
Male	8.20 ± 1.98	7.70 ± 1.91	8.08 ± 1.74
Female	7.88 ± 2.1	7.68 ± 2.23	7.32 ± 1.53

All values expressed as mean \pm standard error mean (mean \pm SEM).

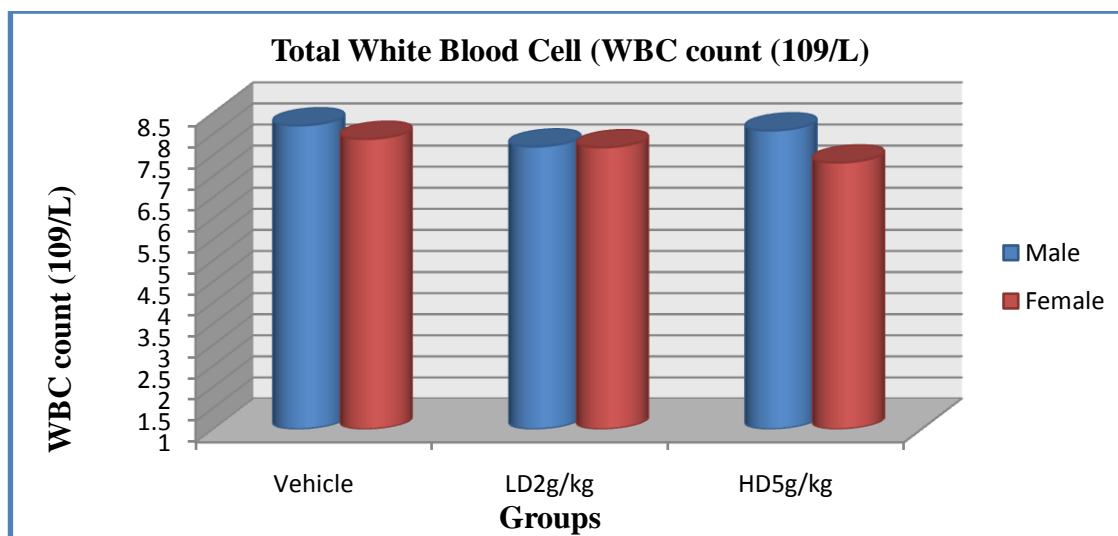


Figure 4.3: Total white blood cell (WBC count (10⁹/L).
All values expressed as mean \pm standard error mean (mean \pm SEM).

4.1.1.4 Serum Biochemistry of Acute Toxicity Test

The parameters of liver function that have been tested were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total protein, Albumin, Globulin, Total bilirubin, Conjugated bilirubin, Alkaline phosphatase, G-Glutamyl Transferase. Levels of all groups were analyzed as indication of liver function as compared to their vehicle groups. Urea, Creatinine, CO₂, anion gap and serum electrolytes (Potassium, Sodium and Chloride) levels of all groups were determined as markers of renal function.

In rats groups given the ethanolic extract of *P.niruri*, no significant changes were found in all tested parameters levels of all rats groups as compared to their respective vehicle group (Table 4.5 and 4.6) and (Figures 4.4 to 4.10). Moreover, there was no significant decrease or increase in levels of all parameters of male as well as female groups in both dosages.

4.1.1.5 Laboratory (Hematology and Chemistry) Results of Acute toxicity test

Table 4.5. Renal function test of rats in acute toxicity study of *P. niruri* extract.

Dose	Sodium (mmol/L)	Pottasium (mmol/L)	Chloride (mmol/L)	CO ₂ (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (μmol/L)
Vehicle	138.25 ± 0.45	5.03 ± 0.19	104.03 ± 0.15	23.03 ± 0.82	18.16 ± 0.72	5.63 ± 0.41	50.18 ± 1.34
LD (2 g/kg)	137.65 ± 0.43	5.21 ± 0.16	102.61 ± 1.22	21.74 ± 0.17	18.07 ± 1.35	4.96 ± 0.43	48.97 ± 0.81
HD(5 g/kg)	137.21 ± 0.51	4.89 ± 0.15	102.67 ± 0.76	22.8 ± 0.86	17.73 ± 0.51	5.93 ± 0.39	48.60 ± 1.80

Values expressed as mean ± SEM. There are no significant differences between groups. Significant value at p<0.05

Table 4.6. Liver function test of rats in acute toxicity study of extract.

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (μmol/L)	CB (μmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle	71.37 ± 1.44	11.36 ± 0.53	59.91 ± 1.33	1.91 ± 0.17	0.89 ± 0.15	134.78 ± 9.57	53.05 ± 3.27	153.65 ± 9.35	4.91 ± 0.93
LD (2 g/kg)	71.47 ± 0.52	11.61 ± 0.34	59.61 ± 0.35	2.18 ± 0.16	1.00 ± 0.00	133.37 ± 8.63	51.90 ± 1.33	156.07 ± 3.56	5.00 ± 1.23
HD (5 g/kg)	71.81 ± 1.03	11.72 ± 0.16	60.01 ± 0.67	1.88 ± 0.21	1.00 ± 0.00	135.13 ± 6.52	52.27 ± 3.25	155.00 ± 5.35	5.32 ± 1.07

Values expressed as mean ± SEM There are no significant differences between groups. Significant value at p<0.05

TB: Total bilirubin; CB: Conjugated bilirubin; AP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: G-Glutamyl Transferase .

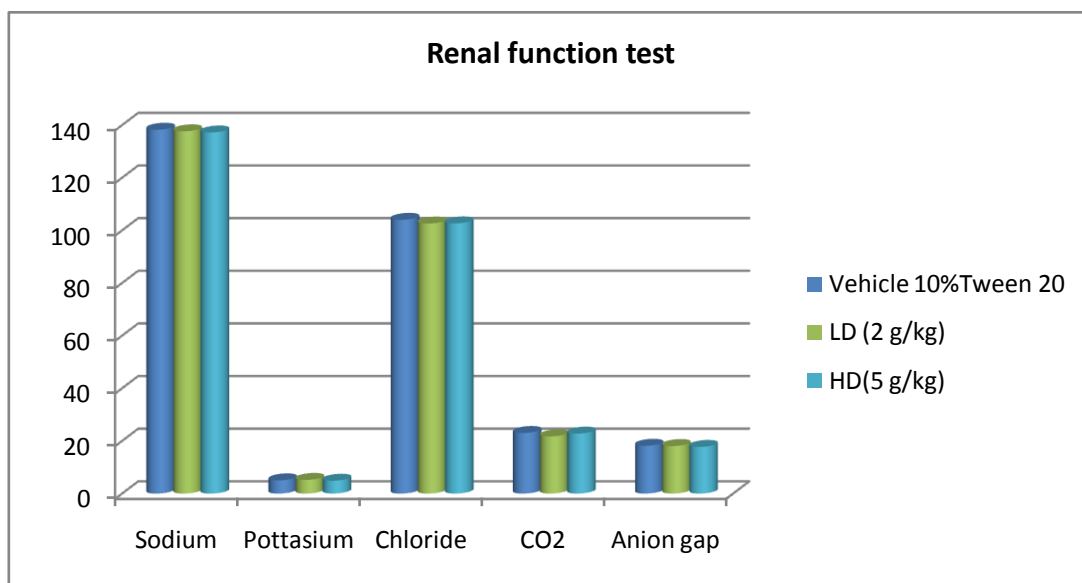


Figure 4.4: Level of (sodium, potassium, chloride, CO2 and anion gap) in rats treated with ethanolic extracts of *P.niruri*.

Values are expressed as mean \pm SEM

No significant difference as compared to vehicle group ($p < 0.05$).

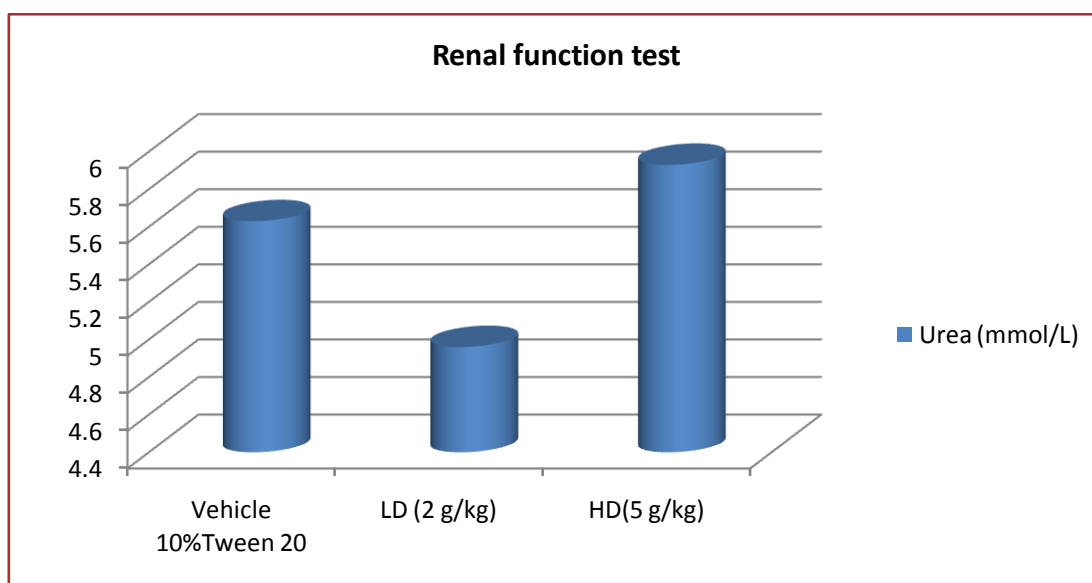


Figure 4.5: Level of urea in rats treated with ethanolic extracts of *P.niruri*.

Values are expressed as mean \pm SEM

No significant difference as compared to vehicle group ($p < 0.05$).

