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Combined Hepatoprotective Effect of Leaves and Flowers of Bassia latifolia Roxb in Paracetamol Hepatotoxic Rats.

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ABSTRACT

The liver is an energetic organ shows important role in the body. It is the chief gland of the body the weight of liver between 1-2.3 kg. The liver plays a chief role in the metabolism and excretion of the many xenobiotics from the body. It has a surprising role in conservation and performance of homeostasis of the body. It also plays important role in the organic pathways to growth, fight, in contradiction of disease, nutrient supply, energy provision and reproduction. The liver injury caused by many environmental toxins, and many communicable diseases like tuberculosis, hepatitis, cancer, and other major health problems affected the liver function known as liver dysfunction. Many antibiotics and over the counter drugs cause liver cell injury. Other hepatotoxins similar alcohol, carbon tetra chloride ccl4, paracetamol, aflatoxins is causing cell injury in the liver. When liver is not worked properly the digestion, metabolism and many functions of the body are affected.so many hepatoprotective plants are used in the handling of liver toxicity like, Annoma squomosa, chamomile capitula, Coccinia grandis, Ficus carica Lepidium sativum Madhuca longifolia, curcuma longa, Andrographis paniculata etc. The plant bassia latifolia also having the hepatoprotective activity. For the better result be studying combined leaves and flower extract for hepatoprotective activity. It gives synergestic hepatoprotective effect in the hepatotoxic animal model.

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1. Introduction

The liver stands a large meaty organ and a chief gland in the body. The weight of liver between 1-2.3 kg. It is located in the upper part of the intestinal cavity in the right and left hypochondriac region. The liver is surrounded by a thin inelastic capsule and moderately covered by a layer of paritonium.it has four lobes. The hepatic route and portal vein stream blood to the liver.

It has many functions which are mentioned here: - Carbohydrate metabolism, Fat metabolism, Protein metabolism, It also synthesizes blood clotting factors, It transforms nitrogenous amino acids in non-essential amino acids in the body. Many hormones like insulin, glucagon, aldosterone, sexharmones are inactivated by the liver in the body. It produces heat in the body of metabolic reaction. It secretes bile which contains bile salts, bile dyes and cholesterol.

Bilirubin cause haemolysis of erythrocytes by hepatic macrophages similar kuffer's cells, in the liver and by other microphages in the spleen and bone marrow.

Poisonous liver injury formed by drugs and chemicals are similar to a natural liver disease. Nonstop use of agents like paracetamol, tetracycline, antitubercular drugs, oral contraceptives. Chemicals used as food stabilizers and Agrochemicals are destructive the integrity of liver. Further, addiction of alcohol and added drugs serious the problem and malnutrition also an important cause of liver damage.

1. Leaves of B. latifolia

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2. Flowers of B. latifolia

Traditional Uses:

The plant of Bassia latifolia is a forest tree found in central and northern India and Malaysia. It is a medicinal plant, various parts of the plant like bark, flowers, leaves, latex, oil, fruits having different pharmacological activity like, antituberculosis, antidiabetic, anti-inflammatory, antipyretic, antiulcer, anti-rheumatic etc. The oil of Mahua used as additional of cocoa butter, emulsifier, moisturizer, in soaps industries, and as a lubricant. The seeds cake used as a pesticidal and insecticidal. The sugar syrup of bassia latifolia flowers used as a sweetening agent in various medicinal syrup preparations. It is also cast-off in preparation of biodiesel because of its less polluting nature. It's also having antibacterial, antiepileptic, and hepatoprotective activity.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant of Bassia latifolia is collected in the month of September, 2018 from the area of Mathura, UP, were dried in shade at room temperature and then subjected to size reduction (coarse powder) with the help of mixer grinder.

2.2 Preparation Of Alcoholic Extract

The leaves, flowers and combination of leaves and flowers of B. latifolia powder packed in soxhlet apparatus was extracted with 95% alcohol for 18 h and appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker, it was kept in a water bath, maintained at 50^{o}C and evaporated to a thick paste. The extract was thoroughly air dried to remove all traces of the solvent, then the percentage yield was calculated.

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2.3 Animals

Wistar rats of both sexes (150-200gm) were maintained under uniform laboratory conditions in standard propylene cages and provided food and water. The experimental protocol approved by the Institutional Animal Ethics Committee.

