Innovations

Comparative Evaluation Against Acetaminophen Induced Hepatotoxi city of Ximenia Americana and Lindera Communis Extract

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Abstract:

To assess the hepatoprotective activity in acetaminophen-induced hepatotoxicity in rats, the Extracts of Ximenia americana Linn and Lindera communis. According to the blood marker enzymes and liver tissues, the plant Extracts (200 and 400 mg/kg, p.o.) show ED a remarkable hepatoprotective efficacy against acetaminophen-induced hepatotoxicity. The amount of total protein was decreased whereas acetaminophen significantly increased the following enzymes: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, and gamma glutamate transpeptidase (GGTP). As comparison to acetaminophen-treated rats, treatment of rats with different doses of plant Extracts of Ximenia Americana and Lindera communis (200 and 400 mg/kg, p.o.) significantly (P 0.05) changed blood marker enzyme levels to normal. Standard liver samples and histopathological liver samples were compared (Silymarin treated). According to the findings of this investigation, Ximenia Americana and Lindera communis Extract exhibited strong liver-protective effects against acetaminophen induced hepatotoxicity in rats.

Key words: *Ximenia americana, Lindera communis, amino transferase, hepatoprotective activity.*

Introduction:

Ximenia americana[1] is one of the Olacaceae family edible wild fruits[2,3]. The fruit's medicinal value makes it suitable for novel medication development. In general, Africa, India, New Zealand, Central America, and South America are where you can get the fruit. The fruit potential of X. americana is well known in Ethiopia[4,5]. The fruit can be found throughout the nation, although the southern region dominates with its abundant supply. It is a rambling, variegated shrub or small tree with spiky branches that can grow up to 4.5 cm tall. With deep cracks and reddish-brown bark, the leaves are ovate to broadly elliptic-oblong or spherical. Flowers are scented supplementary racemes that are white or yellowish white in color. The ripe fruits have a sweet, tangy aroma. Phytochemical composition has been the subject of a few reports, including the presence of oleanene palmitate[6] and oleonolic acid saponin[7]. There have been reports of pharmacological actions with regard to wound [8] healing, antibacterial[9], antioxidant[10], and antitrypanosomal effects. A member of the Lauraceae family, the plant Lindera communis[11] is a synonym for Lindera Formosana Hayata. The hypocarpium at the base of Lindera fruit[12] can occasionally form a cup that encloses the fruit's lower portion. The fruit, which is a tiny red, purple, or black drupe with a solitary seed within, is primarily disseminated by birds. Many species use stolons for vegetative reproduction. Most notably, numerous Lindera plants have historically been employed in traditional medicine[13] for their capacity to treat a variety of health-related issues, including pain, colds, urinary tract problems,

rheumatoid arthritis, gastric ulcers, abdominal pain, cholera, and beriberi. There are reports of numerous biological activities in the roots, bark, leaves, and flowers of Lindera communis Hemsl, including antidiabetic, hepatotoxic, and anti-inflammatory properties.

The liver is an essential organ of the body that performs a variety of metabolic tasks, such as storing glycogen, breaking down red blood cells, synthesising plasma proteins, producing hormones, and detoxifying Xenobiotics, environmental toxins, and chemotherapy drugs. Only a few of these therapeutic plants and their preparations have had their efficacy tested scientifically. The current study was carried out using the plant Ximenia americana and Lindera communis as a component of our ongoing search for new natural compounds with hepatoprotective effectiveness.

Materials and Methods:

Extraction:

20 g from powdered plant sample of X. americana and Lindera communis were Extracted with 200 ml water at 80 °C in a water bath for 12 hr. and then filtered. Water was evaporated using a lyophilizer. Residue was dissolved in sterile distilled water in order to obtain a final concentration of 100 mg/ml. All Extracts were sterilized by filtering through a 0.22 µm filter (Millex). Plant materials, designation of treatments and yield (%) for aqueous Extraction is 9.58%.

Experimental animals: Male Wistar albino rats of weighing about 250 ± 25g body weight were collected. The animals were fed a commercial pellet diet supplied from Pranav agro Industry, Pune.

Experimental design: Wistar Albino rats were randomly divided into six different groups of six rats each.

Group I served as control group- treated with 0.5% gum acacia

Group II administered with 0.5% gum acacia for 45 consecutive days, initially 20% ethanol v/v p.o. was administered for 30 consecutive days and there after maintained for 45 consecutive days with normal rat pellet and water ad libitum and served as toxic control group.

Group III and IV considered as reference standard group – initially 20% ethanol v/v p.o. was administered for 30 consecutive days and there after treated with Silymarin 50mg/kg and Liv 52, 5ml/kg body weight respectively for 15 consecutive days.

