Hepatoprotective Properties and Quality Assessment of Two Medicinal Plants: Moringa Oleifera and Curcuma Longa

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ABSTRACT

Background: Paracetamol is used abundantly as an anti-pyretic and analgesic drug. As this drug has beneficial effects, it is also the major reason of liver problems in humans as well as in animals used in the experiments.

Objective: The objective of this study was to evaluate the hepatoprotective effect of *Moringa oleifera* and *Curcuma longa* and to provide the knowledge of *Moringa oleifera* and *Curcuma longa* as hepatoprotective so people may use herbal drug despite synthetic medicine to treat hepatic diseases.

Methodology: Hepatotoxicity was induced by giving a single oral dose of paracetamol (3g/kg). To assess the hepatoprotective effect for 14tract of *Moringa oleifera* leaves (125mg, 250mg, & 500mg/kg) and extract of *Curcuma longa* rhizomes (125mg, 250mg, & 500mg/kg) or Silymarin (200mg/kg) was orally administered, for 14 days.

Results and Discussion: Paracetamol significantly raised the level of serum ALP, ALT, AST, and serum bilirubin. Oral dose of *Moringa oleifera* and *Curcuma longa* extract reversed paracetamol – induced hepatotoxicity and reduce the level of all the liver enzymes because both plants have antioxidant activity, which helps to reduce the oxidative stress and showed are markable hepatoprotective effect which prevents the harmful cascade of event induced by Paracetamol. Results of present study showed hepatoprotective effect of *Moringa oleifera* extract is better than the *Curcuma longa* extract.

Conclusion: Treatment with *Moringa oleifera* leaves extract and *Curcuma longa* rhizomes extract reduced raised level of ALP, ALT, AST, and Serum bilirubin because of its antioxidant activity and therefore both drugs have hepatoprotective effect but *Moringa oleifera* has better hepatoprotective effect that is why it should be preferred.

Keywords: *Hepatoprotective effect, Moringa oleifera, Curcuma longa, Paracetamol.*

INTRODUCTION

Liver, the largest and main organ of our body, responsible for the removal of waste metabolites and acts as a center of drugs, xenobiotics, and nutrients [1]. It maintains homeostasis, body's corporeal processes and inhibits many substances which cause liver disease [2]. These days, hepatic issues are the major problems being induced by certain toxic substances, medications, autoimmune disorders, infections, food & excessive intake of alcohol [3].

Paracetamol is widely used as an anti-pyretic and analgesic drug. As this drug has many beneficial effects, but is also the major cause of liver problems in human as well as in experimental animals [4]. The normal dose of paracetamol is 325-650 mg or 1 g after 4-6 hours or 1 g, but it should not exceed 4g/day. Paracetamol is the drug that is the exclusive cause of liver transplant at overdose [5].

A liver disease that leads to death includes hepatitis, liver cirrhosis, alcoholic & non-alcoholic hepatic diseases and other drug induced hepatic diseases [6]. Liver enzymes which include albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate amino transferase (AST), and Gama-glutamyl transferase (GGT) are the biomarkers used to determine hepatocytes integrity [7, 8].

Moringa oleifera

There are some modern medicine (e.g.; Acetylcystine, Methionine) that are used to treat hepatotoxicity and are used as antidote of paracetamol [9]. Due to side effects, majority of the people use natural therapeutic substances which improve liver functioning [10]. *Moringa oleifera* is used to treat diseases and it has other medicinal benefits, that's why it is named as "**miracle plant**". This plant has been used as; hepatoprotective, cardiovascular diseases, antioxidant, anti-inflammatory, antiviral antimicrobial, etc. [11]. *Moringa oleifera* has another name, *Moringa pteyrgo sperma*, and belongs to Moringaceae family [12].

