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Effect of Total Extract of Bunchanian Lanzan Leaves against Hepatocellular Carcinoma in Diethyl Nitrosamine induced Mice Tumor Model

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Abstract

Aim: The present study was focused on anticancer effect of total extract (70% ethanol) of *Bunchanian lanzan* leaves against Diethylnitrosamine (DEN) induced hepatocarcinoma in male wistar rats. **Method**: Antitumor potential was estimated via oral administration of ethanolic leaves extract of *Bunchanian lazan* (ELEBL) at the dose of 200 mg/kg and 400 mg/kg once daily for 2 weeks. The doses were fixed after performing acute toxicity study according to OECD guideline-423. 5-flurouracil (10mg/kg) was administered to the standard group. After treatment with the extract the serum samples were collected for estimation of various parameters like SGOT, SGPT, total protein, bilirubin, alkaline phosphatase and antioxidants LPO, SOD and Catalase which are considered as biomarkers in hepatocarcinoma. After determination of all the parameters the obtained values of ELEBL treated groups were compared with the control group and 5-flurouracil treated group. **Result**: A significant decrease in SGOT and SGPT label in all ELEBL treated groups was observed as compared to the DEN treated group(P<0.001) and in case of antioxidant enzymes a significant(P<0.001) increase in SOD, Catalase label and a significant(P<0.001) decrease in LPO was observed in all drug treated groups compared to DEN treated group. Whereas in the animal treated with extract, a decrease in total protein, ALP and bilirubin was observed compared to the DEN treated group. **Conclusions**: The ethanolic extract of Bunchanian lanzan leaves showed a significant dose dependent reduction in DEN induced hepatocarcinoma.
Keywords: *Bunchanian lanzan*, Diethyl nitrosamine (DEN), Hepatocarcinoma, Antioxidant, 5-flourouracil, Biochemical parameters.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common type of liver tumor occurring worldwide irrespective of gender [1], especially in Asia and Africa [2]. It is common in 80-90% of liver tumors and it is the fourth most common cause of cancer mortality. More than half million peoples are affected every year by HCC. Various factors can induce HCC such as environmental factors, hepatitis viral infection, food additives, excessive alcohol consumption and smoking, alfatoxins and also by exposure with chemical carcinogens, air and water pollutants [3]. HCC can be cured surgically, though most of the cases are not diagnosed in time as its propagation is asymptomatic in nature. In this study the researchers had focused their attention towards herbal medicine to counteract the adverse effects and secondary disorders induced by allopathic system of medicine.

Buchanania lanzan (*Anacardiaceae*family) has folklore evidence for its effective role in curing asthma, cough, skindiseases, antioxidants and antitumoreffect.[4]. Diethylnitro samine (DEN) is a potent hepatocarcinogenic agent. It can damage the structure of DNA repairing enzymes and is used to induce cancerous tumors in experimental models. It can be obtained from tobacco smoke, agricultural chemicals, cosmetics and various pharmaceutical products [5-7].

Materials and methods

Plant material

The leaves of *Buchanania lanzan* were collected from Mirjapur region, Utter Pradesh in the month of January, 2011 and authenticated by Dr. Jayaraman, Botanical Survey of India, Chennai, Tamil Nadu, India. The leaves of *Buchanania lanzan* were dried in the air and then milled to a fine powder of 1 mm mesh size.

Extraction procedure

The dried and powdered plant material (100 g) was extracted successively with 600 ml, of ethanol (1:6 w/v) by using soxhlet extractor for 48 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman No. 1 filter paper and then concentrated in vacuum at 40^0C using a rotary evaporator. The percentage yield of extract was calculated. [8]

Animals

Adult male Wister albino rats weighing 100-150gs were purchased from King Institute, Guindy, Chennai. First 30 male rats selected were acclimatized for a period of 2 weeks in laboratory condition and a 12 hour day/night cycle was maintained. Animals had free access to good quality pallet food and tap water ad libitum. The experimental procedure and protocol of the study was approved by Institutional Animal Ethics Committee (IAEC) and the allotted register no is X11/VELS /PCOL/36/2000/CPCSEA/IAEC/11.03.11 and were in accordance with the guidelines of the CPCSEA.