Group V & VI were considered as test groups, initially 20% ethanol v/v p.o. was administered for 30 consecutive days and there after administered with EAGT at a dose of 200mg/kg & 400mg/kg of body weight respectively for 15 consecutive days.

On 45th day an hour after group specific drugs, body weight was recorded. Blood was withdrawn from retro-orbital plexus under light ether anesthesia. Later the serum was tested for serum biochemical parameters such as Aspartate aminotransferase (AST), Alanine transaminase ((ALT), Alkaline phosphatase (ALP), total protein, high density lipoproteins (HDL), cholesterol, total bilirubin, serum lipid profile. The rats were sacrificed under cervical dislocation method and the liver tissues were rapidly isolated washed with ice cold saline and weighed. Antioxidant such as catalase activity, lipid peroxidation, and glutathione peroxidase were estimated.

Determination of catalase activity: The catalase activity was determined by mixing 1mL of liver homogenate with 5ml of phosphate buffer, 4 mL of 0.2 M H_2O_2 in phosphate buffer and time was recorded. Exactly, After 3 minutes of H_2O_2 addition, a set of 1mL of the aforesaid reaction mixture was taken in 2mL dichromate acetic acid and then placed in a boiling water bath for about 10 minutes. The test tube containing reaction mixture was cooled under running water and reading was noted at 570nm against reagent blank using UV- visible spectrophotometer (Systronics 2201, India). Catalase activity in the liver tissue homogenate was expressed in micromoles H_2O_2 consumed/mg protein/minute.

Determination of glutathione peroxidase: The amounts of glutathione peroxidase in the liver homogenate were determined using standard protocol. The 0.2 ml of liver homogenate was added to the test tube containing 0.2 mL of EDTA, sodium azide, reduced glutathione, hydrogen peroxide was added, mixed thoroughly, and incubated at 37 ºC for 10 minutes. The reaction was stopped by adding 0.5 mL of TCA and centrifuged. 67 Supernatant about 0.5 mL was pipetted into test tube containing 4mL of disodium phosphate and 0.5mL of 5, 5-

Dithiobis 2-nitrobenzoic acid (DTNB). The produced color was instantly read at 420nm using UV- visible spectrophotometer (Systronics 2201, India), and the standard was handled in the same way. The glutathione peroxidase level was expressed as micromoles of glutathione utilized /milligram protein/minutes at 370C.

Determination of lipid peroxidation: The lipid peroxidation in the liver homogenate was determined by measuring the MDA using thiobarbituric acid test. Liver homogenate (0.1 mL) was taken in a test tube containing 0.2 mL of 0.1% SDS, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8 N aqueous solution of TBA. The volume of reaction mixture was made up to 4 ml by adding distilled water and heated at 950C for 60 minutes. The reaction mixture was allowed to cool and 1mL of distilled water, 5 mL of mixture of n-butanol and pyridine (15:1v/v) were added and shaken well. Finally, it was centrifuged at 4000 rpm for 10min and read at 532 nm, absorbance was determined using UV- visible spectrophotometer (Systronics 2201, India). In the same way, the standard malondialdehyde was treated. The degree of lipid peroxidation was measured as µmoles of MDA formed /g of wet tissue

Results:

Table 1.- Effect of Extract on serum Alanine transaminase (ALT) in paracetamol induced liver toxicity

Group	Alanine transaminase activity (U/L)	Percent Extract change (%)
Normal control	179.33±14.68	--
Paracetamol (Toxic control) 3g/kg p.o	621.6±29.914	246.62
Paracetamol (3g/kg) +Liv52 (5ml/kg)	433.5±20.179##	30.26 †
Paracetamol (3g/kg) + Silymarin 50 mg/kg	401.2±20.517##	35.45 †
Paracetamol (3g/kg) + EXTRACT 200mg/kg	479.4±19.114##	22.87 †
Paracetamol (3g/kg) + EXTRACT 400mg/kg	467.5±16.550##	23.34 †

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #-when compared to paracetamol control.

Table 2.- Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum Aspartate amino trasferase (AST) in paracetamol induced liver toxicity

Group	Aspartate amino transferase (AST) activity (U/L)	Percent Extract change (%)
Normal control	90±5.046	--
Paracetamol (Toxic control) 3g/kg p.o	319.8±41.281@@	255.33
Paracetamol (3g/kg) +Liv52 (5ml/kg)	205.50±13.732##	35.74†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	209.6±20.094##	34.45†
Paracetamol (3g/kg) + EXTRACT 200mg/kg	244.2±23.135##	23.63†
Paracetamol (3g/kg) + EXTRACT 400mg/kg	236.66±19.770##	25.99†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When Compared to normal control, #- When compared to paracetamol control, Ethanolic Extract.

