



Biological Screening of Triherbal Formulation on Chemically Induced Hepatocellular Carcinoma

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ABSTRACT: The hepatoprotective activity of a triherbal formulation (LAB) comprising of 50 % ethanolic extract of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* was studied using Swiss albino rats. The animals received a single intraperitoneal injection of N-nitrosodiethylamine 200mg/kg body wt followed by subcutaneous injection of CCl₄ in a dose of 3 ml/kg body wt. The administration of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capability of extracts in respect with anticancer potency in comparison with the very much effective in preventing NDEA-induced multistage hepatocarcinogenesis possibly through antioxidant and antigenotoxic nature, which was confirmed by various liver injury and biochemical tumor markers enzymes and molecular events. LAB dose dependently and significantly the increase in serum hepatic enzyme levels after NDEA& CCl₄ treatment compared to the toxin control group. On administration of LAB, it dose dependently resulted in an elevation in the levels of serum CAT, GPX, GSH, GST, SOD and total protein and decrease in lipid peroxidation compared to the toxin control group. It also increased the attenuated the total protein concentrations compared to the toxin control group. The results of this study confirmed the antioxidant and hepatoprotective activity of the triherbal formulation against carbon tetrachloride& N-nitrosodiethylamine induced hepatotoxicity in Swiss albino rats. © 2011 IGJPS. All rights reserved.

KEYWORDS: Carbon Tetrachloride; N-nitrosodiethylamine; Hepatocellular Carcinoma; *Lawsonia innermis*; *Aeagel marmelos*; *Boerrhavia diffusa*.

INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the elements in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and main

compounds in synthetic drugs. Plants have long served as a useful and natural source of therapeutic agents. Almost all plants have medicinal values and their uses differ from place to place. Liver diseases pose an enormous health problem in

spite of tremendous strides in modern medicine. There is hardly any drug that can effectively control inflammation, protect the liver from the damaging effects of hydrophobic bile acids which are retained in cholestatic disorders, promote protein synthesis, manifest antioxidative, anti-lipid peroxidative and antifibrotic properties, prevent fat from infiltrating the liver, enhance glucuronidation, decreases intestinal absorption and suppresses hepatic synthesis and storage of cholesterol, stabilize hepatocyte membranes, help the liver to replace damaged tissue and regenerate itself, promotes effective metabolism of drugs by maintaining levels of CYP or Cytochrome P450, protect the liver from damage and also regulate the liver enzymes. Because of the affordability, availability and accessibility of dependable hepatoprotective drugs in scientific/ conventional medicine, plants play an important role in the management of various liver disorders and in meeting the demands of primary health care in many developing countries. These plants are commonly used in foods as vegetables and spices for flavouring and in medicine they are used for the treatment of various ailments such as diarrhea, headache, fever, hepatitis, dysentery, malaria, nausea, diabetes . They have been used singly and in combination with other plants for treatment of different disorders. The hepatoprotective potentials of a triherbal formulation containing these three plants are evaluated using carbon tetrachloride and NDEA.

MATERIALS & METHODS

Animals

Healthy Swiss albino rats weighing 140 -160g and mice weighing 17-22g were used for this study. The animals were housed in polypropylene cages at controlled temperature, well ventilated with a 12-12 h light dark cycle. The rats and mice were fed with standard laboratory diet and water was provided *ad libitum*. The animals were maintained as per the CPCSEA guidelines and regulations and the study was approved by the institutional animals ethics committee at AIPR, Lucknow.(CPCSEA Registration No: 1146/ac/07/CPCSEA) .

Plant Materials

The leaves of *Aeagel marmelos* whole plant of *Boerrhavia diffusa* and *Lawsonia innermis* were collected from the National Botanical Research Institute Garden, Lucknow in August-September 2011. The plant materials were authenticated and identified by Dr. Sayeeda Khatoon, chemotaxonomist and compare with Voucher specimens (NAB 99229, 98070 and 94888) and voucher specimens (NAB 300696,300697 and 300698) were deposited in the NBRI herbarium and museum for future reference.

Preparation of plants extract(s)

The fresh plant materials of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* and were washed with distilled water and air -dried at 30+₋ 2 C. The dried plant materials of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* (1000g) was exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol (50% EtOH) and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour and then dried in lyophilizer under reduced pressure.

Efficacy of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* (LAB) on N-nitrosodiethylamine induced hepatocellular carcinoma in animal- in vivo model

Animals were randomized and grouped into experimental control rats. Group I rat were treated with 0.9% normal saline. Group II rats received single intraperitoneal injection of N-nitrosodiethylamine(200mg/kg body wt). Group III rats received subcutaneous injection of carbon tetrachloride(3ml/kg body wt) once a week for 6 weeks. Group IV rats received single intraperitoneal injection of N-nitrosodiethylamine(200mg/ kg body wt) followed by subcutaneous injection of carbon tetrachloride(3ml/kg body wt) as Group III. After 20-25 weeks hepatocellular carcinoma(HCC) was confirmed in Group IV(NDEA+CCL4) rats with the help of bio-chemical and histopathological

studies and Group IV (NDEA+CCL4) rats were used for investigation of HCC experimentation.