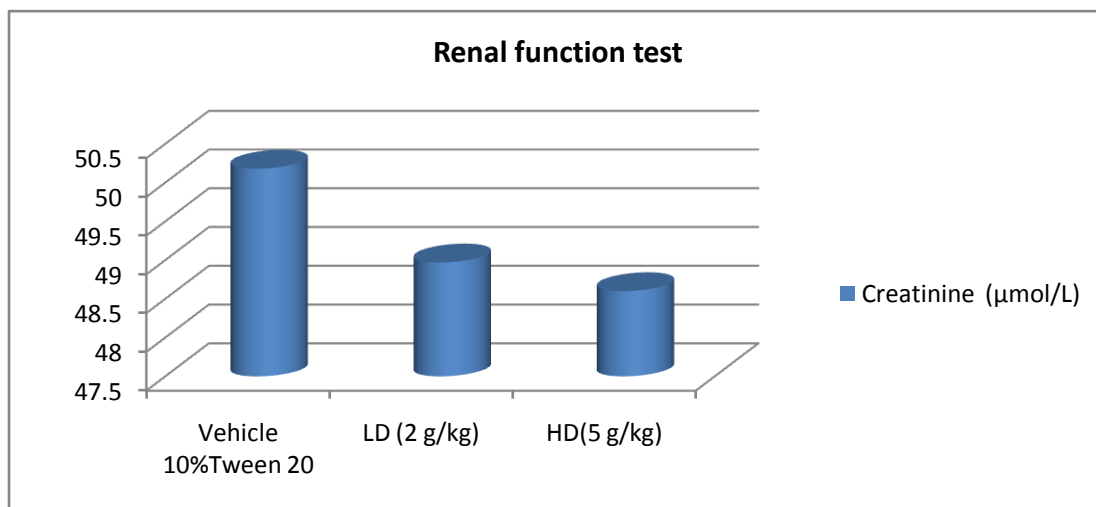


Figure 4.6: Level of creatinine in rats treated with ethanolic extracts of *P.niruri*
 Values are expressed as mean \pm SEM
 No significant difference as compared to vehicle group ($p < 0.05$)

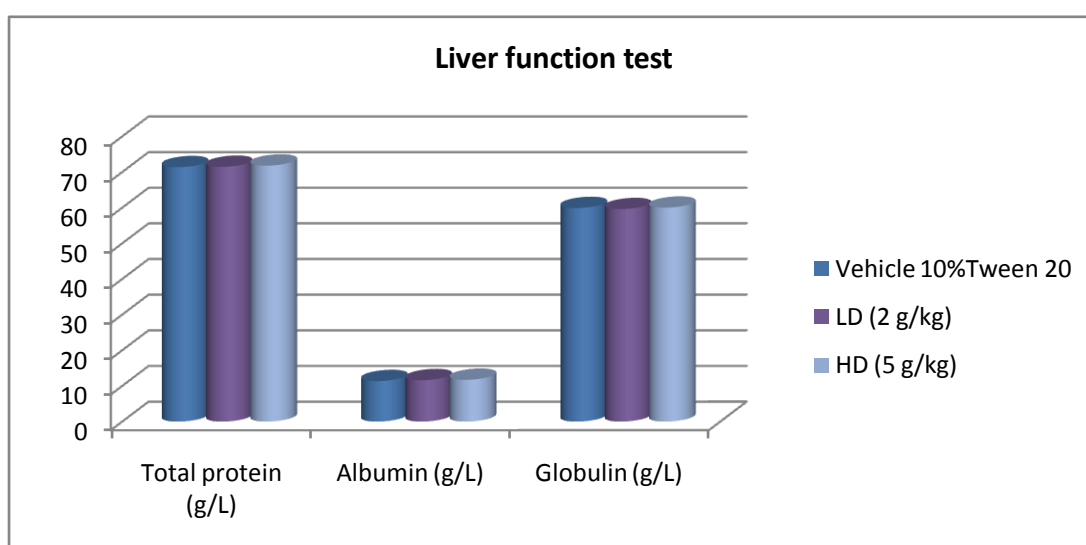


Figure 4.7: Level of Total protein, albumin, and globulin in rats treated with ethanolic extracts of *P.niruri*
 Values are expressed as mean \pm SEM
 No significant difference as compared to vehicle group ($p < 0.05$).

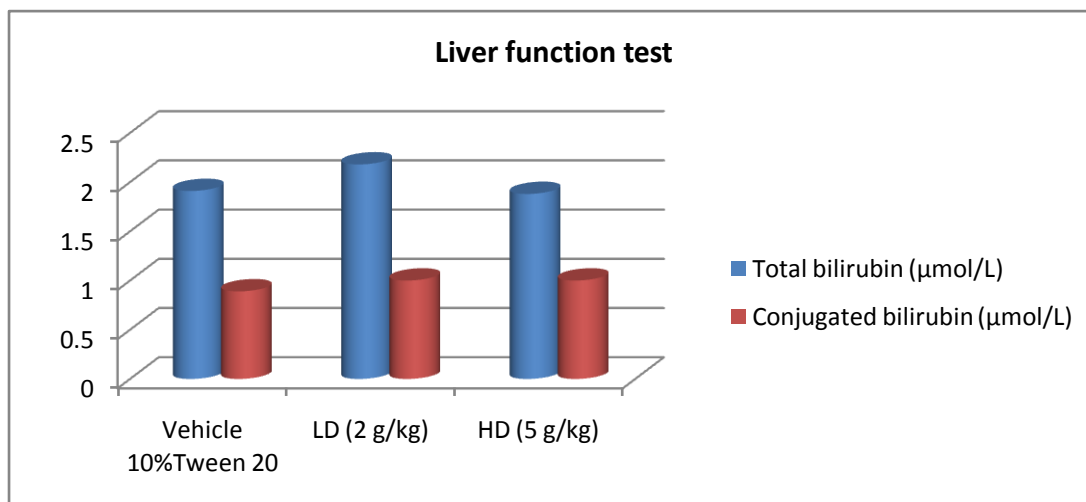


Figure 4.8: Level of Total bilirubin and conjugated bilirubin in rats treated with ethanolic extracts of *P. niruri*

Values are expressed as mean \pm SEM

No significant difference as compared to vehicle group ($p < 0.05$)

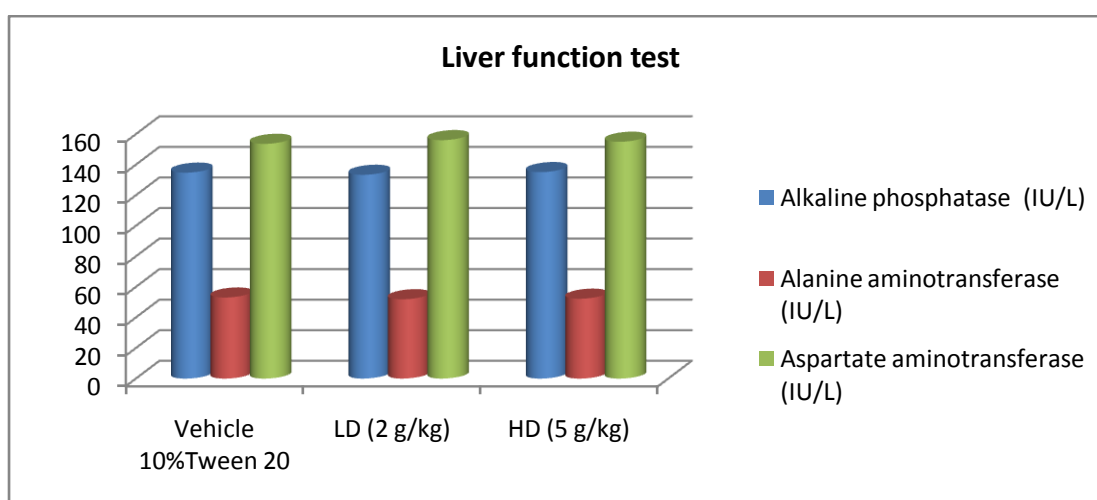


Figure 4.9: Level of (AP, ALT, and AST) in rats treated with ethanolic extracts of *P. niruri*. Ap: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Values are expressed as mean \pm SEM

No significant difference as compared to vehicle group ($p < 0.05$)

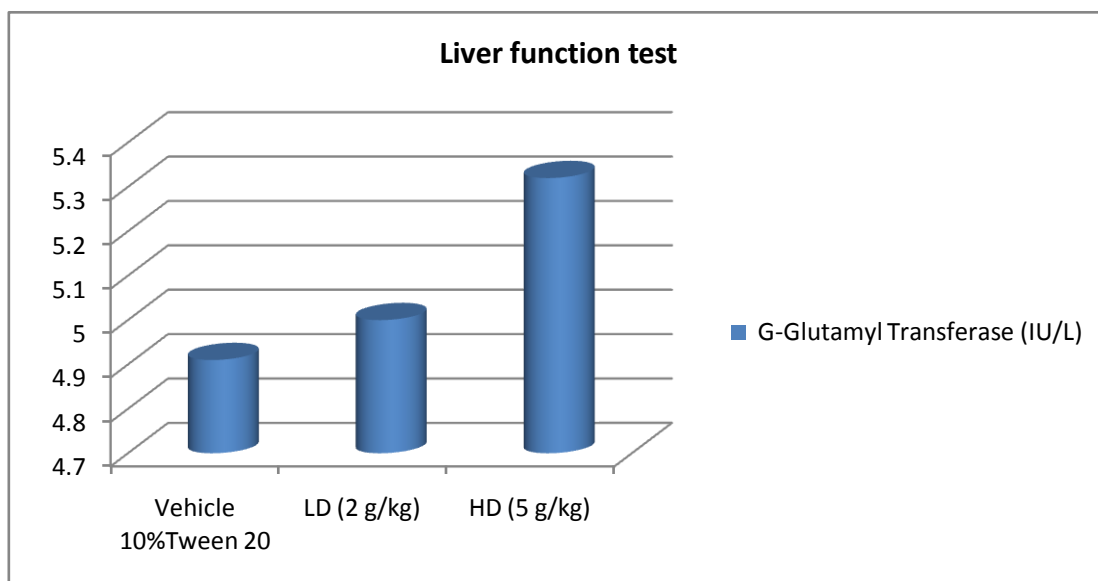


Figure 4.10: Level of G-Glutamyl Transferase in rats treated with ethanolic extracts of *P. niruri*

Values are expressed as mean \pm SEM

No significant difference as compared to vehicle group ($p < 0.05$).