Experimental design

The experimental rats were then randomly divided into 5 groups and each group consisted of 6 rats which were housed three per cage. Liver carcinogenesis was induced in group II, III, IV and V by injecting DEN (in DMSO) intraperitonially at a dose of 50 mg/kg once in a week for a period of three weeks. Group 1 served as control; group II served as positive control (DEN alone); group III was treated with 200mg/kg and group IV was treated with 400mg/kg dose of total extract of *Buchananian lazan* leaves. After DEN administration, plant extract were simultaneously given orally to group III and group IV at the dose of 200 mg/kg and 400 mg/kg. Group V served as standard and the standard drug was 5- flurouracil (10 mg/kg).

Biochemical estimation

The rats were weighed periodically and their body weights were recorded. After 21 days of observation the overnight fasted animals were sacrificed by chemically induced euthanasia using chloroform. Blood samples were collected by cardiac puncture and the serum was obtained from the blood by centrifugation at 10,000 rpm for 10 minutes to analyze antioxidant parameters and the liver was isolated from the animals and homogenized to estimate enzyme levels.

Serum Glutamate Oxaloacetate Transferase (SGOT) [9]

This enzyme is also known as Aspertate Amino Transferase (AST) and its level will be increased in serum if there is an injury in liver and heart or it can be increased when liver function is impaired in case of necrosis or cell damage. The serum levels of AST were measured using commercial kits by (Reitman S& Frankel S1957). The enzyme activity is expressed in IU/ liter.

Serum Glutamate Pyruvate Transaminase (SGPT) [9]

This enzyme also known as Alanine AminoTransferase (ALT) and its level will be increased in serum if there is an injury in liver or in case of necrosis or cell damage. It was estimated by the reagents and methods used were the same as those used for the assay of SGOT but substrate solution was different and the incubation time was reduced to 30 minutes.

Alkaline Phosphatase [10]

Alkaline phosphatase is an enzyme responsible for removing phosphate groups from nucleotides, alkaloids and proteins. In adults, the level will be elevated in case of liver and biliary tract disease.

Total Bilirubin [11]

Total serum bilirubin consists of conjugated (mostly with glucouronic acid) and unconjugated (free) form. Conjugated bilirubin content was determined by subtracting unconjugated bilirubin from total bilirubin.

Total Protein [12]

For estimating total protein, 0.1 ml of serum suitably diluted with 0.9 ml of water and 4.5 ml of alkaline copper reagent were added and kept in room temperature for 10 min. and then 0.5 ml of Folin's reagent was added and the color developed was read after 20 min at 640 nm. The level of protein was expressed as mg/dl of serum.

Tissue Lipid Peroxidation [13]

The basal lipid peroxidation system consisted of 1.2 ml of 0.3 M Tris - HCl buffer, 0.2 ml of sodium pyrophosphate and 0.2 ml of diluted tissue homogenate. The inducing system contained 0.2 ml ferrous sulphate, 0.2 ml ascorbate (as an inducer), 0.2 ml sodium pyrophosphate and 0.2 ml of diluted tissue homogenate. The volume was made upto 2.0 ml with water. The tubes were incubated at 37^0 C with constant shaking for 20 minutes. The reaction was stopped by the addition of 1.0 ml of 10 % TCA. The tubes were shaken well then 1.5 ml TBA reagent was added and was heated at 90^0 C for 20 minutes. The tubes were centrifuged and the colour developedin the supernatant was read at 532 nm. Standard (1-5 nmoles) was taken in 2.0 ml volume and was processed as above along with blank containing 2.0 ml water.The basal and inducers added lipid peroxidation in the experimental setup was compared with respective controls. Level of lipid peroxidation was expressed as amount of MDA formed / mg protein.

Superoxide Dismutase (SOD) [14]

In SOD estimation, 0.5 ml of the tissue homogenate, 0.25 ml of absolute ethanol and 0.15 ml of chloroform were added. After 15 minutes of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant was taken. This supernatant liquid consisted of the enzyme extract.