Experimental Design

Effect of 50% EtOH extract of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* (LAB) on hepatocellular carcinoma

Group I- Normal Control

Group II- Chemical induced HCC (NDEA+CCL4)

Group III- 50% EtOH (LAB) (100mg/kg.body wt p.o) in HCC rats

Group IV- 50% EtOH (LAB) (200mg/kg.body wt p.o) in HCC rats

Group V- 50% EtOH (LAB) (400mg/kg.body wt p.o) in HCC rats

Group VI- Cisplatin (6mg/kg. body wt i.p. weekly once for 3 weeks) 50%EtOH in HCC rats

Effect of 50% EtOH extract of (LAB) on hepatocellular carcinoma

The animals were treated with the test drugs and standard for 28 days. After completion of treatment, animals were anaesthetized with anesthetic ether and blood was removed from the retro-orbital puncture with the help of capillary, collect, then after 30 min centrifuge at 3000 rpm for 15 min then separate serum/plasma for biochemical estimation.

Estimation of tumour marker enzymes

1. Determination of Serum glutamic oxaloacetic transaminase(SGOT)
2. Determination of Serum glutamate pyruvate transaminase(SGPT)
3. Determination of Serum alkaline phosphatase(SALP)
4. Determination of Serum γ glutamyl transaminase(GGT) activity
5. Determination of Serum bilirubin(SB)
6. Determination of Serum total protein

6. Estimation of free radical generation

- 6.1 Measurement of Lipid peroxidation(LPO)
- 6.2 Measurement of Superoxide dismutase(SOD)
- 6.3 Measurement of Catalase(CAT)
- 6.4 Determination of glutathione-S-transferase(GST)
- 6.5 Determination of reduced glutathione(GSH)
- 6.6 Determination of glutathione peroxidase(GPX)

7. Determination of Nucleic Acids

- 7.1 Extraction of Nucleic Acids
- 7.2 Estimation of Deoxyribonucleic acid (DNA)
- 7.3 Estimation of Ribonucleic acid (RNA)

8. Haematological studies

Red blood cells(RBC), White blood cells(WBC) and Haemoglobin(Hb) were estimated with the help of hematology analyser. The RBC and WBC were expressed as million/mm³ of blood and Hb as g/dl of blood.

9. Histological studies

At the end of each scheduled duration the control as well as treated rats were sacrificed by using cervical dislocation and the liver was dissected out and changes in liver weight and tumour incidence was noted and a part of liver tissue was immediately fixed in Bouin's fluid for 24 hr and washed in running tap water to remove the color of Bouin's fluid and dehydrated in alcohol in ascending and descending order, embedded in paraffin and cut at 5 μ m in a rotary microtome. These sections were then deparaffinized in xylene and stained with hematoxylin-eosin using routine method. The sections were then stained with haematoxylin-eosin dye and mounted with Canada balsam. The histopathological slides were examined and photographs were taken with a digital stereomicroscope.

10. Statistical analysis

Data are expressed as mean \pm SEM (standard error of mean). The difference among means has been analysed by unpaired student's t-test.

RESULTS

Pharmacological Investigation

The present study was undertaken to investigate the hepatocellular carcinoma effects of (LAB) with special emphasis on the molecular mechanisms involved in the curative activity against N-nitrosodiethylamine (NDEA) – induced hepatocellular carcinoma in rats.

1. General behavior and acute toxicity Studies

50% ethanolic extracts of selected plants *Lawsonia inermis*, *Aeagal marmelos*, *Boerhavia diffusa* (LAB) up to 2000mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc. during the period of observation.

2. Per se effect of the 50% ethanolic extracts of LAB on SGOT, SGPT, SALP and Bilirubin level (BL) in serum

50% ethanolic extracts of LAB at a dose of 100, 200mg and 400mg (O.D x 28 days) were subjected for se effect by studying various biochemical parameters like SGOT, SGPT, SALP AND BL serum. The 50% ethanolic extracts of LAB did not show any significant effect on liver biochemical markers viz SGOT, SGPT, SALP AND BL levels (Table 1)

3. Per se effect of the 50% ethanolic extracts of LAB on lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in rats

50% ethanolic extracts of LAB at a dose of 100, 200mg and 400mg (O.D x 28 days) were subjected for se effect by studying LPO, SOD, CAT and GPx in liver homogenate of rats. This is a significant decrease in the levels of lipid peroxidation product malondialdehyde (LPO) at higher dose

levels of 200 and 400 mg/kg. However, the levels of SOD and GPx were increased at 200 and 400 mg/kg whereas the catalase (CAT) showed significant increase on 100mg/kg of LAB. (Table 2).