4.1.2. Antioxidant Activity Results

4.1.2.1. Ferric-reducing antioxidant power (FRAP) assay results

The antioxidant activity of *P. niruri* extract was studied by Ferric-reducing antioxidant power (FRAP) assay was evaluated and Figure 4.12 showed the antioxidant of *P. niruri* which was quit low in comparison to the standards Figure 4.11 (Ascorbic acid, trolox & quercetin).

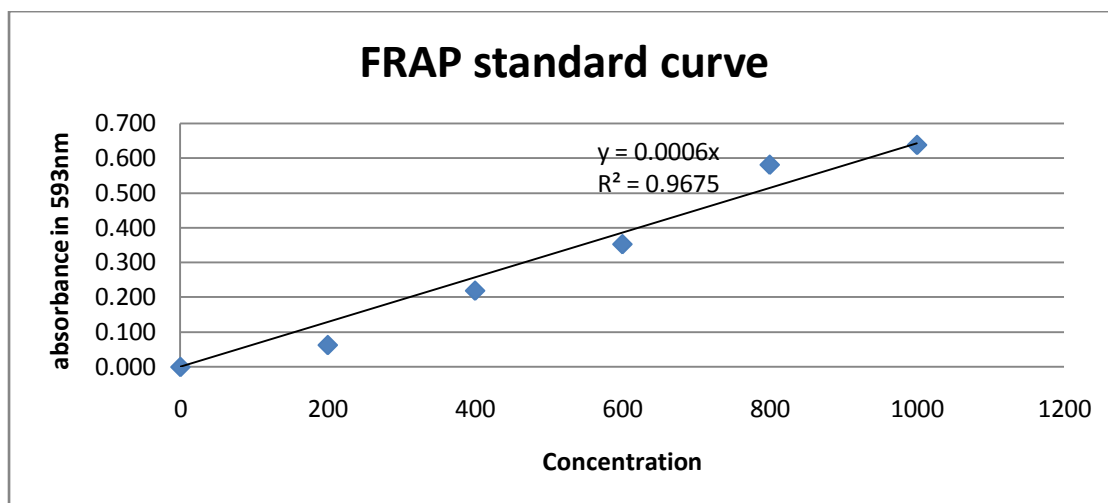


Figure 4.11: FRAP assay standard curve
STD: Ferrous Sulfate Hepta Hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)

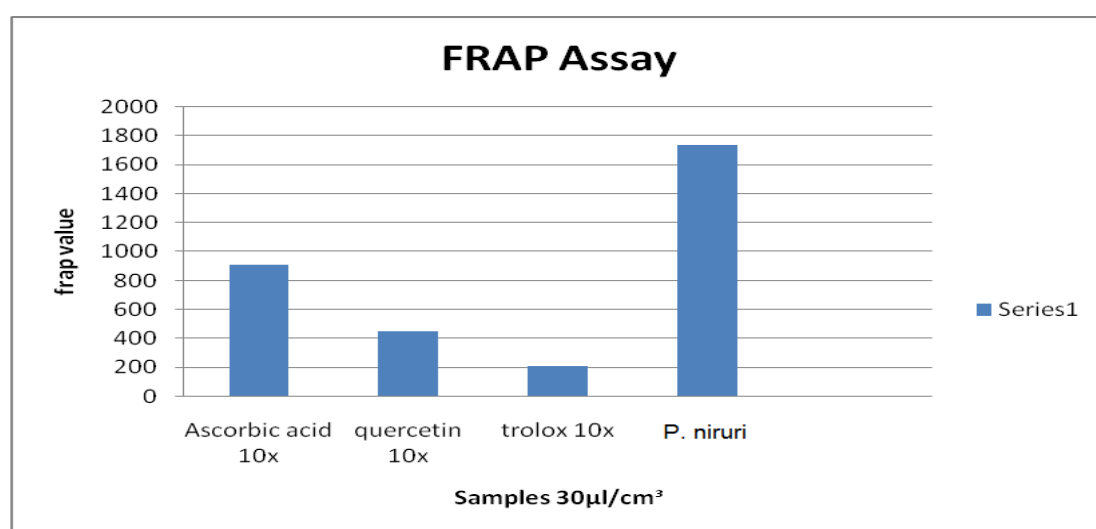


Figure 4.12: Data of FRAP assay of *P. niruri* extract

4.1.2.2. DPPH Free Radical Scavenging Activity Test results

P. niruri extract in DPPH Free Redical Scavenging Activity assay as shows in (Figure 4.13) was studed and IC_{50} for *P.niruri* was 37.61 $\mu\text{g}/\text{Cm}^3$ consider high in compare to ascorbic acid was 5.3 $\mu\text{g}/\text{cm}^3$ (Figure 4.14).

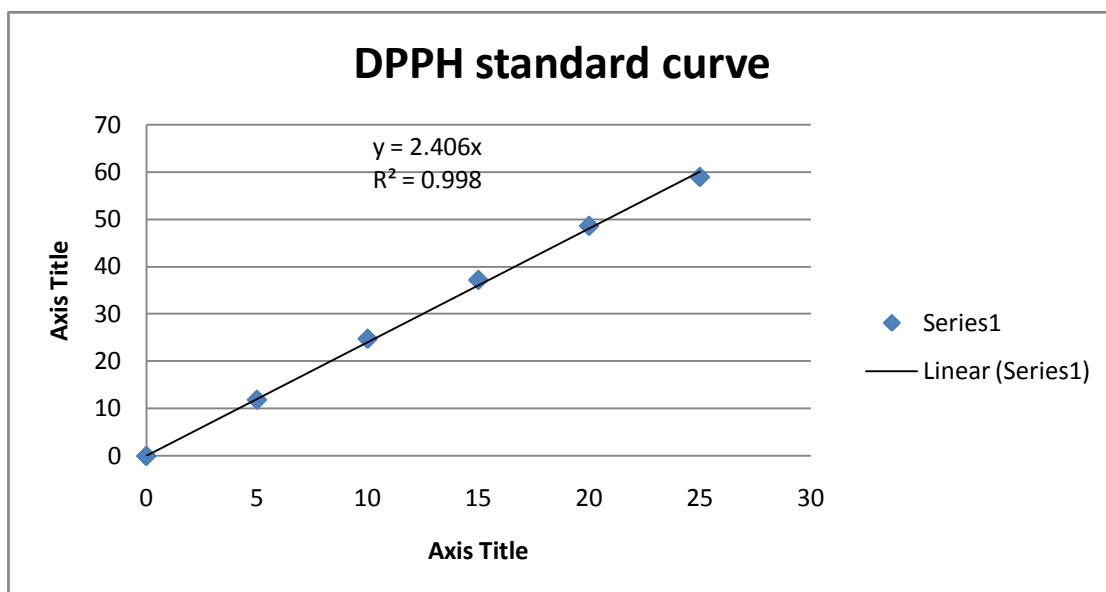


Figure 4.13: DPPH assay standard curve
Values are expressed as mean \pm SEM

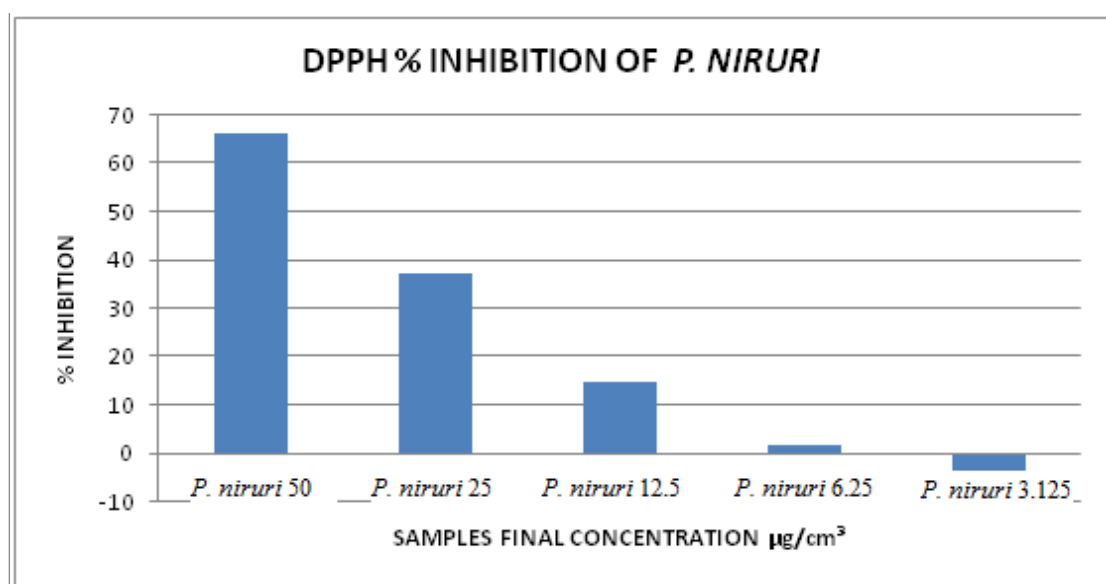


Figure 4.14: Data of DPPH assay of *P. niruri* extract
Values are expressed as mean \pm SEM

4.1.3. Experimental Animals Results

4.1.3.1 Analysis of body weight

The rat's body weight of all groups (high, low, and control group) throughout the 10 weeks had observed and the average was compared in week 1 and 10 (Table 4.7 and Figure 4.15) there was no significant observed in any of rates groups when compared to their respective vehicle groups.