The assay mixture for the enzyme contained 2 ml of 0.1M Tris-HCl buffer, 0.5 ml of pyrogallol, 0.5 ml of aliquots of the enzyme extracts and water to give a final volume of 4 ml. The rate of inhibition of pyrogallol auto-oxidation after the addition of the enzyme was noted at 470 nm at an interval of a minute for 3 minutes.

The enzyme activity was expressed in terms of units/mg protein and one unit corresponds to the amount of enzyme required to inhibit the auto oxidation reaction by 50 %.

Catalase [15]

For estimation of catalase enzyme concentration in serum,0.1 ml of the tissue homogenate was taken to which 1.0 ml of phosphate buffer and 0.5 ml of hydrogen peroxide was added in each tube and the reaction was started. The reaction was assessed by the addition of 2 ml of dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 2 µM was taken and treated similarly. The tubes were then heated in a boiling water bath for 10 minutes. The green colour developed was read at 570 nm in a colorimeter.

Catalase activity was expressed as µ moles of H_2O_2 utilized/min/mg protein under incubation condition.

Statistical Analysis

All data were presented as the mean ±SD. One way ANOVA was used for multiple comparisons of groups with post hoc Bonferroni's test.

Table 1. Data showing preliminary Phytochemical screening of the ethanolic extract of *Buchanania lanzanleaves extract.*

Sl. No.	Phytochemical Test	Ethanolic Extract
1.	Test for Alkaloids	+ve
2.	Test for Steroids	-ve
3.	Test for Tannins	-ve
4.	Test for Saponin	-ve
5.	Test for Gallic acid	+ve
6.	Test for flavonoids	+ve
7.	Test for carbohydrates	-ve
8.	Test for Gum and Mucilages	-ve
9. .	Test for Glycoside	+ve

Result & Discussion

Extraction and Preliminary Phytochemical study

The percentage yield of total extract of *BuchananiaLanzan* was found to be 18.50 %. Preliminary phytochemical analysis of the ELEBL of plant revealed the presence of alkaloid, flavonoid, glycosides and gallic acid. After purifying the extract, the yield was completely dried and subjected for acute toxicity study to determine the therapeutic dose using rats in controlled environment.

Acute toxicity studies

According to OECD guideline-423 acute toxicity study was done. The total extract of the *Bunchanian lanzan* leaves were administered orally to different group of rats. No deviation was observed from any of the group within a span of 24 hours. There was no sign of occurring toxicity up to the dose of 2000 mg/kg body weight. Based on the result obtained from this study, the dose for anti-cancer activity was fixed to be 200 mg/kg and 400 mg/kg for dose dependent study.

Estimation of SGPT and SGOT Level:

Necrosis or membrane damage release the enzyme into circulation, therefore it can be measured in serum. High level of SGOT and SGPT indicates liver damage. Its label also increased in group II (DEN alone). Conversely, the administration of ELEBL in the dose manner of 200 and 400 mg/kg reduced the SGPT and SGOT level in Group III and IV treated animal. In DEN induced cancerous rats, significant ($P<0.001$) increase in serum SGOT and SGPT were found to be 103.33±0.45 mg dl^{-1} and 255.51±0.51 mg dl^{-1} respectively. Whereas the animal treated with total extract exhibited a decrease in SGOT (91.21±0.23 in 200 mg/kg and 84.45±0.65 in 400 mg/kg) and SGPT (242.98±0.51 in 200 mg/kg and 180.43±0.33 in 400 mg/kg) and the data is tabulated in Table-2. The graphical representation of activity shown by the extract is depicted in Figure1.

Estimation of ALP, Bilirubin and Total protein Level:

DEN intoxicated normal rat showed elevated label of alkaline phosphatase, bilirubin and also the total protein level compared to control group. Serum ALP, BB and TP are related to the function of hepatic cell. Increase in serum level of ALP is due to increase synthesis in presence of increasing biliary pressure. In DEN induced cancerous rat, significant ($P<0.001$) increase in serum total protein, ALP and BB were found to be 832.16±0.52

mg/100 g, 295.33±0.62 IU/L and 1.517±0.093 mg/dl respectively (Table 3). Whereas in the animal treated with extract, a decrease in TP (818.32±0.43 in 200 mg/kg and 792.21±0.21 in 400 mg/kg) and ALP (288.12±0.33 in 200 mg/kg and 252.65±0.22 in 400 mg/kg) and BB (0.510±0.06 in 200 mg/kg and 0.536±0.12 in 400 mg/kg) were found (Table-3). The The graphical representation of activity shown by the extract is depicted in Figure - 2 and Figure - 3.