4. Effect of 50% ethanolic extract of LAB on body weight, Liver weight and average liver weight in (NDEA+CCL4) induced HCC rat

50% ethanolic extracts of LAB, at a dose of 100, 200 mg and 400 mg once daily for 28 days and standard Cisplatin at a dose of 6mg/kg were subjected for studying the body weight, liver weight and Average liver weight in HCC rats. The study showed that the liver weights were significantly increased from 4.3 \pm 0.26 to 7.8 \pm 0.68 in NDEA plus CCL4 group. However, 50% ethanolic extracts of LAB showed a dose dependent and significant protection in liver weight from 7.8 \pm 0.68 in NDEA plus CCL4 group to 6.1 \pm 0.38 to 4.8 \pm 0.40 in LAB treated animals. Whereas, standard drug cisplatin (6mg/kg) showed significant reduction in liver weight compared to NDEA+CCL4 group (Table 3)

5. Effect of 50% ethanolic extract of LAB on SGOT, SGPT, SALP AND BL and GGT against NDEA+CCL4- induced Hepatocellular Carcinoma

It is clearly evident from the table 4 that NDEA+CCL4 caused significant elevation of liver serum markers. In the NDEA+CCL4 treated group, the level of SGOT (192.20 – 354.21, p<0.001), SGPT (83.21 – 371.53, p<0.001), SALP (234.14 – 439.31, p<0.001), BL (0.72 – 1.26, p<0.001) and GGT (32.4 – 154.2, p<0.001). In contrast, the groups treated with LAB extract at dose of (100 – 400 mg/kg) once daily for 28 days prevented the cancer in a dose related manner. The range of protection in the serum marker were found to be SGOT (354.21 – 202.81, p<0.05 to p<0.01), SGPT (371.53 – 109.06, p<0.05 to p<0.001), SALP (439.31 – 249.96, p<0.05 to p<0.001), BL (1.26 – 0.82, p<0.01 to p<0.001) and GGT (154.2 – 78.1, p<0.001) respectively. The protection of cisplatin ranged for SGOT (354.21 – 198.32, p<0.01), SGPT (371.53 – 92.34, p<0.001), SALP (439.31 – 242.26, p<0.001), BL (1.26 – 0.78, p<0.001), GGT (154.2 – 52.4, p<0.001) respectively as

shown in table 4. The histological observations also basically support the results obtained from serum enzyme assays (Figure.1)

6. Effect of 50% ethanolic extract of LAB on LPO, SOD, CAT, GPX, GST and GSH against NDEA+CCL4-induced Hepatocellular Carcinoma

Administration of NDEA+CCL4 led to increase in the levels of LPO (0.46, $p<0.001$), and decrease in SOD (114.4 – 48.2, $p<0.001$), CAT (28.8 – 6.24, $p<0.001$), GPX (3.54 – 1.42, $p<0.001$), GST (1.06 – 0.47, $p<0.001$) and GSH (0.36-0.04, $p<0.001$) levels in the 5% w/v liver homogenate. Treatment of rats with 50% ethanolic of LAB at a dose of (100 – 400mg/kg) markedly prevented the NDEA+CCL4 induced alterations of various parameters LPO (4.64 – 1.02, $p<0.05$), SOD (48.20 – 98.52, $p<0.05$ to $p<0.001$), CAT (6.24 – 21.39, $p<0.05$ to $p<0.001$), GPX (1.42 – 3.24, $p<0.05$ to $p<0.001$), GST (0.47 – 0.94, $p<0.05$ to $p<0.001$), GSH (0.04 – 0.27, $p<0.05$ to $p<0.001$) respectively. The protection of cisplatin ranged for LPO (4.64 – 0.88, $p<0.01$), SOD (48.2 – 102.31, $p<0.001$), CAT (6.24 – 24.09, $p<0.001$), GPX (1.42 – 3.42, $p<0.001$), GST (0.47- 0.97, $p<0.001$) AND GSH (0.04-0.32, $p<0.001$) respectively as shown in the table 5.