Table 4.7 The average of body weight of rats

Group	week 1	week 10
Low dose	143.4	290.64
High dose	132.2	302.85
Control group	140	274

L.D: Low dose, H.D: High dose

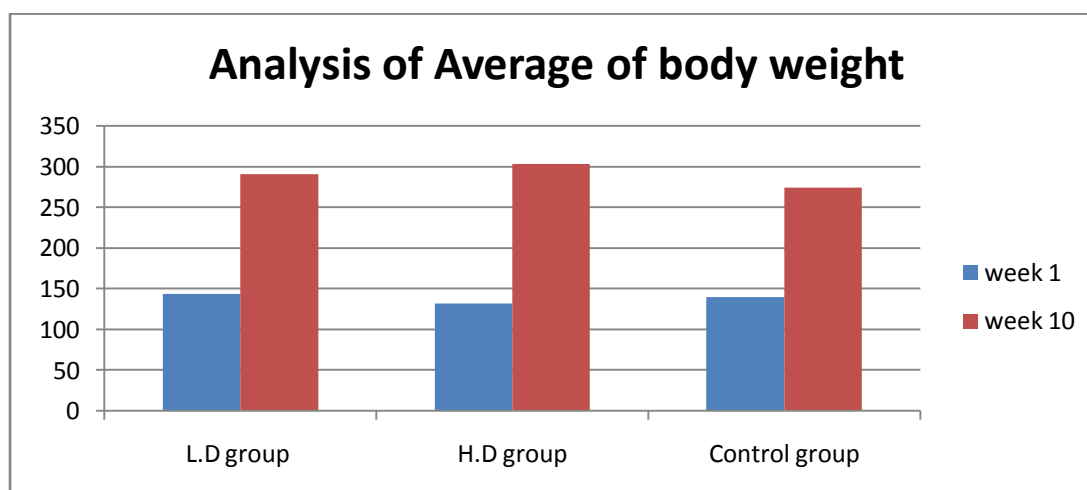


Figure 4.15: Average of rat's body weight

All values expressed as mean \pm standard error mean (mean \pm SEM).

No significant body weight changes observed in all rates group

4.1.3.2 Serum biochemistry of colon cancer

Liver and kidney function have been tested; levels of all groups were analyzed and compared to their vehicle groups (Table 4.8 and 4.9).

Table 4.8. Renal function test of rats in acute toxicity study of *P. niruri* extract.

Group	Urea (mmol/L)	Creatinine
L.D	5.4±0.457	41.8±5.435
H.D	6.12±0.553	29.2±1.933
Control	6.14±0.610	39±4.335
Normal	5.16±0.286	67.8±0.599

Values expressed as mean ± SEM There are no significant differences between groups. Significant value at p<0.05

Table 4.9. Liver function test of rats in acute toxicity study of extract.

Group	TP (g/L)	Albumin (g/L)	Globulin (g/L)	TB (μmol/L)	CB (μmol/L)	AP (IU/L)	AAT (IU/L)	AST (IU/L)	G-GT (IU/L)	TC (mmol/L)
L.D	60±1.04	12±0.89	48±1.14	2±0.00	1±0.00	88.2±1.34	60.8±2.10	154.8±1.99	4.2±0.05	1.3±0.00
H.D	63±1.70	11.2±0.73	50±1.04	2±0.00	1±0.00	94.4±0.13	88.2±1.67	170.2±1.56	2.94±0.02	1.3±0.00
Control	60.4±1.46	11.4±0.24	49±1.30	2±0.00	1±0.00	91.6±1.17	64.2±0.11	193.6±1.49	3±0.00	1.44±1.13
Normal	67.8±1.11	10.2±0.37	57.6±1.43	2±0.00	1±0.00	69.4±1.21	57.2±0.42	168.6±1.29	3±0.00	1.52±0.06

Values expressed as mean ± SEM There are no significant differences between groups. Significant value at p<0.05

TB: Total bilirubin; CB: Conjugated bilirubin; AP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;GGT: G-GlutamylTransferase.

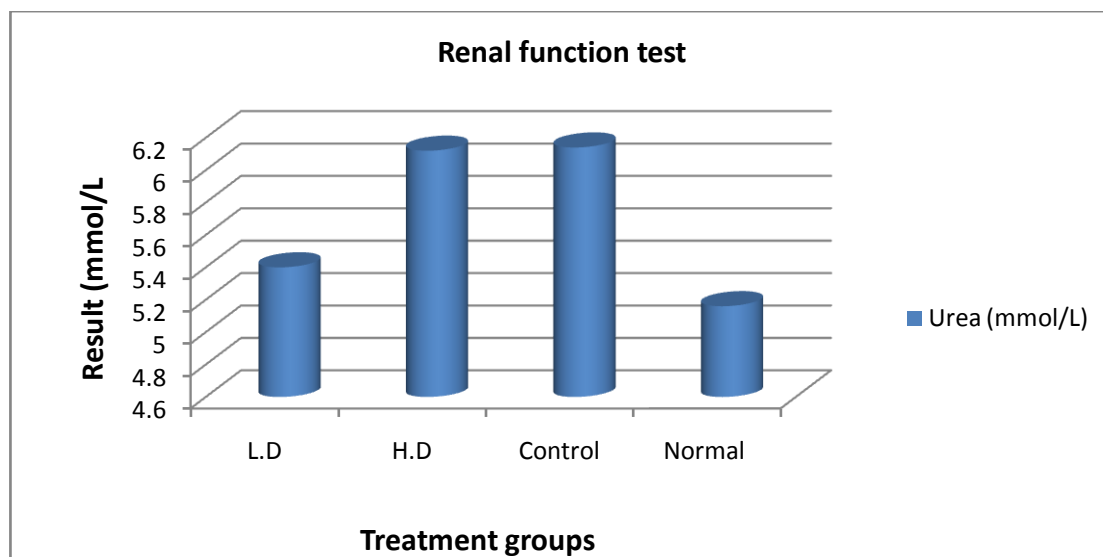


Figure 4.16: Level of urea in rats treated with ethanolic extracts of *P.niruri*
Values are expressed as mean \pm SEM

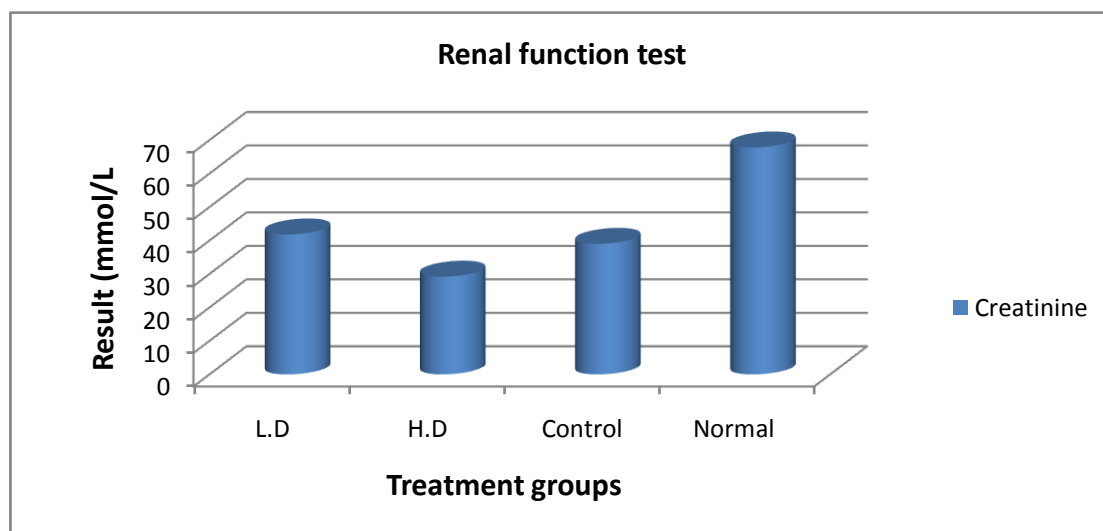


Figure 4.17: Level of creatinine in rats treated with ethanolic extracts of *P.niruri*
Values are expressed as mean \pm S.E.M.

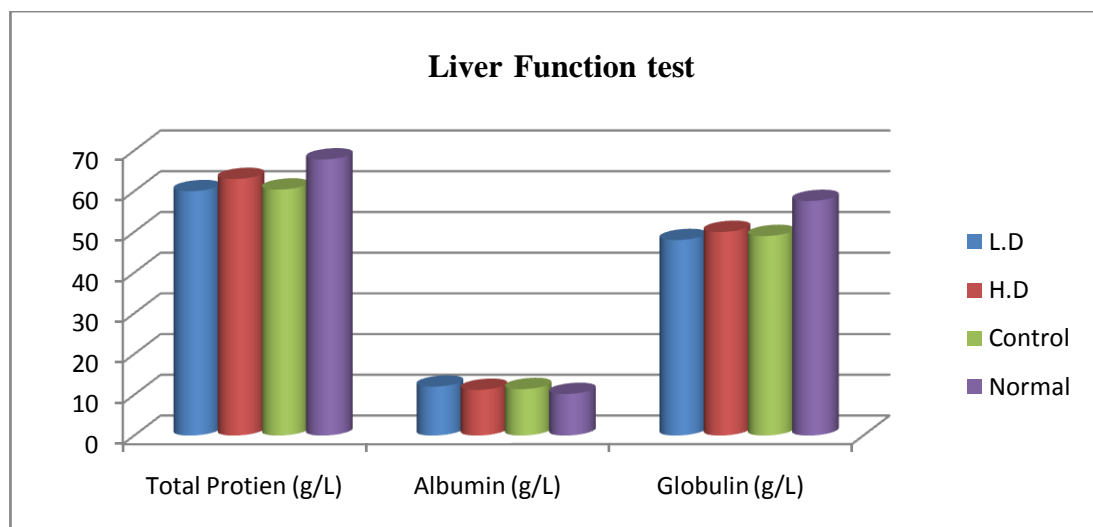


Figure 4.18: Level of Total protein, albumin, and globulin in rats treated with ethanolic extracts of *P.niruri*
Values are expressed as mean \pm SEM