Table 2. Effect of *Buchanania Lanzan* on Biochemical parameters in DEN induced Hepatocarcinoma in rats.

Sl. no.	Groups (n=6)	SGOT (IUL^{-1})	SGPT (IUL^{-1})
1.	Normal control	51.47±0.33	98.25±0.27
2.	DEN alone	103.13±0.45	255.51±0.15
3.	DEN+200 mg/kg extract(T1)	91.21±0.23	242.98±0.34
4.	DEN+400 mg extract(T2)	84.45±0.65	180.43±0.33
5.	DEN+ 5FU	81.11±0.54	167.55±0.56

All values are presented as mean ± SEM. Group II was compared with group I and all values were significant ($p<0.001$); group III, group IV and group V were compared with group II and all values were significant. ($p<0.001$). P values were calculated by one way ANOVA analysis with post hoc Bonferroni's test. (N=6)

Estimation of LPO, SOD and CAT

LPO and SOD and CAT are also a very useful biomarker which shows the biochemical changes in serum. Because in DEN intoxicated animal, the lipid peroxidation (LPO) in the liver tissue was slightly elevated in the group III, IV and V. However, DEN treatment alone (GP II) caused a significant two fold increase respectively in the status of LPO in the liver tissue of rats. The level of LPO decreased in the ELEBL treated group significantly ($P>0.001$) and also in the standard drug treated group. In the DEN intoxicated animal group, its level was significantly ($P<0.001$) found to be 0.219±.004 whereas the treated group with extract was significantly found to be 0.115±0.013 in 200 mg/kg and 0.178±0.021 in 400 mg/kg (Table-4). The graphical representation of activity shown by the extract is depicted in Figure – 4, Figure – 5.

In case of SOD and CAT, a moderate decrease of activity was shown in the liver tissue of rats compared to control group. Group II (DEN alone) showed a significant decrease ($P>0.01$) in activity and a significant increase was seen in the drug treated as well as the test groups (ELEBL treated). The values of SOD and CAT in DEN treated were 4.44±0.10 and 41.85±1.33 respectively. When the test groups were treated with the extract, the SOD (5.11±0.21 in 200 mg/kg and 7.65±0.33 in 400 mg/kg) and CAT (48.55±1.09 in 200 mg/kg and 56.09±0.99 in 400 mg/kg) were significantly ($P>0.01$) found.

Statistics report

In table-1all the values were presented as mean ± SEM. Group II was compared with group I and all values were significant ($P<0.001$); group III, group IV and group V were compared with group II and all values were significant. ($P<0.001$). P values were calculated by one way ANOVA analysis with post hoc Bonferroni's test. (N=6).

In table-2 all the values were presented as mean ± SEM. Group II was compared with group I and all values were significant ($P<0.001$)Group III ($P<0.01$) vs Group II, Group IV ($P<0.01$) vs Group II, Group V ($P<0.01$) vs Group II, N=6.

SOD = µ moles of MDA/min/mg protein

CAT = µ moles of H_2O_2 consumed/min/mg protein

LPO = µ moles of MDA/min/mg protein.

Table 3 . Effect of *Buchanania Lanzan* on Biochemical parameters in DEN induced Hepatocarcinoma in rats.