2.4. Determination of acute toxicity (LD_{50}) [7] Method

The oral acute toxicity of ALEBL was determined in albino mice (18-20 g) those maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment and "Up and Down" procedure of OECD Guidelines No. 425 methods of CPCSEA were assumed for toxicity studies. Animals were administered with single doses of ALEBL in different groups of animals and observed for the mortality during 48 h study period (short term) toxicity. Based on the short-term profile the doses for the next group of animals were determined as per OECD Guidelines No. 425. All the animals were observed for long term toxicity (14 days) and from the observed LD_{50} doses of ALEBL $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ doses were selected for the present study.

2.5. Determination of Hepatoprotective Activity

(a) Paracetamol induced hepatotoxicity[8, 9] (preventive aspect)

Albino rats weighing between 150-200g and each group containing six animals will be divided into 8 groups.

Group A - Normal control vehicle treated, p.o for 21 days

Group B - Toxicant Paracetamol 2gm/kg daily, p.o for 03 days

Group C - ALEBL leaves in low dose (200 mg. / Kg.) daily, p. o. for 21 days

Group D - ALEBL leaves in high dose (400 mg. / kg.) daily, p. o. for 21 days

Group E - ALEBL flowers in low dose (200 mg. / kg.) daily, p. o. for 21 days

Group F - ALEBL flowers in high dose (400 mg. / kg.) daily, p. o. for 21 days

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Group G - ALEBL leaves + flowers in low dose (200 mg. / kg.) daily, p. o. for 21 Days

Group H - ALEBL leaves + flowers in high dose (400 mg. / kg.) daily, p. o. for 21 Days

Experimental Procedure

Wistar rats weighing between (150-200 g) will be divided into 8 groups of 6 rats in each. Group A will be served as normal control which will be given vehicle only and Group B with Paracetamol (2 gm/kg p.o). Animals of Group C and D will be treated with alcoholic extract of B. latifolia leaves in low and high dose. Animals of Group E and F will be treated with alcoholic extract of B. latifolia flowers in low and high dose. Similarly, animals of Group G and H will be treated with alcoholic extract of B. latifolia leaves + flowers in low and high dose.

Group B, C, D, E, F, G and H will be intoxicated with Paracetamol (2 gm/kg p.o) for 3 days daily, 30 min after treatment with the extract. Blood will be collected through retroorbital puncture, on 21st day later sacrificed by an overdose of ether. Livers removed will be washed with saline, weighed and stored in 10% formaldehyde for histological studies.

(b) Histopathological Studies

Processing of isolated livers (Modified Luna's method 1960)

The livers isolated from each animal was cut into small pieces, preserved and fixed in 10% formalin for 2 days. Then the liver piece was washed in running water for about 12 h to remove the formaline followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 h each. Then finally dehydration is done using absolute alcohol with about three changes for 12 h each.

Dehydration was performed to remove all traces of water. Further, alcohol was removed by chloroform and chloroform by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 min each.

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Embedding Of Paraffin Vacuum:

Hard paraffin was melted and poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allowed to cool.

Sectioning:

The blocks were cut using Microtome to get sections a thickness of, 5 (, taken on a microslide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in oven at 60^0C for 1 h. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide.

Staining:

Eosin is an acid stain; hence it stains all the cell constituents pink which is basic in nature i.e., cytoplasm. Haematoxylin, a basic stain which stains all the acidic cell components blue i.e., DNA in the nucleus.

The stained sections were observed microscopically for its histological changes produced by alcohol intoxication. The ability of the ALEBL and AQEBL in preventing the chemical induced hepatic changes was determined histologically.

3. RESULTS.

Administration of Bassia latifolia combined extracts of leaves and flowers in two different dosages remarkably prevented pcm induced elevation of serum enzyme in a dose dependent manner. As given in Table 1, compared with control group and it attained an almost near the normal value in groups which were treated with bassia latifolia leaves and flowers combined extract.

4. DISCUSSION

The present study demonstrates the hepatoprotective activity of alcoholic extract of leaves and flowers and combined extract of leaves and flowers of B. latifolia against various models of liver injury in rats. The liver is one of the vital organs in our body responsible for detoxification of toxic chemicals and drugs. The target organ for all toxic chemicals. Numerous studies noted that PCM, is widely used to induce liver damage because it is metabolized in hepatocytes by

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cytochrome P450, generating a highly reactive carbon cantered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis [10,11, 12] Paracetamol is a commonly used as an analgesic and antipyretic agent. Hepatotoxic doses of paracetamol diminish the normal levels of hepatic glutathione, when NAPQI covalently binds to cysteine groups on proteins to form 3-(cysteine-S-yl) acetaminophen adducts[13]. The glutathione protects hepatocytes by combining with the responsive metabolite of paracetamol thus preventing their covalent binding to liver proteins[14].