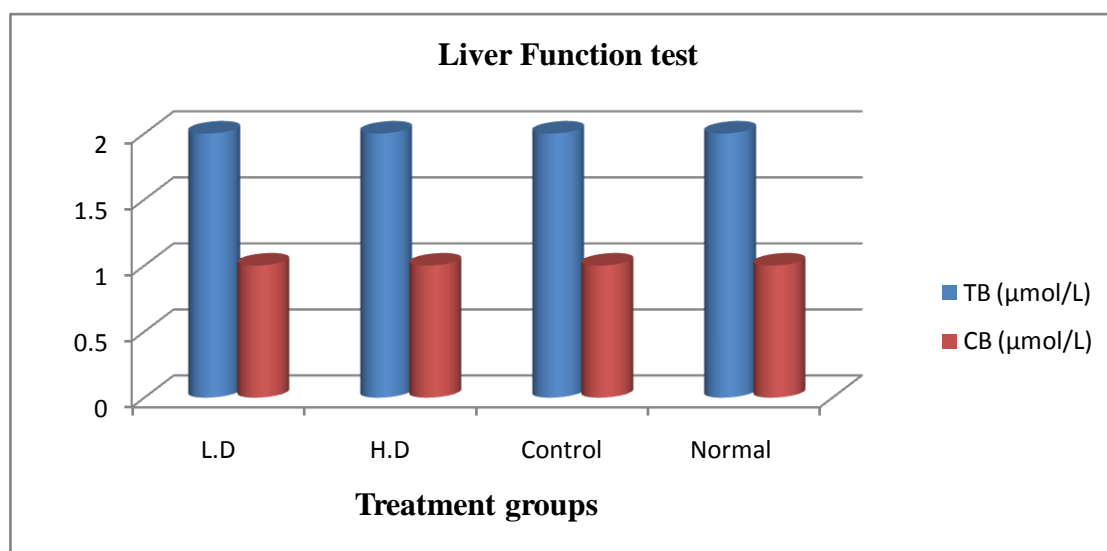


Figure 4.19: Level of Total bilirubin and conjugated bilirubin in rats treated with ethanolic extracts of *P.niruri*.
Values are expressed as mean \pm S.E.M.

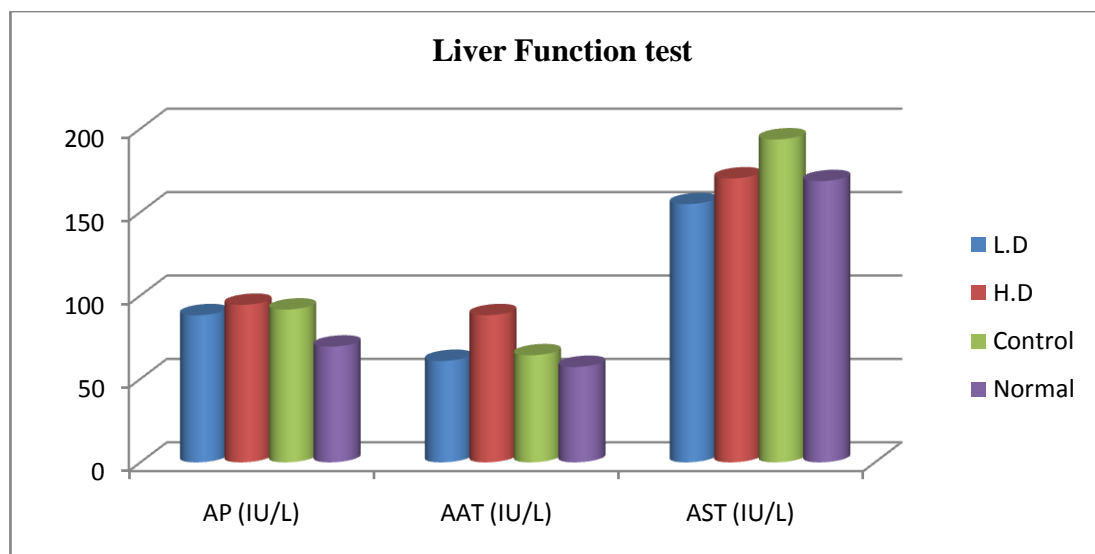


Figure 4.20: Level of AP, ALT, and AST in rats treated with ethanolic extracts of *P.niruri*. Ap: Alkaline phosphatase; ALT: Alanin aminotransferase; AST: Aspartate aminotransferase.

Values are expressed as mean \pm SEM

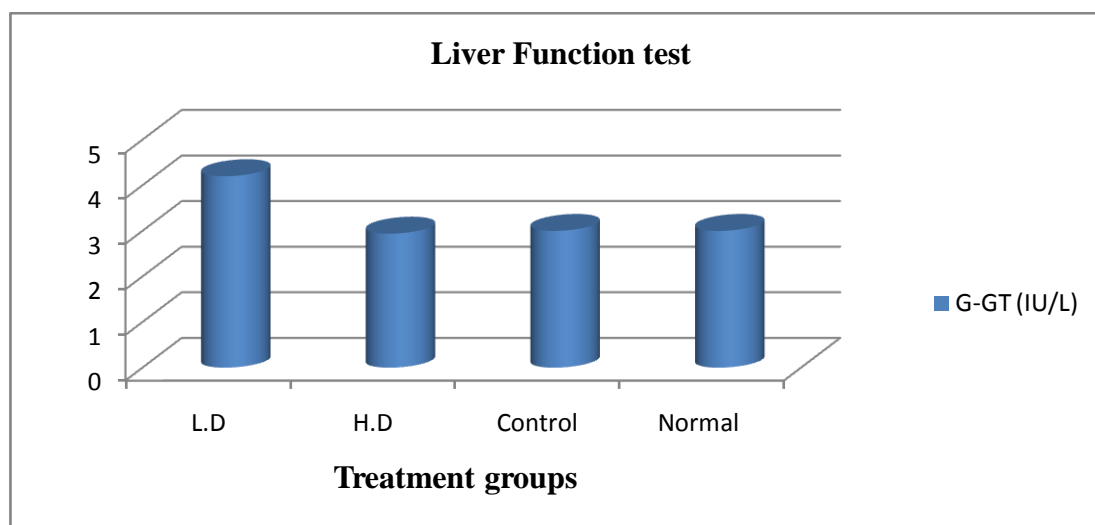


Figure 4.21: Level of G-Glutamyl. Trasferase in rats treated with ethanolic extracts of *P.niruri*

Values are expressed as mean \pm SEM

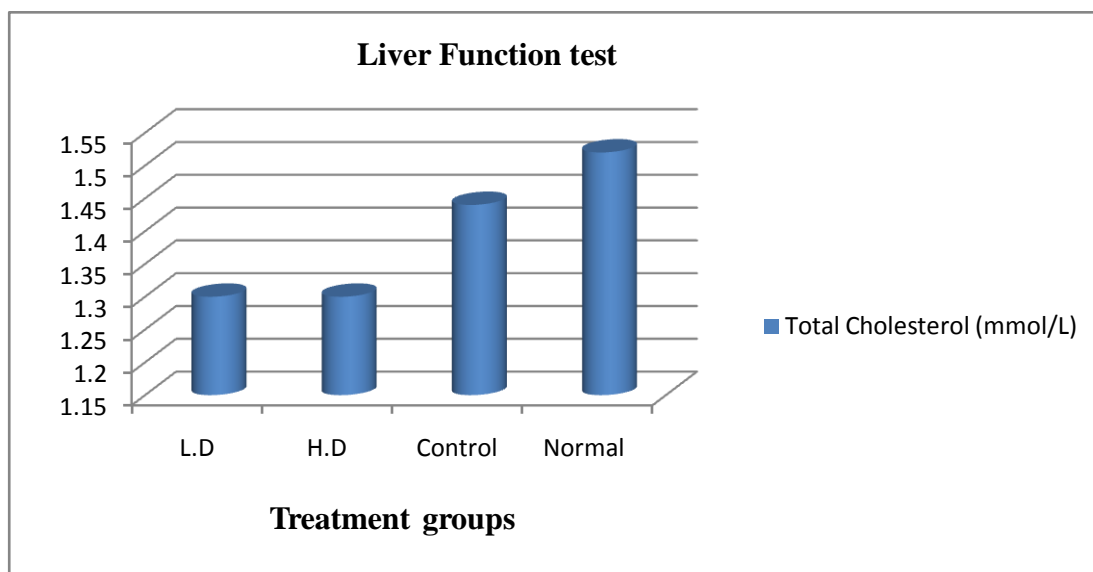


Figure 4.22: Level of Total cholesterol in rats treated with ethanolic extracts of *P.niruri*

Values are expressed as mean \pm SEM

4.1.3.3 Counting the ACF

ACF in the colon were counted and the average of total number of ACF as well as the number of crypts per focus was obtained (Table 4.10) and (Figure 4.16 to 4.27)

Table 4.10: average of ACF number in colon tissue

group	1 crypt	2 crypt	3 crypt	4 crypt	5 & more crypt	Total ACF	conc. Of inhibition
Control	14.6 \pm 8.84	18.2 \pm 8.56	18 \pm 5.98	11.4 \pm 2.77	14 \pm 5.14	107.6 \pm 25.53	0.00%
LD	5 \pm 0.71	8 \pm 1.14	10.8 \pm 1.39	9.2 \pm 3.84	8 \pm 3.65	41 \pm 7.79	66.60%
HD	3 \pm 0.89	8 \pm 1.52	8 \pm 2.43	4.6 \pm 1.5	6.8 \pm 2.52	30.4 \pm 5.38	77.20%

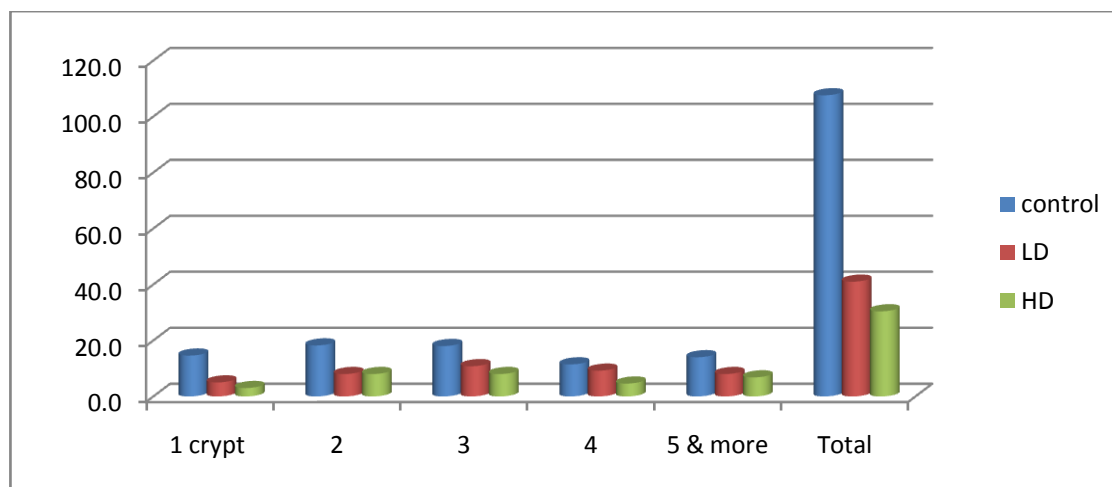


Figure 4.23: average of ACF number in colon tissue

Values are expressed as mean \pm S.E.M.