Chemical constituents of *Moringa* reported from different studies include; 4-[(4'-O-acetylalpha-L rhamnosyloxy) benzyl isothiocyanates, nitrites, quercitin, Niaziminin A&B, Isothiocyanate, propylundecanoate, benzylcarbamate, vanillin, cysteine, D-glucose, methyl-p-hydroxybenzoate, beta-sitosterol, 4-hydroxylmellein, kaemopherol, kaempferitin and ascorbic acid, octacosonoic acid, Methionine, protein, Moringine, moringinine, spirachin, 1,3-dibenzylurea, p-cymene, alpha-phellandrene, Deoxy-niazimicine [13]. The methanolic extract of *Moringa* has hepatoprotective effect and it is due to the presence of quercitin [14].

Studies have shown that *Moringa* leaves have antioxidant activity, in scavanging free radicals and provide protection in degenerative diseases and inhibition of lipidperoxidase [15].

Curcuma longa

*Curcuma longa (*turmeric) belongs to zingiberaecea family. Three major curcuminoids present in curcuma are Curcumin, demethoxycurcumin, bis-demethoxy curcumin. It is a natural dye which is yellow and orange in color and is derived from the rhizomes of this plant. It has various chemical, biological, and pharmacological properties like antioxidant, anti-inflammatory, anticarcinogenic activity. Curcumin administration also showed the hepatoprotective, and antimicrobial activity and to be protective against diverse disease such as atherosclerosis, ischemia, cystic fibrosis [16] [17].

The chemical constituents present in fresh rhizome of *Curcuma* include aromatic-turmerone, alpha and beta turmerone, and in rhizome oil aromatic-turmerone, alpha-santalene and aromatic curcumene are present [18]. Other chemical constituents present in *Curcuma longa* includes; Flavanoids, Phenols, Tannins, Curcumin, Terpenoids, Saponins, D-cymarose, β-Elemenone, Aceetic acid, Cinnamic acid, Curzerenone, 14-hydroxy-δ-cadineneandγ-eudesmolacetate [19].

To our knowledge, no attempt has been made to compare the hepatoprotective effect of *Moringa oleifera* and *Curcuma longa.* The major aim of this study is to compare the hepatoprotective effect of both plants on paracetamol induced hepatotoxicity in rats in comparison with silymarin as a standard hepatoprotective drug.

MATERIALS AND METHODS

Chemicals

Paracetamol (GSK, Pakistan) and Silymarin from (Abbott Laboratories) were purchased and employed during the entire protocol.

Equipment

UV Spectrophotometer (U2020, IRMECO, Germany), Freezer (Haier, HF-240T), Water Bath (China), Centrifuge Machine (Hettich-EBA20), Digital Weighing Balance (Shimadzu, AY62), Light Microscope (XSZ-107BN, China), Homogenizer (WiseTis-HG-15D).

Assay

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and serum bilirubin levels were assayed by using assay kits, purchased from Human Diagnostics, Germany.

Plant Collection

Dried leaves of *Moringa oleifera* and rhizomes of *Curcuma longa* were purchased from the local market of Rahim Yar Khan. A dried sample of each plant material was deposited in the Herbarium section of department of Botany, Khwaja Fareed University of Engineering and Information Technology and voucher number for leaves of *Moringa oleifera* (MO-CL-06-22-80) and for rhizomes of *Curcuma longa* (CL-CR-06-22-81) were obtained. The leaves of *Moringa oleifera* were then washed with tap water to remove oil particles and other dirt and then air dried (30±2°C) for 12-days. The dried leaves were grounded into fine powder form and stored at 4°C until further use [20]. Similarly, the rhizomes of *Curcuma longa* were cleaned, dried, grounded weighed for further use [21].

Preparation of Crude Extract

The leaves of Moringa *oleifera* were powdered (1kg) and then subjected to soaking in 80% hydroalcoholic solvent (70% ethanol:30% distilled water) followed by vigorous shaking at room temperature on daily basis for 3-days [22]. The filtrate obtained will then be filtered by using Whatman's filter paper (No.1). The residue obtained will then be subjected to second and third soaking in ethanol for 3-days followed by same procedure mentioned earlier. The filtrate obtained was then dried at room temperature until a semi-solid form was achieved, weighed, and stored at -4°C for future use. The crude extract was re-suspended in distilled water before administration to the animals. Stock solution of *Moringa oleifera* (MO) extract was prepared by using 7.5 g in 30 ml of distilled water and given to the animals according to their body weight in concentrations of 125mg, 250mg and 500mg/kg [1]. A similar procedure was adopted for ethanolic extract of *Curcuma longa* and the extract was achieved, weighed, and stored at -4°C for future use. Stock solution of *Curcuma longa* extract was prepared by using 7.5 g in 30ml of distilled water and given to the animals according to their body weight in concentrations of 125mg, 250mg and 500mg/kg.