Chronic administration of drugs (paracetamol, alcohol and ranitidine) to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and rise the levels of these marker enzymes in serum indicate damage to the liver cells. Pre-treatment with leaves and flowers and combined extract of leaves and flowers of ALEBL decreased the levels of ALT, AST, ALP, BILD, BITD, CHO, TG levels, an indication of the hepatoprotective activity of these extracts against drug induced hepatotoxicity Intoxication with the drugs cause increase in cholesterol and triglyceride levels. ALEBL prevented elevated cholesterol and triglyceride levels due to hepatic lipid peroxidation occurred after drug intoxication.

In this present work, it was planned to verify the therapeutic helpfulness of locally available plants. The literature survey revealed that the plant B. latifolia apart from other medicinal uses was used as an ethnic folklore medicine for hepatic disorder.

Hence, the combined extracts of leaves and flowers of B. latifolia have been selected to study their hepatoprotective activities in experimental animals, albino wistar rats, shows more synergestic hepatoprotective activity, The Chemical constituents present in leaves and flowers of ALEBL are carbohydrates, glycosides, sterols, starch, resins, flavonoids, proteins and saponins. These chemical constituents are already reported for their hepatoprotective activities

5. CONCLUSION

The alcoholic extracts of leaves and flowers of Bassia latifolia studied for hepatoprotective activity against Wistar rats with liver damage induced by paracetamol. It was found that the

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alcoholic extract of bassia latifolia at a dose of 200 and 400mg/kg body weight exhibited moderate protective effect by lowering the serum levels of glutamate pyruvate transaminase (SGPT), SGOT, SALP, BILD, BILT, Triglyceride, and cholesterol to a significant extent. The present finding demonstrated the alcoholic extract of combined leaves and flowers of Bassia latifolia could afford a significant dose dependent protection against pcm induced hepatocellular activity.

Table o-1 Effect of various Extracts on SGOT, SGPT, and SALP in PCM induced hepatotoxicity in rats.

GROUP	SGPT	SGOT	SALP	BILD	BILT	TG	CHO
Normal control	41.38±1.10	95.35±3.o2	108.04±2.16	0.193±0.009	0.63±0.008	98.14±5.48	149.16±2.52
Toxicant control(pcm)	106.5±2,90	350.51±1.63	157±1.69	0.795±0.017	1.29±0.008	203.48±2.9	205.58±2.9
ALEBL of leaves in low dose(200mg/kg)	88.94±1.84	338.21±4.11	175.70±2.68	0.73±0.018	1.23±0.02	194.31±4.82	198.53±5.34
ALEBL of leaves in high dose(400mm/kg)	65.70±3.08	229.52±3.86	128.66±6.00	0.57±0.01	1.011±0.024	159.26±1.53	181.12±2.21
ALEBL of flowers in low dose(200mg/kg	85.78±3.98	248.67±4.13	114.08±2.17	0.69±0.01	1.011±0.028	195.26±2.66	196.28±3.62
ALEBL of flowers in high dose(400mm/kg)	65.16±1.84	200.52±2.63	145.86±1.41	0.45±0.01	0.86±0.04	119.6±4.20	164.56±3.62
ALEBL combined in low dose(200mg/kg)	53.58±1.72	244.25±6.19	119.11±2.79	0.493±0.016	0.981±0.04	145.46±2.94	183.71±2.41
ALEBL combined in high dose(400mg/kg)	50.73±1.35	190.73±2.11	104.18±1.56	0.31±0.022	0.76±0.027	107.14±3.65	165.16±2.86

One-way ANOVA followed by Dunnett's Multiple Comparison test Values are expressed as mean□SEM; n=6. p<0.05 is considered as significant, ns non-significant, ## p< 0.01 compared to normal control, *** p< 0.001 compared to toxicant control.