There are significant differences between control group and Low and High groups.

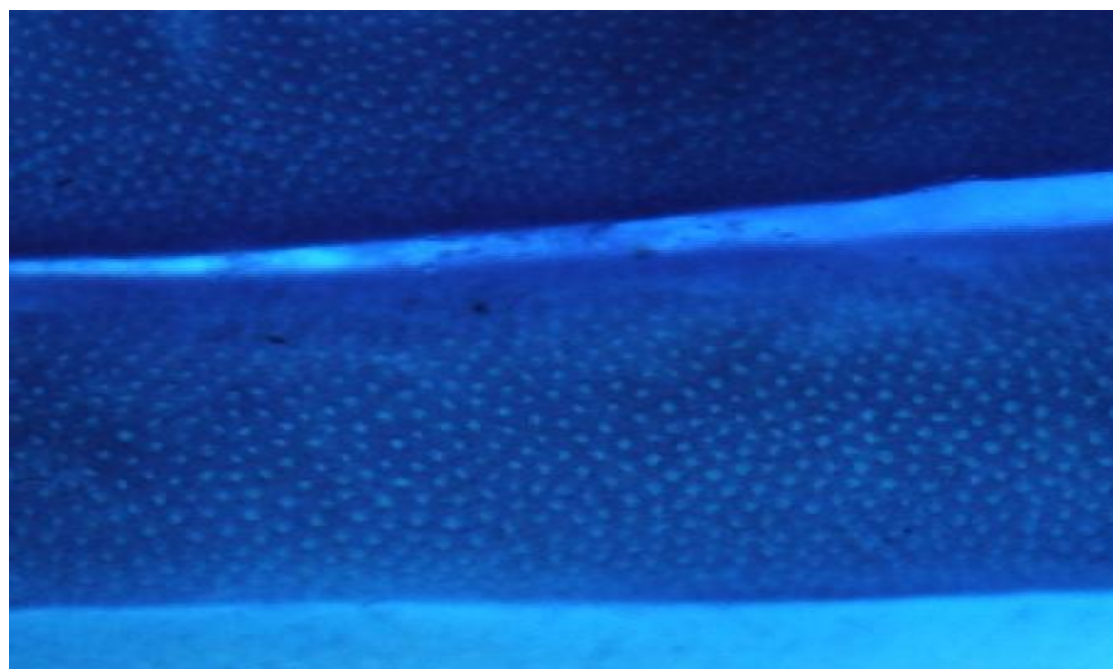


Figure 4.24: Light microscopy of methylene blue staining of rat colonic tissue (gross) showing the normal crypts from rats treated with vehicle (2x)



Figure 4.25: Light microscopy of methylene blue staining of rat colonic tissue (gross) showing the normal crypts from rats treated with vehicle (10x)

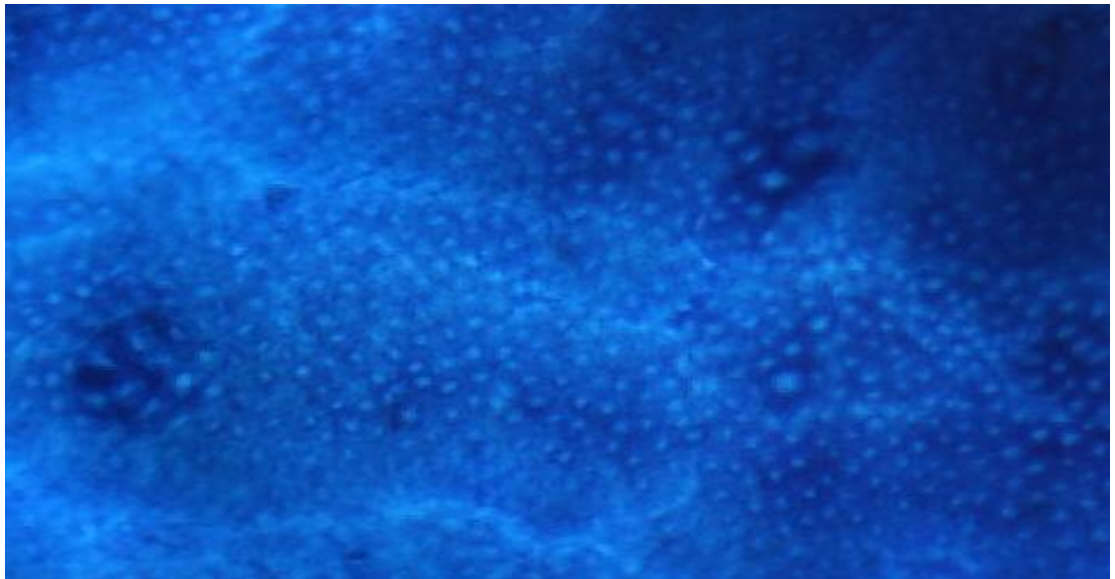


Figure 4.26: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at low dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discernible pericryptal zone (2x)

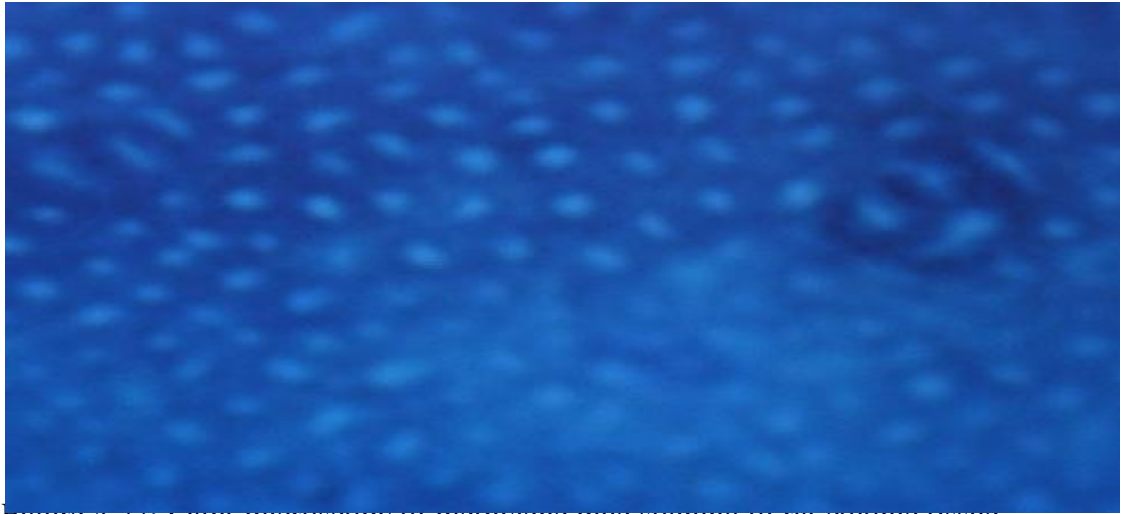


Figure 4.27: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at low dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discemible pericryptal zone (4x)

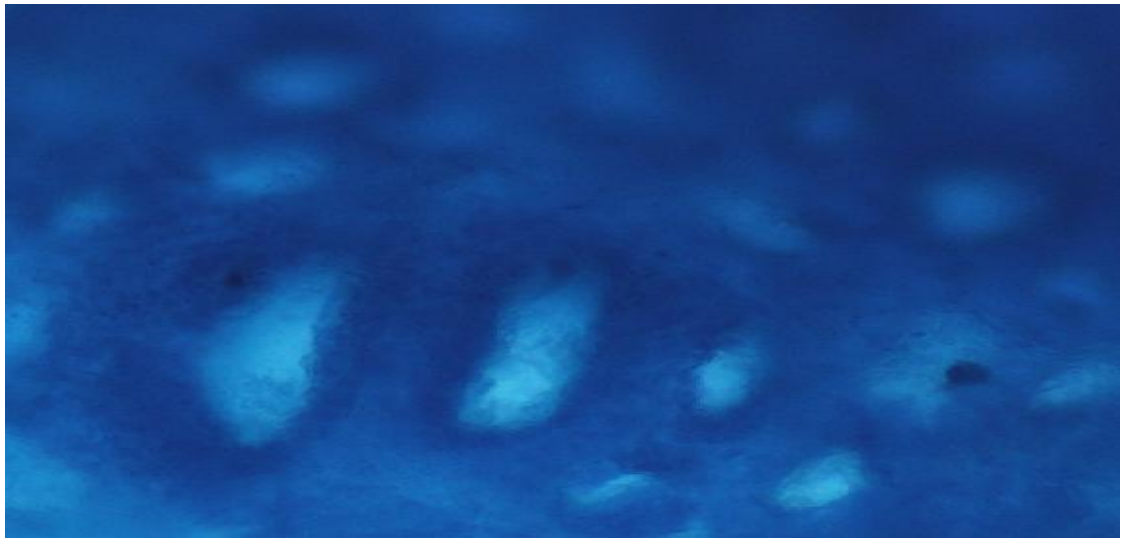


Figure 4.28: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at low dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discemible pericryptal zone (10x)