7. Effect of 50% ethanolic extract of LAB on DNA, RNA and Protein against NDEA+CCL4- induced Hepatocellular Carcinoma

It is clearly evident from the table 6 that NDEA+CCL4 caused significant elevation of DNA and RNA and decrease in protein level. In the NDEA+CCL4 treated group, the level of DNA, RNA were increased to (5.41 – 7.58, $p<0.001$) and (7.52 – 9.92, $p<0.001$) and protein decreased to (8.04 – 6.27, $p<0.05$) respectively. In contrast, the groups treated with LAB extract at dose of (100 – 400 mg/kg) decreased the elevated levels of DNA (7.58 – 5.52, $p<0.001$) and RNA (9.92 – 7.62, $p<0.05$ to $p<0.01$) and increase the protein level towards normalization (6.27 – 7.87). Upon treatment with cisplatin the level of DNA, RNA and protein shows (7.58 – 5.48, $p<0.001$), (9.92 – 7.58, $p<0.01$) and (6.27 – 7.55) respectively.

8. Effect of 50% ethanolic extract of LAB on haematological parameter (RBC, WBC and Hb) of

control and NDEA+CCL4- induced Hepatocellular Carcinoma in rat

The table 7 shows the levels of red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb). However, the Hb and RBC levels were decreased (11.97 – 8.52, $p<0.001$) and (8.12 – 6.42), respectively with a concomitant increase in WBC (6.22 – 8.79, $p<0.05$) with respect to control. In contrast, the groups treated with LAB Extract at dose of (100 – 400 mg/kg) once daily for 28 days prevented the cancer in a dose related manner. The range of protection in the Hb, RBC and WBC shows (8.52 – 10.67, $p<0.05$), (6.42 – 7.89) and (8.79 – 6.54, $p<0.05$). The protection of cisplatin ranged for Hb (8.52 – 11.45, $p<0.01$), RBC (6.42 – 7.97) and WBC (8.79 – 6.24, $p<0.05$) respectively.

DISCUSSION

Epidemiological studies have shown that fruits, vegetables, beverages, spices, tea and medicinal herbs rich in antioxidants and other micronutrients protect against diverse forms of chemically-induced hepatic damage, carcinogenesis, mutagenesis, DNA-damage and lipid peroxidation .

An attempt has been made to investigate plants and plant product used to treat Hepatocellular carcinoma. An attempt has been made in scientifically validated experiment animal models to investigate a novel herbal drug based anticancer agents from plants viz. (LAB) *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* against hepatocellular carcinoma.

The 50% ethanolic extracts of the selected plants were further assessed to understand the molecular defensive mechanism involved in safety and efficacy in treatment of Hepatocellular carcinoma.

The 50% ethanolic extracts of LAB per se showed dose dependent antioxidant activity as evidenced by their effect on elevated levels of SOD, CAT, GPX and depleted levels of LPO in liver homogenate.

Oral treatment (mg/kg, od x 28 days)	SGOT	SGPT	SALP	BL
Control (Normal rats)	196.0 ± 1.49	80.2± 1.11	232.3± 1.12	0.64± 0.08
LAB 100mg	196.1± 1.51	79.8± 1.43	232.1± 8.23	0.61± 0.07
LAB 200mg	197.3± 1.48	80.3± 1.54	231.2± 1.02	0.63± 0.12
LAB 400mg	196.2± 1.40	81.9± 1.01	232.6± 0.83	0.64± 0.06

Table 1 *Per se* effect of the 50% ethanolic extracts of LAB (100, 200 and 400 mg) on SGOT (U/l), SGPT (U/l), SALP (U/l), and Bilirubin level (U/l), (BL) in serum of rat. Values are mean ± SEM of rats in each group.

Oral treatment (mg/kg, od x 28 days)	LPO	SOD	Catalase	GPx
Control (Normal rats)	0.41± 0.04	112.0± 8.9	21.4± 1.2	3.1± 0.05
LAB 100mg	0.39± 0.01	124.2± 3.2	27.8± 0.9c	3.3± 0.14
LAB 200mg	0.30 ± 0.02a	141.4 ± 3.2b	30.8± 1.4c	3.4± 0.06b
LAB 400mg	0.18± 0.04	152.2± 6.2b	34.3± 2.1c	3.5± 0.04

Table 2 *Per se* effect of the 50% ethanolic extracts of LAB (100, 200 and 400 mg) on lipid peroxidation, LPO(MDA nmoles/mg of protein), superoxide dismutase, SOD (units/mg of protein), catalase, CAT (units/mg of protein) and glutathione peroxidase, GPx (mmol/g tissue) in rats. Values are mean ± SEM of 8 rats in each group. P values: a <0.05, b<0.01, c<0.001 compared with control group.