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Abbreviations:

ALEBL- alcoholic extract of bassia latifolia**,**

SGPT- serum glutamate pyruvate transaminase (ALT)

SGOT-serum glutamate oxaloacetate transaminase (AST)

SALP-serum alkaline phosphate**,**

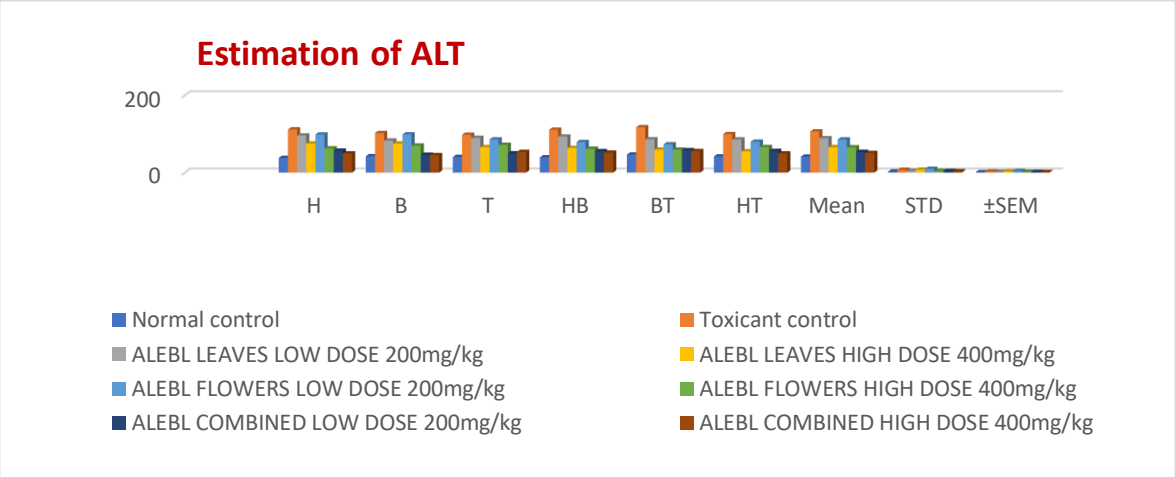
BILD – serum direct bilirubin**,**

BILT-serum total bilirubin**,**

TG- serum triglyceride,

CHO- serum cholesterol

Graphical representation of results



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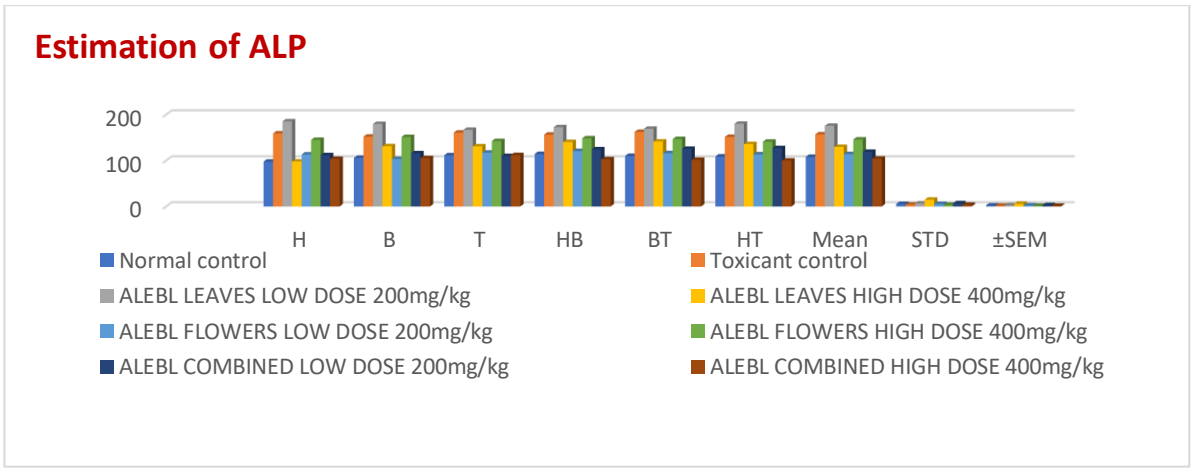