Table-3. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum alkaline phosphatase (ALP) inparacetamol induced liver toxicity

Group	Alkaline phosphatase activity (U/L)	Percent Extract change (%)
Normal control	346.16±18.953	--
Paracetamol (Toxic control) 3g/kg p.o	792±46.840@@	128.79
Paracetamol (3g/kg) +Liv52 (5ml/kg)	559.5±16.099##	29.35†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	502±13.048##	36.61†
Paracetamol (3g/kg) + EXTRACT 200mg/kg	555.6±20.892##	29.84†
Paracetamol (3g/kg) + EXTRACT 400mg/kg	564.83±22.434##	28.68†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control.

Table-4. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum Total bilirubin in paracetamol induced liver toxicity

Group	Total bilirubin (mg/dl)	Percent Extract change
Normal control	0.27±0.0237	--
Paracetamol (Toxic control) 3g/kg p.o	0.708±0.07365@@	162.22
Paracetamol (3g/kg) +liv52 (5ml/kg)	0.57±0.1208##	19.49†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	0.512±0.1033##	27.683†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	0.48±0.1158##	32.20†
Paracetamol (3g/kg) +EXTRACT 400mg/kg	0.45±0.07638##	36.44†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control.

Table-5. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum direct bilirubin in paracetamol induced liver toxicity

Group	Direct bilirubin (mg/dl)	Percent Extract change
Normal control	0.13 ±0.0237	--
Paracetamol (Toxic control) 3g/kg p.o	0.126±0.01887@@	3.07†
Paracetamol (3g/kg) +liv52 (5ml/kg)	0.1192±0.0347##	5.39†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	0.106±0.0248##	15.87†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	0.132±0.04271##	4.76
Paracetamol (3g/kg) +EXTRACT 400mg/kg	0.115±0.01708##	8.730†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control, - Ethanolic Extract.

Table-6. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on Serum albumin in paracetamol induced liver toxicity

Group	Albumin (g/dl)	Percent Extract change
Normal control	3.635±0.0335	--
Paracetamol (Toxic control) 3g/kg p.o	4.0634±0.2349@@	11.73
Paracetamol (3g/kg) +Liv52 (5ml/kg)	3.49±0.23##	14.11†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	3.3684±0.2249##	17.103†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	3.726±0.1499##	8.303†
Paracetamol (3g/kg) +EXTRACT 400mg/kg	3.386±0.09404##	16.67†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control, Ethanolic Extract.

Table-7. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) drug on serum globulin level in paracetamol induced liver toxicity

Group	Globulin (g/dl)	Percent Extract change
Normal control	3.574±0.233	--
Paracetamol (Toxic control) 3g/kg p.o	3.007±0.301@@	15.86†
Paracetamol (3g/kg) +Liv52 (5ml/kg)	3.447±0.268##	14.63
Paracetamol (3g/kg) + Silymarin 50 mg/kg	2.762±0.189##	8.147†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	2.758±0.04##	8.28†
Paracetamol (3g/kg) +EXTRACT 400mg/kg	2.59±0.236##	13.86†

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control.

Table-8. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum total protein in paracetamol inducedliver toxicity

Group	Total protein (g/dl)	Percent Extract change
Normal control	7.11±0.2615	--
Paracetamol (Toxic control) 3g/kg p.o	7.0096±0.2407	1.41†
Paracetamol (3g/kg) +Liv52 (5ml/kg)	6.9458±0.07351##	0.91†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	5.916±0.1831##	7.49†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	6.484±0.1751##	7.54†
Paracetamol (3g/kg) +EXTRACT 400mg/kg	5.96±0.2108##	14.97†

Data expressed in mean ± SEM, ##P<0.01, # When compared toparacetamol control.

Table-9. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum total cholesterol in paracetamol induced liver toxicity

Group	Total cholesterol (mg/dl)	Percent Extract change
Normal control	50.86±1.835	--
Paracetamol (Toxic control) 3g/kg p.o	102.514±7.123@@	101.56
Paracetamol (3g/kg) +Liv52 (5ml/kg)	76.143±5.524##	25.74†
Paracetamol (3g/kg) + Silymarin 50 mg/kg	78.54±6.465#	23.40†
Paracetamol (3g/kg) +EXTRACT 200mg/kg	67.218±3.680##	34.44†
Paracetamol (3g/kg) +EXTRACT 400mg/kg	66.04±3.207##	35.59†

Data expressed in Mean ± SEM, @@P<0.01, #p<0.05, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control.