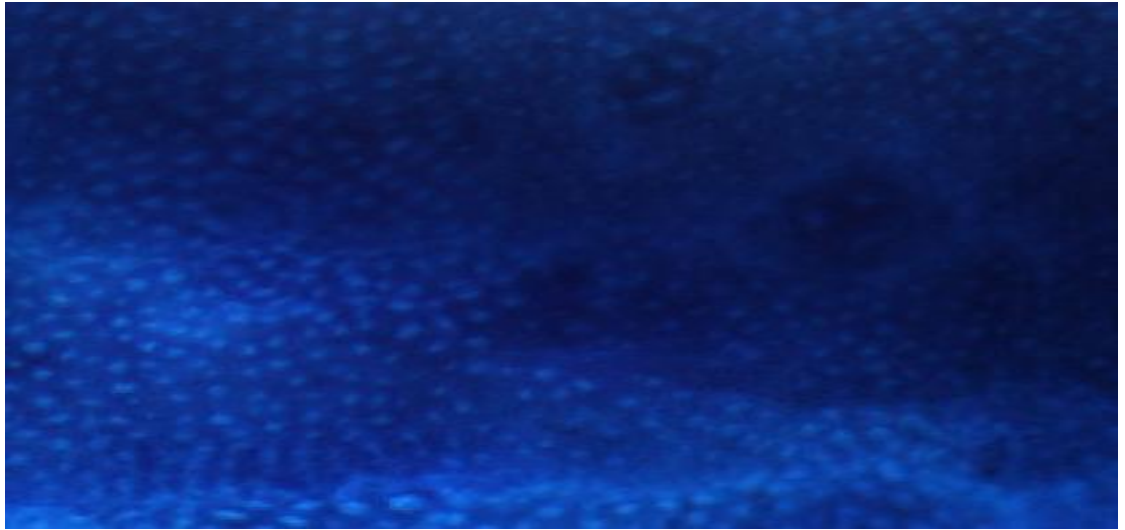


Figure 4.29: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at high dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discemible pericryptal zone (2x)

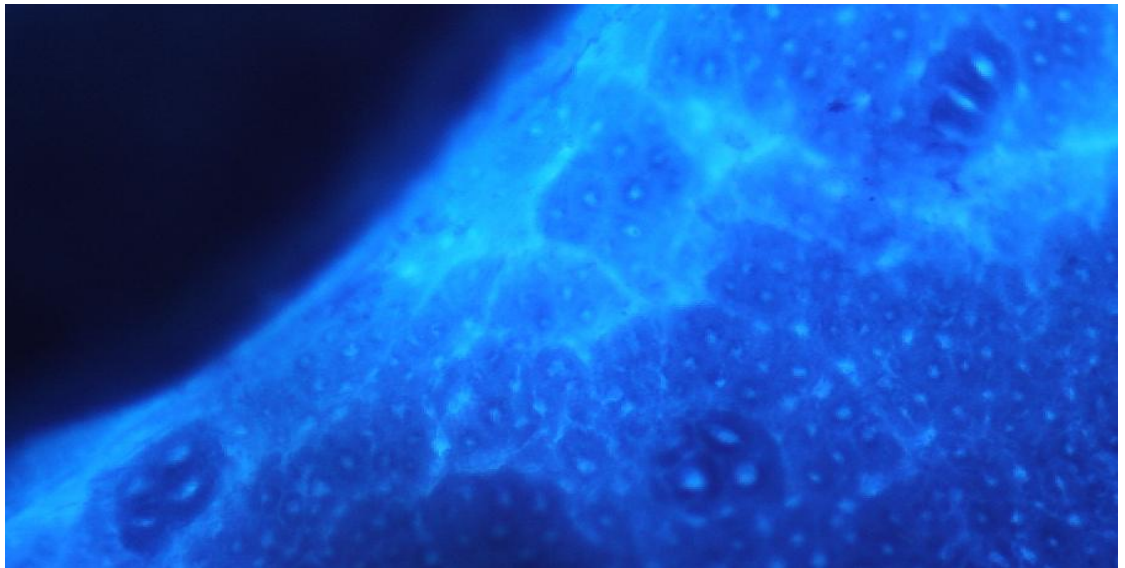


Figure 4.30: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at high dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discemible pericryptal zone (4x)

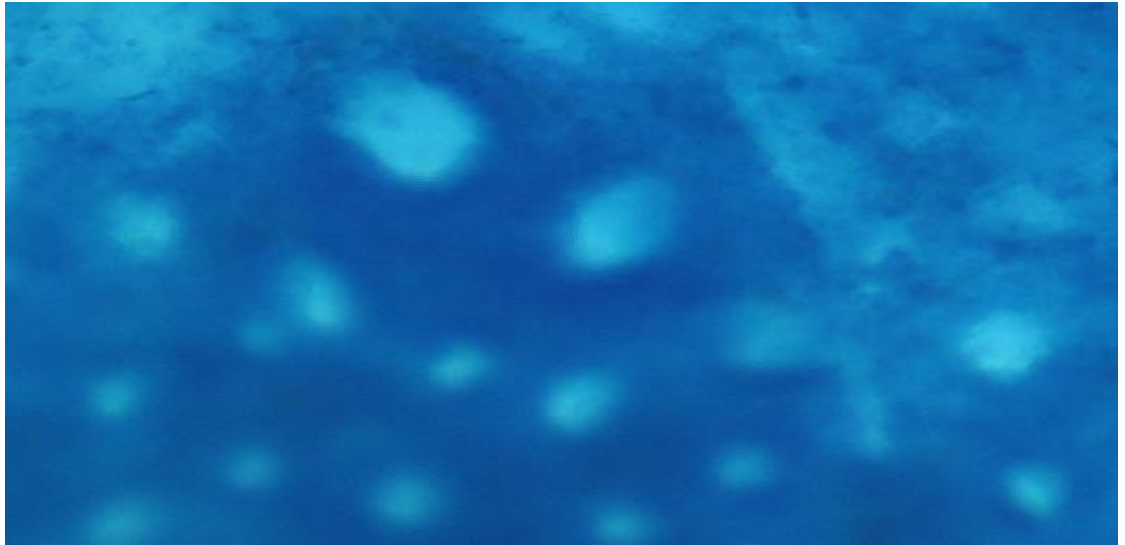


Figure 4.31: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract at high dose. ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discernible pericryptal zone (10x)

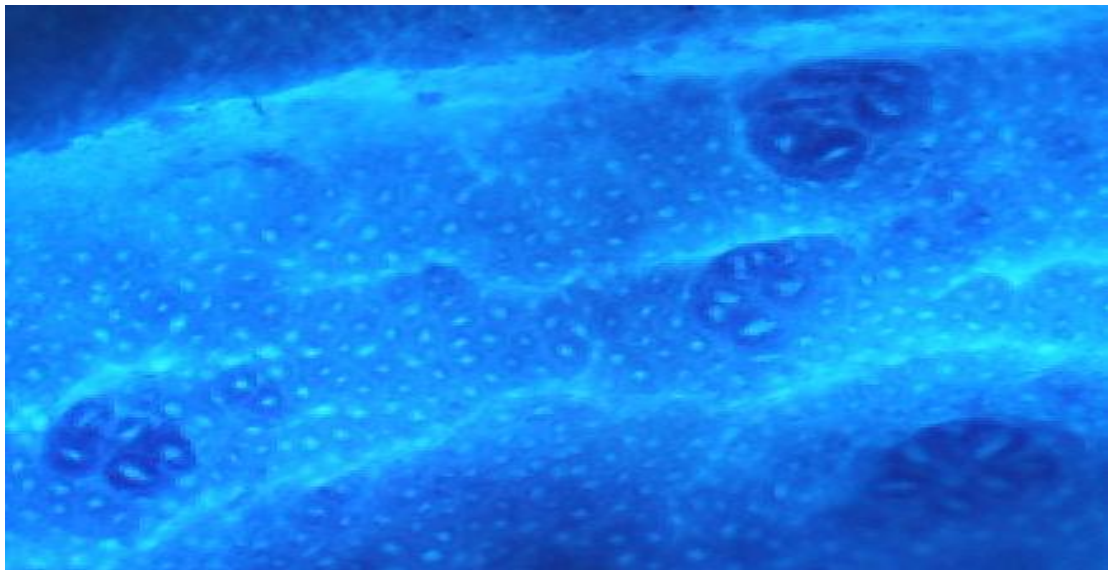


Figure 4.32: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane (cancer control group). ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy discernible pericryptal zone (2x)

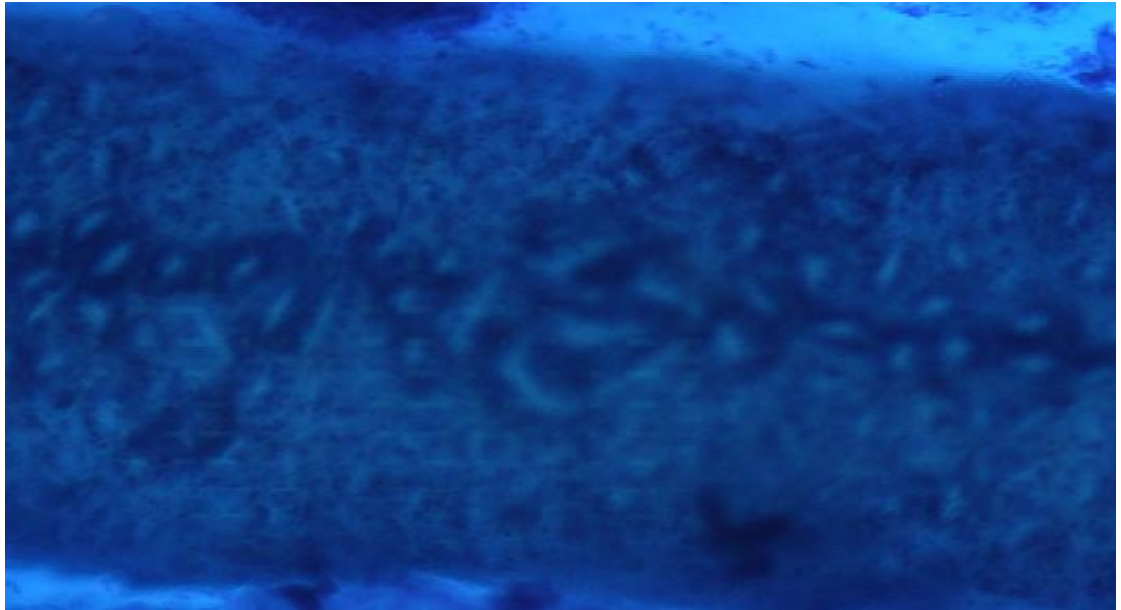


Figure 4.33: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane (cancer control group). ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy disceivable pericryptal zone (4x)



Figure 4.34: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane (cancer control group). ACF were distinguished from normal crypts by their increase size, increase distance from lamina to basal surface of cells, and easy disceivable pericryptal zone (10x)

Under light microscope, the total number of ACF as well as the number of crypts per focus was counted and the main was founded to be compared in each group with the control group. Numbers of crypts per focus vary (Figure 4.35 to

4.39). Aberrant crypts were distinguished from the surrounding normal crypts by their increased size and figure. Nondysplastic crypts are 1.5 – 2.5 times larger than surrounding crypts, often with rounded lumens and mucin depletion. ACF possess larger luminal openings and flatter mucosal surfaces. The latter occurs because of loss of microvilli (Vaccina et al., 1998).