Sl. no.	Groups(n=6)	ALP (IUL^{-1})	TP (mg/100g)	Bilirubin (mg/dL)
1.	Normal control	220.25±0.25	767.90±0.23	0.460±0.022
2.	DEN alone	295.33±0.62	832.16±0.52	1.517±0.093
3.	DEN+200 mg extract(T1)	288.12±0.33	818.32±0.43	0.510±0.06
4.	DEN+400 mg extract(T2)	252.65±0.22	792.21±0.21	0.536±0.12
5.	DEN+ 5FU	247.55±0.93	788.34±0.99	0.510±0.05

All values are presented as mean ± SEM. Group II was compared with group I and all values were significant ($p<0.001$); group III, group IV and group V were compared with group II and all values were significant. ($p<0.001$). P values were calculated by one way ANOVA analysis with post hoc Bonferroni's test. (N=6)

Table 4. Effect of *Buchanania Lanzan* on Antioxidant level in DEN induced Hepatocarcinoma in rats.

Sl. no.	Groups (n=6)	SOD	CAT	LPO
1.	Normal control	8.89±0.12	63.21±1.45	0.095±.003
2.	DEN alone	4.44±0.10	41.85±1.33	0.219±0.004
3.	DEN+200mg extract(T1)	5.11±0.21**	48.55±1.09**	0.115±0.013
4.	DEN+400mg extract(T2)	7.65±0.33	56.09±0.99	0.178±0.02**
5.	DEN+ 5FU	7.99±0.55	57.99±1.93	0.099±0.003

All values are presented as mean ± SEM. Group II was compared with group I and all values were significant ($p<0.001$)

Group III ($p<0.01$) vs Group II

Group IV ($p<0.01$) vs Group II

Group V ($p<0.01$) vs Group II, N=6

SOD = µ moles of MDA/min/mg protein

CAT = µ moles of H_2O_2 consumed/min/mg protein

LPO = µ moles of MDA/min/mg protein

Graphical representation of post treatment change in SGOT, SGPT, Total protein, ALT, superoxide radical, lipid peroxidation, catalase, bilirubin labels on different group of rat