Groups	Treatment	Dose	Initial body wt (gm)	Final body wt (gm)	Liver weight (gm)	Average liver wt (liver weight/100gbw)
I	Control	---	145± 7	182± 7	4.3± 0.26	2.8± 0.54
II	NDEA + CCL4	200 mg/kg	157± 8 (NDEA)+3ml/kg bw(CCL4)	141± 8z	7.8± 0.68z	6.9± 1.10
III	LAB	100 mg/kg	147± 6	162± 7	6.1± 0.38	4.8± 0.74
IV	LAB	200 mg/kg	154 ± 7	175± 8	5.5± 0.41	3.9± 0.46
V	LAB	400 mg/kg	157± 6	178± 7	4.8± 0.40a	3.2± 0.20
VI	Cisplatin	6 mg/kg	142 ± 8	169± 7	4.5± 0.41a	3.0± 0.10

Table 3 Effect of 50% ethanolic extract of LAB on Body weight and Average liver weight in (NDEA+CCL4) induced HCC rats. Values are mean ± SEM of rats in each group. P values: z<0.001 compared with respective control group. P values: a<0.01 compared with group II (NDEA+CCl4).

Groups	Treatment	Dose	SGOT	SGPT	SALP	BL	GGT
I	Control	---	192.20± 1.64	83.21 ± 1.60	234.14± 10.34	0.72± 0.02	32.4± 0.54
II	NDEA + CCL4	200 mg/kg (NDEA)+3ml/kg bw(CCl4)	354.21± 36.31z	371.53± 42.72z	439.31± 28.31z	1.26± 0.08z	154.2± 12.8z
III	LAB	100 mg/kg	256.34± 22.52a	246.38 ± 34.29a	354.24± 24.34a	0.91± 0.07b	148.8± 10.8
IV	LAB	200 mg/kg	218.51± 36.31z	181.14 ± 36.29b	299.28± 21.54b	0.87± 0.06b	121.4± 9.6
V	LAB	400 mg/kg	218.51± 15.71b	109.06± 21.34c	249.96 ± 18.28c	0.82± 0.04c	78.1± 7.7c
VI	Cisplatin	6 mg/kg	198.32 ± 10.78b	92.34 ± 23.48c	242.26 ± 18.72c	0.78± 0.03c	52.4± 8.1c

Table 4 Effect of 50% ethanolic extract of LAB on SGOT (U/l), SGPT (U/l), SALP (U/l), and Bilirubin level (U/l), (BL) and Gamma glutamyl transpeptidase, GGT(U/l) in serum of rat. Values are mean ± SEM of 6 rats in each group. P values: z<0.001 compared with respective control group. P values: a<0.05, b<0.01, c<0.001 compared with group II (NDEA+CCl4).

Groups	Treatment	Dose	SOD	CAT	LPO	GPx	GST	GSH
I	Control	---	114.4± 9.1	28.8± 1.2	0.46± 0.04	3.54± 0.04	1.06± 0.12	0.36± 0.02
II	NDEA + CCL4	200 mg/kg (NDEA)+3ml/kg bw(CCl4)	48.20± 9.14z 2.2z	6.24 ± 1.21z	4.46 ± 0.02z	1.42± 0.02z	0.47 ± 0.01z	0.04 ±
III	LAB	100 mg/kg	78.12± 5.31a	12.19± 1.14	3.62± 0.02a	1.50± 0.04a	0.60± 0.02a	0.10±
IV	LAB	200 mg/kg	91.21± 6.26b	16.19 ± 0.98	2.16 ± 0.03c	2.94± 0.05c	0.82± 0.03b	0.18±
V	LAB	400 mg/kg	98.52± 4.21c	21.39 ± 0.93c	1.02± 0.36a	3.24± 0.02c	0.94 ± 0.08c	0.27± 0.04c
VI	Cisplatin	6 mg/kg	102.31± 5.24c	24.09 ± 2.12c	0.88 ± 0.22b	3.42± 0.02c	0.97 ± 0.09c	0.32± 0.05c

Table 5 Effect of 50% ethanolic extract of LAB on liver superoxide dismutase, SOD(units/mg of protein), catalase, CAT (units/mg of protein), lipid peroxidation, LPO (MDA nmoles/mg of protein), glutathione peroxidase, GPx (ug/mg), glutathione-S-transferase, GST (ug/mg of protein) and reduced glutathione, GSH(ug/mg of protein). Values are mean± SEM of 6 rats in each group. P values: z<0.001 compared with respective control group. P values: a<0.05, b<0.01, c<0.001 compared with group II (NDEA+CCl4).