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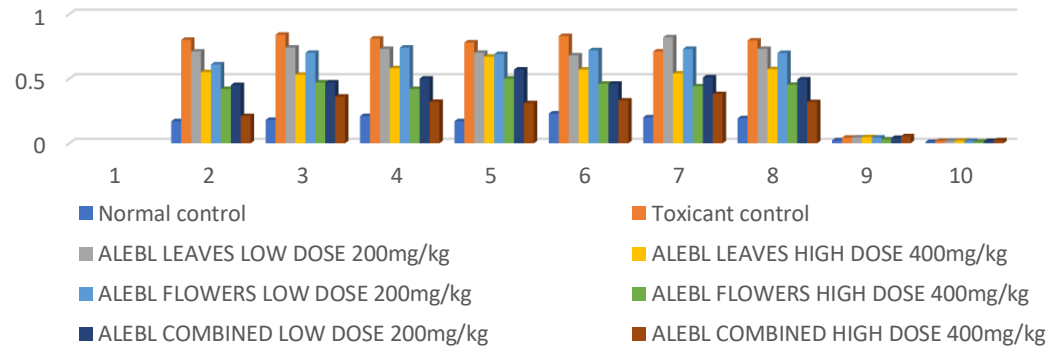
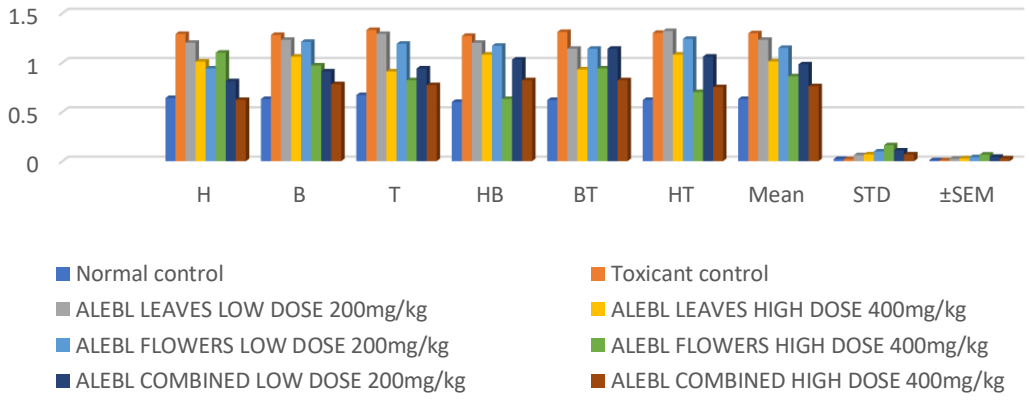
Estimation of BILD



Estimation of BILT



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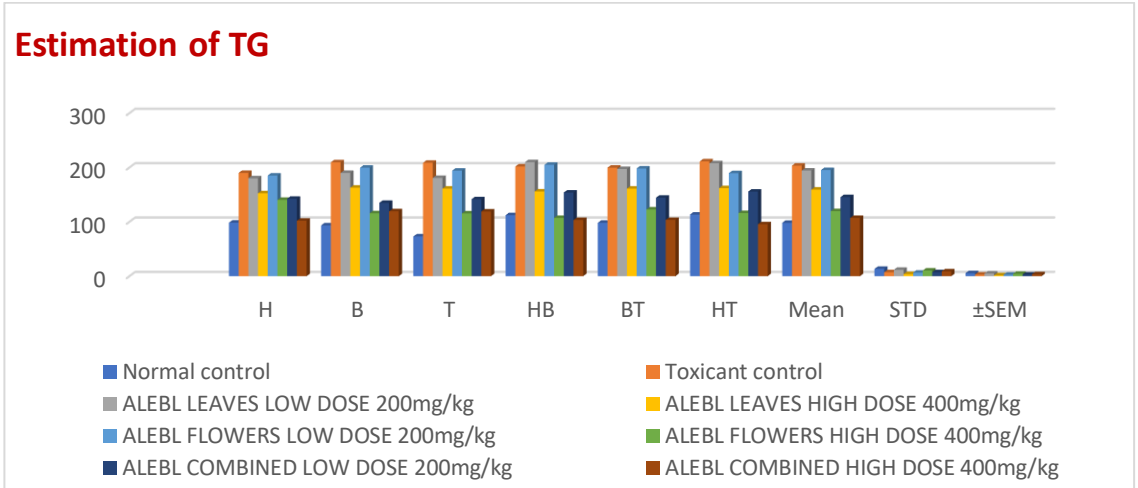
Estimation of TG



Estimation of CHO

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