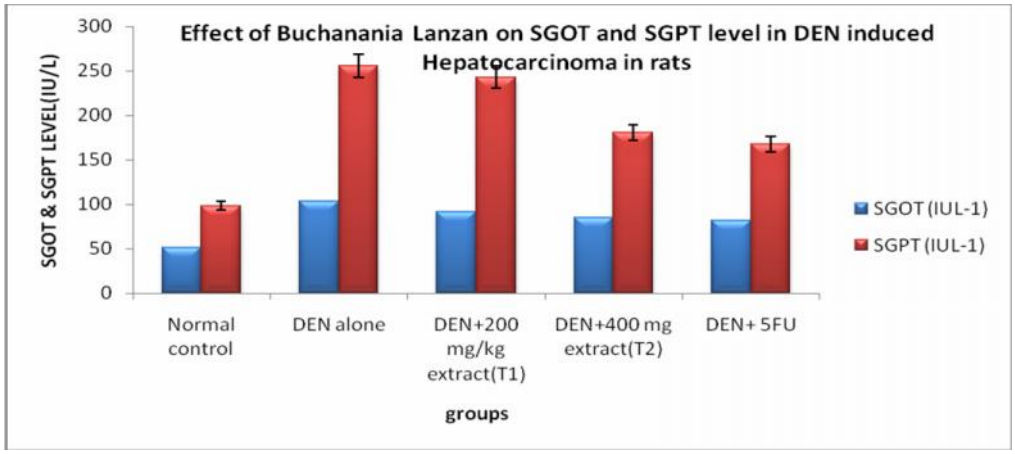

Figure – 1

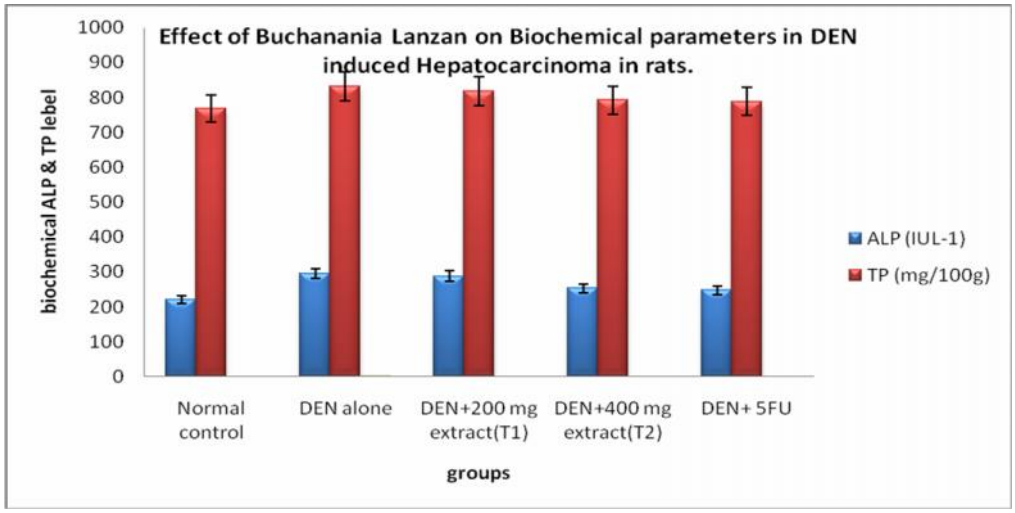

Figure – 2

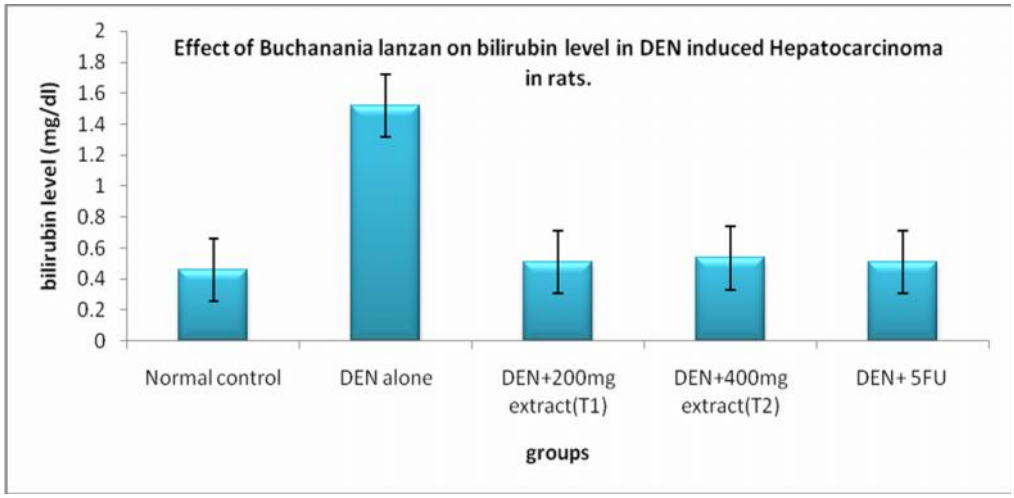

Figure – 3

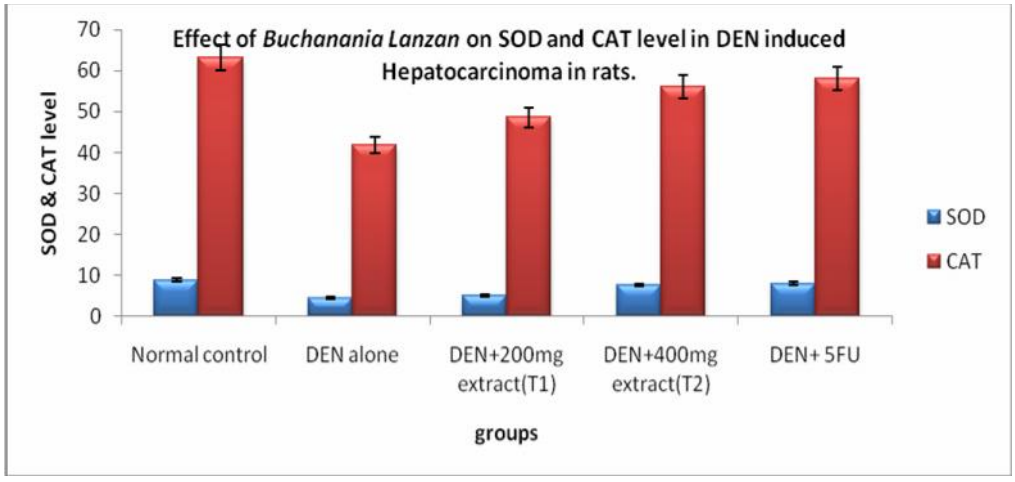

Figure – 4

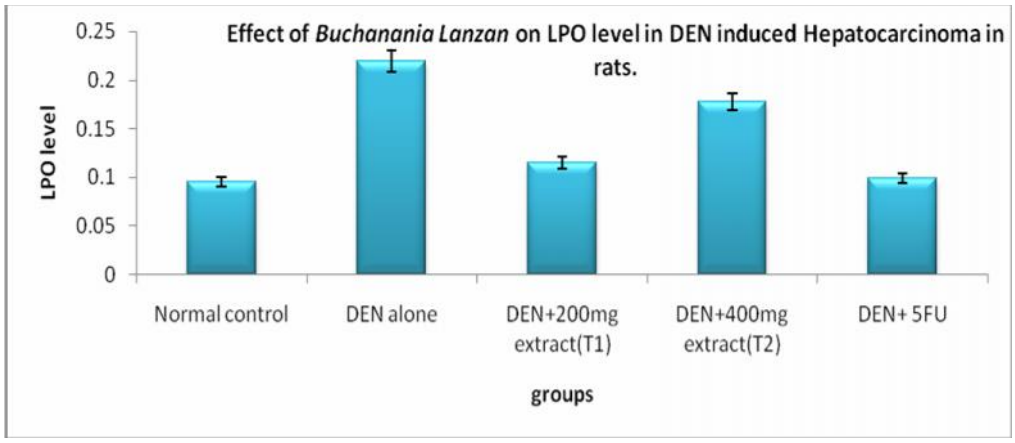

Figure -5

Discussion

In Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agent. One of the most versatile plant used intreating HCC is *BuchananiaLanzan*, a member of Anacardiaceae family, and it was taken for anticancer evaluation in DEN induced rats.

Hepatocellular carcinoma (HCC) is the seventh most common cancer in men and the ninth most common cancer in women, within an estimated incidence of 250,000 to 1.2 million per year worldwide [16]It is a highly malignant tumor with poor prognosis, the poor prognosis has been attributed to late diagnosis. An effective screening system to detect HCC at an early stage may result in more effective treatment. DEN a hepatocarcinogen, is known to cause perturbations in the nuclear enzymes involved in DNA repair/replication and is normally used as carcinogen to induced liver cancer in animal models. DEN has been shown to be metabolized to its active ethyl radical metabolite, and the reactive product interacts with DNA causing mutation, which will lead to carcinogenesis.