Group	Treatment	Dose	DNA	RNA	Protein
I	Control	---	5.41± 0.44	7.52± 0.24	8.04± 1.03
II	NDEA + CCL4	200 mg/kg (NDEA)+3ml/kg bw(CC14)	7.58± 0.24z	9.92± 0.46z	6.27± 0.72x
III	LAB	100 mg/kg	6.87± 0.28	8.14± 0.38a	6.73± 0.95
IV	LAB	200 mg/kg	5.68± 0.32	7.88± 0.42b	7.45± 1.02
V	LAB	400 mg/kg	5.52± 0.26c	7.62± 0.30b	7.87± 1.23
V	Cisplatin	6 mg/kg	5.48± 0.22c	7.58± 0.28b	7.55± 1.44

Table 6 Effect of the 50% ethanolic extracts of LAB (100, 200 and 400 mg) on the level of Deoxyribonucleic acid, DNA(mg/g wet tissue) and Ribo nucleic acid, RNA (mg/g wet tissue) and Protein(g/dl). Values are mean ± SEM of 6 rats in each group. P values: x <0.05, z<0.001 compared with respective control group. P value: a<0.05, b<0.01, c<0.001 compared with group II (NDEA+CCL4).

Group	Treatment	Dose	RBC (million/mm3)	WBC (million/mm3)	Hb (g/dl)
I	Control	---	8.12± 0.76	6.22± 0.06	11.97± 0.42
II	NDEA + CCL4	200 mg/kg (NDEA)+3ml/kg bw(CC14)	6.42± 0.52z	8.79± 0.82x	8.52± 0.32z
III	LAB	100 mg/kg	6.91± 0.58	7.57± 0.71	8.91± 0.72
IV	LAB	200 mg/kg	7.12± 0.37	7.02± 0.53	9.76± 0.4a
V	LAB	400 mg/kg	7.89± 0.82	6.54± 0.43a	10.67± 0.82a
V	Cisplatin	6 mg/kg	7.97± 0.58	6.24± 0.39a	11.45± 0.82b

Table 7 Effect of the 50% ethanolic extracts of LAB (100, 200 and 400 mg) on haematological parameter(RBC, WBC, and Hb) of control and (NDEA+CCL4) induced HCC in rat. Values are mean ± SEM of 6 rats in each group. P values: x <0.05, z<0.001 compared with respective control group. P value: a<0.05, b<0.01 compared with group II (NDEA+CCL4).