Figure 4.35: : Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract. Aberrant crypt foci lesions (One crypt per focus) in colon tissue (4x)



Figure 4.36: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract. Aberrant crypt foci lesions (Two crypt per focus) in colon tissue (4x)



Figure 4.37: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered plant extract. Aberrant crypt foci lesions (Three crypts per focus) in colon tissue (4x)



Figure 4.38: Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered with vehicle. Aberrant crypt foci lesions (four crypts per focus) in colon tissue (4x).



Figure 4.39: A Light microscopy of methylene blue staining of rat colonic tissue (gross) treated with colon carcinogen azoxymethane and administered with vehicle. Aberrant crypt foci lesions (four crypts per focus) in colon tissue. Aberrant crypt foci lesions (more than five crypts per focus) in colon tissue (4x).

ACF in the colon were counted as described by Bird (1987). Briefly, each colon was split open longitudinally and placed on a filter paper with the luminal surface open and exposed. Another filter paper was placed on top of the luminal

surface and fixed overnight using 10% buffered formalin. Each fixed colon was cut into proximal and distal portions of equal length and each portion was further cut into 2-cm long segments. Each segment was placed in a Petri dish and stained using 0.5% methylene blue solution for 5 min. The segments were transferred to another Petri dish containing buffer to remove excess stain, and then

CHAPTER FIVE

5.1. DISCUSSION

Administration of *P. niruri* significantly reduced AOM-induced total colonic ACF formation and multicrypt aberrant crypt growth. To date, there are no studies with *in vivo* (colon) cancer models in evaluating anticarcinogenic potential of *P. niruri*. Thus, our investigation in well-established models of chemically induced colon carcinogenesis is the first study to validate the chemopreventive effects of *P. niruri* in animal models.

It has been well established that excessive proliferation and lack of apoptosis leads to colon tumor growth, and our results clearly suggest that *P. niruri* can be used as a potential antitumorigenic agent.

In the present study, administration of *P. niruri* provided up to 77.2 % inhibition of AOM-induced total ACF formation and suppression of five or more crypts growth up 72%, which clearly suggests the potential colon tumor inhibitory properties of *P. niruri*. Previous studies have established that ACF containing five or more aberrant crypts correlate with colon tumor outcome. In this context, previous studies with naturally occurring agents, such as curcumin, caffeic acid ester (Rao et al., 1993) , and diosgenin (Jayadev et al., 2004), significantly suppressed AOM-induced colon ACF and colon adenocarcinoma in male F344 rats. Also, it is possible that *P. niruri* may provide some antioxidant activities that may help in prevention of carcinogenesis.

ACF were identified in human colonic mucosa 4 years after initial reports of these lesions in carcinogen-treated animals (Pretlow et al., 1991). In resected colonic surgical specimens fixed flat in formalin, human ACF were found to have similar morphological characteristics to those in experimental animals: 1) component ACF crypts were larger than normal crypts, 2) pericryptal spaces were expanded, 3) crypts stained darker than adjacent crypts, and 4) crypt orifices differed in configuration from circular openings of normal crypts (Shpitz et al., 1998, Bouzourene et al., 1999). ACF varied in size from a single crypt to multiple abnormal crypts, sometimes with more than 150 – 200 crypts per focus. ACF are usually elevated, but can be depressed relative to adjacent mucosa. It is important to emphasize that ACF must be identified in situ in the colon, and not from histological sections. The criteria that define ACF require identification of specific surface features that cannot be reliably appreciated in histological sections. Investigators have described dysplastic crypts that differed from conventional ACF in the colons of carcinogentreated rodents. These included β -catenin-accumulated ACF (Yamada et al., 2001), flat-appearing ACF (Paulsen et al., 2005), or mucin-depleted ACF (Caderni et al., 2003). These newly described lesions appear to exhibit stronger associations with the development of tumors than conventional ACF. Some of these lesions appear to be variants of conventional ACF, while others are reported to have normal surface features when stained in situ, but are dysplastic in histological sections. Conventional ACF are heterotypical in histology, with features ranging from hyperplastic to dysplastic. Nondysplastic crypts are 1.5 – 2.5 times larger than surrounding crypts, often with rounded

lumens and mucin depletion. On scanning electron microscopy, ACF possess larger luminal openings and flatter mucosal surfaces. The latter occurs because of loss of microvilli (Vaccina et al., 1998). Colonic epithelial cells of dysplastic ACF show loss of basal nuclear polarity and increased proliferation (Di Gregorio et al., 1997). Dysplastic ACF often possesses slit-like, irregular, or closed lumens. Dysplastic crypt cells within ACF may be focally localized, with adjacent cells in the same focus showing only hyperproliferative changes. Some ACF exhibit a serrated luminal pattern and may constitute a distinct group from hyperplastic or dysplastic ACF (Shpitz et al., 1998, Otori et al., 1995). Studies suggest there may be staining differences between dysplastic and nondysplastic ACF (Ochiai et al., 2005). Dysplastic ACF might provide ideal premalignant structures to elucidate causally important steps early in colonic carcinogenesis. Differential staining could also allow more rapid and accurate detection of precursor lesions for chemoprevention studies.

Features of a crypt that can be identified by viewing the colons in this manner include the size and shape of the crypt, the thickness of the epithelium lining, and the size and shape of the luminal opening (Bird, 1987).

Criteria used to define an ACF consisting of a single crypt are as follows. The size of the crypt is at least twice that of the normal surrounding crypts; the luminal opening is more elliptical than circular; and the thickness of the epithelial lining is greater than that of the normal surrounding crypts. ACF consisting of more than one crypt are characterized as follows: (a) individual crypts comprising

the ACF have a thicker epithelial lining and an elliptical luminal opening: (b) the crypts have the appearance of forming a distinct focus, i.e., there are no normal-appearing crypts separating the crypts within the ACF; and (c) the total area occupied by the crypts composing the ACF is greater than the area occupied by an equivalent number of surrounding morphologically normal crypts.

Our observation that a reduction in the number of ACF generally consisting of ACF with 1 or 2 crypts is interesting and raises important questions regarding the developmental aspects of ACF and their importance in colon carcinogenesis. Confirmation of the observation that some ACF are indeed transient lesions is needed before any significance is attached to this particular finding. Division of data into ACF consisting of 1, 2, 3, or 4 or more crypts revealed that the number of ACF consisting of 4 or more crypts was significantly greater in cancer control group. A majority of ACF exhibited morphological atypia. Using a grading system to enumerate atypia based on nuclear morphology (elongation and stratification), it became evident that some ACF exhibited a grade 4 nuclear atypia. Nuclear elongation and stratifications are the 2 main characteristics of dysplastic epithelium (Konishi and Morson, 1982). Because dysplasia is generally considered to be a significant finding in the carcinogenic process, our observation that some ACF exhibit dysplasia presents strong evidence that ACF represent precursor lesions. An ACF exhibiting mild dysplasia revealed nuclei with the characteristic "picket-fence" pattern seen in human adenomatous polyps in association with mild dysplasia (Konishi and Morson, 1982). However, the observation that not all ACF exhibited dysplasia raises interesting questions. If dysplasia can be used as a basis

for distinguishing ACF with an increased neoplastic potential from those with no increased potential, the histological appearance of ACF expressed at the time the lesions are formed (i.e., at the single crypt stage) would be of paramount importance. If this is not the case (i.e., if dysplasia can be expressed at later time points), then dysplasia could not be used as a marker of neoplastic potential. To establish a quantitative relationship between number of crypts per ACF and grade of dysplasia, and between nuclear grade (dysplasia) and time, histological features of a large number of ACF with varying crypts numbers at various times will need to be examined. It is essential to establish whether these relationships exist.

5.2. CONCLUSION

Colorectal cancer develops from complex interactions between inherited susceptibility and environmental factors. This study aimed to evaluate the chemoprotective effects of *phyllanthus niruri* against AOM-induced foci of aberrant crypts in rats. Aberrant crypt foci formation is among the observed earliest changes in the colon that may lead to cancer, as they are formed before colorectal polyps. *P. niruri* is an annual herbaceous plant that inhabits the tropical and subtropical regions of both hemispheres. Commonly has been popularly used in traditional medicine either as whole plant, fresh leaves or as fruits in the treatment of various diseases. In the acute toxicity the administration of *P. niruri* on *Sprague Dawley* rats did not cause mortality among all groups and considered quite safe even at high doses. In addition, no behavioral evidence of signs of toxicity was observed throughout the study period among all groups.

In conclusion, Administration of *P. niruri* significantly reduced AOM-induced total colonic ACF formation and multicrypt aberrant crypt growth. Further studies with purified constituents and evaluation in clinical settings are needed to understand the complete mechanism of colon cancer activity of *P. niruri*.