Elevation of the plasma levels of cytoplasmic and mitochondrial enzymes are sensitive indicators of liver damage [17]. Drug induced liver damage has been reported to correlate with an increase in the activity of these enzyme [18]. Among the various phosphatase ALP have attained much attention because of their location in plasma membrane and possible role in active transport. The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanism that are unbalanced by a hepatotoxin. [19]

Elevation of serum SGPT, SGOT, ALP, TP, LPO and bilirubin is known effect of DEN toxicity which specially affects the liver. And activities of ALP, SGPT, SGOT, and LPO are most commonly used biochemical markers of liver damage. Lipid peroxidation plays an important role in carcinogenesis [20]and may lead to the formation of several toxic products, such as malondialdehyde (MDE) and 4-hydroxynonenal. These products can attack cellular targets including DNA, thereby inducing mutagenecity and carcinogenicity [21].Recently, the increase in lipid peroxidation was reported during DEN induced hepatocarcinogenesis. In line with this finding, there was a significant increase in the level of lipid peroxidation in the liver of rats treated with *Bunchanian lanzan* extract. The observed reduction in the level of lipid perioxidation in extract treated animals was presumably due to its ability to scavenge the hydroxyl and peroxyl radicals. Whereas the decrease of the serum level of CAT and SOD are also an indication of the liver damage due to cancer induction which also reversed in extract treated groups.