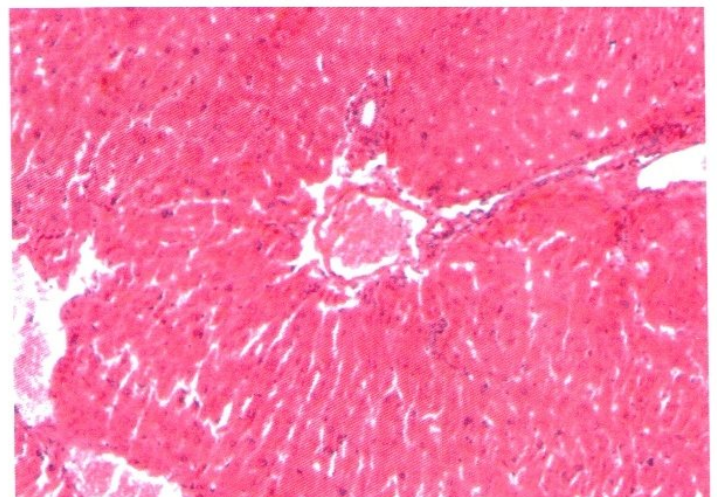
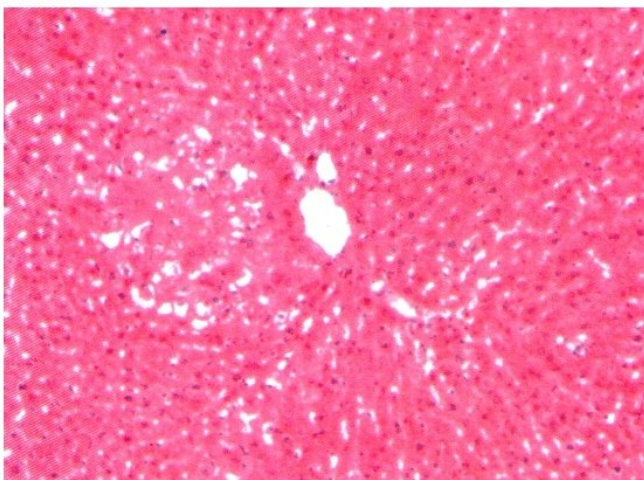
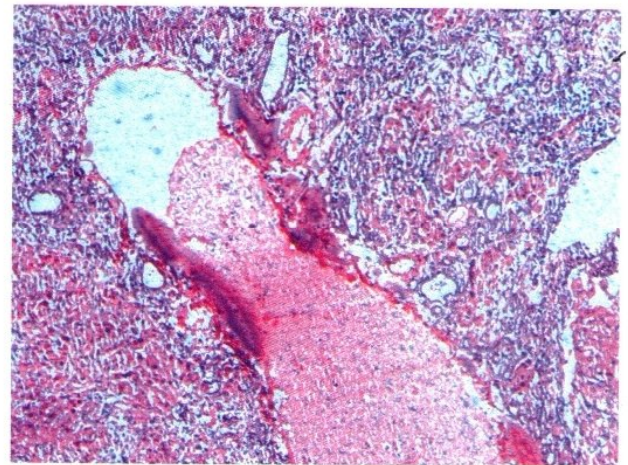
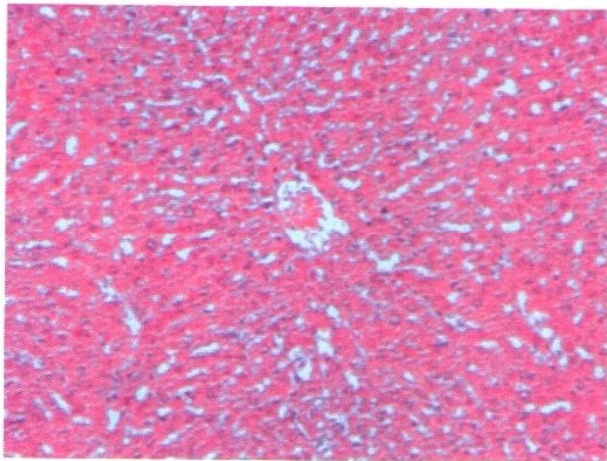
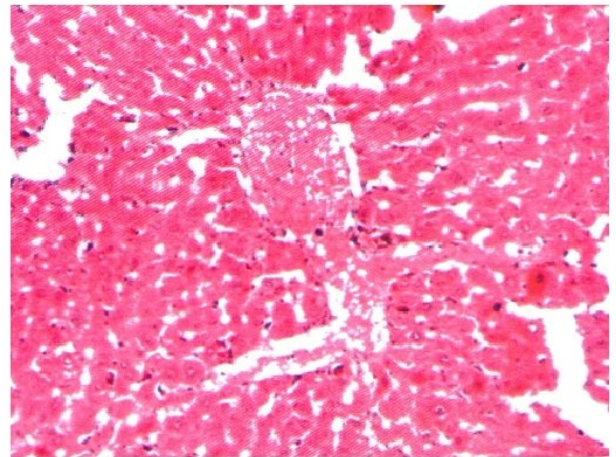
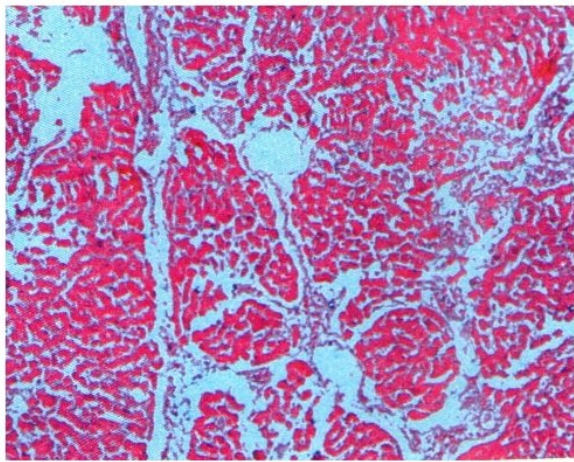


Figure 1

Further there was no change observed in the serum biochemical markers viz, SGOT, SGPT and ALP. This indicates that LAB exert their antioxidant defense mechanism probably by metabolizing lipid peroxides and scavenging endogenous peroxides.

The effect of 50% ethanolic extract of LAB plants on body weight, liver weight and average liver weight changes in HCC in treatment. There is an appreciable loss in body weight in hepatoma bearing rat as compared to control rats and the reduction in body weight correlates well with the decreased food intake.

The body weights were steadily increased after treatment with extracts and compared with standard cisplatin in hepatoma bearing animal which indicate, LAB extracts reduces the tumour incidence and change in energy metabolism and also shows anticancer potency. The administration of LAB extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capacity of extracts in respect with anticancer potency in comparison with the standard drug cisplatin.

The 50% ethanolic extract of the plants treatment showed significant dose dependent (100, 200 and 400 mg/kg) alteration in level of SGOT and SGPT in serum which were recuped back to near normal in HCC bearing animals which shows the anticarcinogenic activity of the plants.(table-4). The levels of SGOT and SGPT in plants extract(100-400 mg/kg) in HCC bearing animals were found to be near to the levels of enzymes when treated with cisplatin. This result shows the antineoplastic effect of plants as with the standard drug cisplatin.

In the present investigation, the elevation in levels of ALP was observed in animal of hepatoma(NDEA and CCl4) induced hepatocellular carcinoma in the serum(Table 4).