Table-10. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum HDL Cholesterol in paracetamol induced liver toxicity

Group	HDL (mg/dl)	Percent Extracted change
Normal control	19.06±1	--
Paracetamol (Toxic control) 3g/kg p.o	23.424±2.102@	22.89
Paracetamol (3g/kg) +Liv52 (5ml/kg)	32.90±2.132#	40.46
Paracetamol (3g/kg) + Silymarin 50 mg/kg	36.328±2.968##	55.08
Paracetamol (3g/kg) +EXTRACT 200mg/kg	29.986±2.916##	28.01
Paracetamol (3g/kg) +EXTRACT 400mg/kg	27.90±1.760##	19.10

Data expressed in mean ± SEM, @P<0.05, #P<0.05, ##p<0.01, @- When compared to normal control, # - when compared to paracetamol control.

Table-11. Effect of Extract (Lindera communis Hemsl (L.) and Ximenia americana) on serum Triglycerides in paracetamol induced liver toxicity

Group	Triglycerides(mg/dl)	Percent Extract change
Normal control	84.44±13.396	--
Paracetamol (Toxic control) 3g/kg p.o	94.312±16.134@@	11.69
Paracetamol (3g/kg) +Liv52 (5ml/kg)	129.59±14.715##	37.49
Paracetamol (3g/kg) + Silymarin 50 mg/kg	141.25±15.269##	49.76
Paracetamol (3g/kg) +EXTRACT 200mg/kg	144.914±13.731##	53.65
Paracetamol (3g/kg) +EXTRACT 400mg/kg	136.83±15.064##	45.08

Data expressed in Mean ± SEM, @@P<0.01, ##p<0.01, @ - When compared to normal control, #- When compared to paracetamol control.

Table-12. Paracetamol induced hepatotoxicity and histopathological changes after treating with standard drugs and Extract (Lindera communis Hemsl (L.) and Ximenia americana)

Histopathological findings	Group -1 Normal control	Group-2 Paracetamol (Toxic control) 3g/kg p.o	Group-3 Paracetamol (3g/kg)	Group-4 Paracetamol (3g/kg) + Silymarin 50 mg/kg	Group-5 Paracetamol (3g/kg)	Group-6 Paracetamol (3g/kg) +EXTRACT 400mg/kg
Glycogen depletion	-	+++	-	+	-	-
Condensation of nuclei	-	++	-	-	-	-
Pyknosis	-	++	+	+	+	++
Dilatation of sinusoids	+	+++	+	++	+	-
Lymphocytic infiltration	-	++	-	+	+	++
Karyolysis	-	+	-	-	-	-
Congestion	-	++	+	-	-	-
Endothelium disruption	-	++	+	-	-	-
Microvesicular steatosis	-	++	-	-	-	-
Macrovesicular steatosis	-	++	-	-	-	-

None (-), Mild(+), Moderate (++) or Severe (+++)

Table No.7.31: Effect of ethanolic extract of *Lindera communis Hemsl (L.) and Ximenia americana* Extract on tissue parameters

S.no	Treatment	TBARS (mmol/g)	GSH (ug/g)	SOD (IU/dl)	Catalase (IU/dl)
1	Group I	29.8±0.7	67.1±5.2	409±35	54.2±0.71
2	Group II	108±8.0*	23.3±6.5**	160±6.3***	19.2±8.06***
3	Group III	33.2±3.6**	56.8±3.3*	171±11***	48.4±3.6***
4	Group IV	24±1.9***	55.3±3.1*	331±22***	48.2±1.9***

All values are expressed as mean ±SEM, one way Analysis of variance, followed by Dunnets,*p<0.05, **p<0.001, ***p<0.0001, ns-non significant when compared Group II with normal control and remaining groups with disease control.

Conclusion:

The analysis of the data acquired during the study shows that Paracetamol, D-Galactosamine and Rifampicin, caused considerable liver impairment at the doses administered, which is evidenced by histopathological studies and biochemical indicator. Injurious effects were reversed by both reference standard and test substance. Our study gave experimental basis for the claim of hepatoprotective activity of Ethanolic extract of Lindera communis Hemsl (L.) and Ximenia americana. In the present study we have used paracetamol and ethanol as toxicant to induce hepatotoxicity in rats Ethanolic extract of Lindera communis Hemsl (L.) and Ximenia Americana at both doses that is 200mg/kg and 400mg/kg has shown hepatoprotective activity in both these models which is evident from histopathological and biochemical parameters.

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