Conclusion

The preliminary phytochemical studies reveal the presence of flavonoids in total extract of *BuchananiaLanzan*. Various flavonoids have been reported for its anticancer activity. Previous study on flavonoid has been shown to possess anti mutagenic and antimalignant effect. Flavonoid has a chemo preventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis. Therefore the possible mechanism of anticancer of *Buchananialanzan* may be due to flavonoid content and further study has to be carried out to study the exact mechanism behind it.

References

1. Anand P, Kunnumakkara AB. Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* 2008; **25**: 2097–116.
2. QianY,Ling CQ. Preventive effect of Ganfujian granule on experimental hepatocarcinoma in rats. *World J Gastroenterol* . 2004; 10:755–757. [PubMed]
3. Ellman GL. Tissue sulfhydryl groups. *Arch BiochemBiophy.*1959; 82: 70–77. [PubMed]
4. Shalini Kapoor Mehta, Swaru pananda Mukherjee and B. Jaiprakash. Preliminary phytochemical investigation leaves of on buchananian lanzan. *International Journal of Pharmaceutical Sciences Review and Research.* 2010; 3:55-60.
5. Brown JL. N-Nitrosamines. *Occu p Med.* 1999; 14:839–848. [PubMed]
6. Sullivan BP, Meyer TJ, Stersh i c MT, Keefer LK. Accelerati on of N-nitrosation reactions by electrophiles. *IARC Sci Publ.* 1991; 14:370–374. [PubMed]
7. Reh BD, Fajen JM. Worker exposurestonitrosamines in a rubber vehicle sealing plant. *Am IndHyg A ssoc J.* 1996; 57:918–923. [PubMed]
8. Jeyachandran R, Mahesh A, Cindrella L. DEN induced cancer and its alleviation by Anisomelesmalabarica(L) R.Br. ethanolic leaf extract in male albino mice. *International Journal of Cancer Research,* 2007; 3(4): 174-179.
9. Reitman s, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transminase, *American J Clinical pathology*, 1957; 28: 56-58.
10. King PR, King EJ, Estimation of alkaline phosphatsae by determination of hydrolysed phenol with antipyrin .*American Jclinical pathology,* 1954; 7: 322 -326.
11. HT, Evelyn KA. Estimation of serum bilirubin level with the photoelectric colorimeter. *J bio chem.*, 1937; 119, 481-484.
12. Lowery OH, Rosenbrough NT, farr AL protein measurement with Folin-phenol reagent. *J bio chem.*, 1951; 173, 265-275.
13. Devasagayam TPA, Tarachand U, decrease lipid peroxidation in the rat kidney gestation. *Biochem Biophys Res Commun,* 1987; 56, 836-842.
14. .Marklund S, Marklund G. Involvement of superoxide anion radical in the auto oxidation of the pyrogallol and a convenient assay for SOD. *European J Biochem*, 1997, 47, 469-474.
15. Sinha AK. Colorimeter assay of catalase. *Analytical Biochemistry*, 1972; 47, 389-394.
16. Kew MC .Tumors of the liver. In Hepatology: A Textbook of Liver Disease, 3rd edn, Zakin D, Boyer TD, editors. WB Saunders: Philadelphia; 1996, p. 1513-1548.
17. Sherlock, S. and J. Dooley. Disease of the Liver and Billiary System. 9th Edn., Blackwell Scientific Publishing, London; 1993, p.-
18. Rhoades R, Pflanzer R. Human physiology. Saunders college Publishing, London; 1996. p. 13-14.
19. Mehendale, H.M., R.A. Roth, A.J. Gandolfi, J.E. Klaunig, J.J. Lemasters and L.R. Curtis,. *Novel mechanisms in chemically induced hepatotoxicity. FASEB. J.*, 1994; 8: 1285-1295.
20. Anand KK, Singh B, Chend D and Bhandan DK,.An evaluation of Lawsoniaalba extract as hepato protectiveagent.*PlantaMedica* 1992;58:22-25.
21. Sturgil M, G Lambart G H. Xenobiotic induced hepatotoxicity:Mechanism of liver injury and methods of monitoring hepatic function. *Clin. Chem* 1997; 43:1512-1526.

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