In the present study, 50% ethanolic extract of the plants tested showed significant decrease in the levels of ALP or in other words it shows the potentiating the level of ALP back to normal and also likely show the level of this enzymes near to the level when treated with the standard drug cisplatin

which indicates , the anti carcinogenic effects of plant in (NDEA and CCl4) treated hepatoma rats (Table 4)

In the present investigation , in (NDEA and CCl4) hepatoma bearing animals there was an elevation in levels of serum bilirubin which may be due to the leakage of plasma membrane and loss of functional integrity of cell membranes in liver(Table 18). In groups treated with 50% ethanolic extract of the plants(100, 200 and 400 mg/kg) showed significant results, reducing the levels of these elevated levels in a dose dependent manner, indicates the restoring serum marker enzymes back to normal.(Table 4).

An increase in GGT activity in serum of hepatoma bearing animals (NDEA and CCl4) induced carcinogenesis in this study suggests its potential role as an indicator of carcinogen exposure and also reflects the toxic effects of drug on microsomal structure in liver cells. Recoupment of tumour marker enzyme (GGT) upon treatment with plants extracts of 50%ethanol showed a significant dose dependent decrease in the levels of GGT in comparisons with hepatoma bearing animals and it also shows the result same as comparisons with the standard drug cisplatin suggest a combinatorial therapy gives protective mechanism against abnormal cell growth by changing the permeability of membrane or affecting cellular growth (Table-4).

The levels of SOD were remarkably decrease in hepatoma bearing animals whereas these status were increased significantly after treatment of extract in a dose dependent manner, which shows an antioxidant potency of plants extract (Table 5).

The levels of CAT in treatment with plant extract in different dose concentration significantly increase which suggest the antioxidant capabilities to scavage radical production produce by NDEA and CCl4 metabolism (Table 5) Upon treatment of extracts, shown a dose dependent increase in activity of GPx, which signify a radical scavenging activity by increasing the level of GPx (Table 5)

The elevation in the level of GST was observed in HCC bearing rats treated plants extract at a dose concentration

(100, 200 and 400 mg/kg) when compared with reduced level of the enzyme activity in the untreated hepatoma bearing animals (Table 5). This potentiation of plant extract upon enzyme level may be a result of decreasing and/or inhibiting lipid peroxide formation one of the main functions in carcinogenic process.

The GSH level has been decreased in hepatoma bearing animals. Upon treatment with our plants extract at (100, 200 and 400 mg/kg) shows a significant result, which revert back to near normal values, which attribute to the utilization of these antioxidants to alleviate free radical induced oxidative stress by NDEA (Table 5).

The level of DNA and RNA of liver found to be progressively increased in hepatocellular carcinoma bearing animals. Among the nucleic acid DNA exhibited prominent increase than RNA.

The increased nucleic acid synthesis in tumour animals was found to decrease when the animals were treated with LAB (100, 200 and 400 mg/kg) in a dose dependent manner and also show the result effective when compared with the standard drug cisplatin (Table 6)

The administration of LAB (100, 200 and 400 mg/kg) to the HCC bearing group resumed the protein level to near normal (Table 6) and also in comparison with the standard drug cisplatin, it shows the anticancer activity of the LAB plant extract (50% ethanolic) on NDEA and CCl₄ induced hepatocellular carcinoma.

The administration of NDEA produced significant decrease in RBC count, Hb content, with simultaneously increase in WBC count. The decreased RBC count may be due to destruction of erythrocytes or the results of adverse effect of NDEA on erythropoietic tissue namely the bone marrow (Table 7)

To prove the anticancer activity of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa*, histopathological studies were carried out. In the present investigation, noticeable changes were observed in the architecture of liver of cancer bearing animals. These indicates

the presence of neoplastic conditions following NDEA and CCl₄ administration. In drug treated animals, the NDEA and CCl₄ damage was recovered due to anticancer potency of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa*. The regression of the tumours in liver may be due to the protective effect of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa*.

CONCLUSION

Recent studies on tumour inhibitory compounds of plant origin have yielded an impressive array of research on medicinal plant. The efficacy of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* in experimental liver cancer described in the present investigation offer the potential for reaching on understanding of anticancer potency.

The administration of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* extracts and cisplatin decreased the liver weight and average liver weight, which shows the rehabilitating capability of extracts in respect with anticancer potency in comparison with the very much effective in preventing NDEA-induced multistage hepatocarcinogenesis possibly through antioxidant and antigenotoxic nature, which was confirmed by various liver injury and biochemical tumour markers enzymes and molecular events. Studies on molecular aspect of cancer therapy will give mechanistic information in cancer therapy and also critical balance should be there between the animal model and clinical research. This holds great promise for future research in human beings. The anticancer properties of *Lawsonia innermis*, *Aeagel marmelos*, *Boerrhavia diffusa* should provide useful information in the possible application in cancer prevention and cancer therapy.

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