Phytochemical Analysis

Phytochemical analysis of *Moringa oleifera* and *Curcuma longa* was done to evaluate the existence of phytoconstituent's such as Saponins, Alkaloids, Glycosides, Steroids, Tannins, Anthocyanins, Coumarin, Proteins, and Amino acids, Flavonoids, Diterpenes, Phytosterols, Phenol, Phlobatannins, Leucoanthocyanin, Anthraquinones and Carbohydrates.

Saponins

Saponins was detected by adding 5ml of crude extract in 20ml of distilled water and then agitated in a cylinder for fifteen minutes. The formation of a foam indicated the presence of saponins.

Alkaloids

Alkaloids were detected by adding 3ml of *Curcuma longa* extract with 1ml of HCl in a test tube and this mixture was heated for twenty minutes, let it cool and then filtered. This filtrate was used with 1ml of Wagner's reagent; precipitates formation indicates the presence of alkaloids.

Glycosides

Legal's test: 1ml pyridine and 2-3 drops of sodium nitroprusside was added in *Curcuma*

longa extract, color was changes from pink to red indicates the occurrence of glycosides.

Steroids

Steroids were detected by adding 10ml of chloroform in 1ml of extract and then an equal volume of concentrated H2SO4 was added in it. The presence of steroid was confirmed by the red layer formation on upper side and H2SO4 showed yellow color with green fluorescence.

Tannins

Tannins were detected by adding 4ml of crude extract were treated with 4ml of Fe $(Cl)^3$. The appearance of green color indicates the presence of tannins.

Anthocyanin

Anthocyanin was detected by adding 2ml of NH3 and 2N HCl in 2ml of aqueous extract, pink, red color was formed and then turned into violet blue color, this color formation confirms the presence of anthocyanin.

Coumarin

Coumarin was detected by adding 2ml of extricate was added in 3ml of 10% NaOH, appearance of yellow color confirms the occurrence of coumarin.

Proteins Tests

Xanthoproteic test: Some drops of concentrated HNO3 were placed in extract; formation of yellow color confirms the presence of proteins.

Amino acids Tests

Ninhydrin test: 2ml of extract were boiled with 2ml of ninhydrin reagent, blue color formation indicates the presence of amino acids.

Flavonoids Tests

Alkaline reagent test: aqueous extract was added in 10% of sodium hydroxide solution, formation of yellow color confirms the existence of flavonoids.

Phytosterol Tests

Salkowski's test: Chloroform was added in extract and then filtered. Then a few drops of concentrated H2SO4 were added and shaken, allow it to stand for few minutes, appearance of golden red confirms the incidence of phytosterols.

Phenol Tests

Ferric Chloride test: Alcoholic Ferric Chloride solution (4 drops) was added in crude extract. The appearance of bluish black color confirms the existence of phenol.

Carbohydrate

Carbohydrates were detected by adding 5ml of distilled water that were dissolved in extract and then filtered. The filtrate was then used for the following test.

a) Molisch's Test: Alcoholic naphthol (2drops) solution was added in filtrate, appearance of violet ring confirms the presence of carbohydrates.

b) Barford's Test: 1ml of Barford's reagent & 1ml of test solution were added in a test tube, and test tube were held in water bath, formation of brick red colored precipitates at the bottom of test tube indicates the presence of carbohydrate.

Pharmacological Section

Animal Handling

27 rats (250–300g) were purchased from the faculty of veterinary medicine. They will be kept in cages and exposed to 12-hour light/darkness cycle in a controlled room (temperature 24±1°C, humidity 60% ± 5%). The animals had free access to drink tap water *ad libitum*. Rats were routinely acclimatized to laboratory conditions for 7 days before the experiment.

Experimental Design

Group-I: Normal Control

The treatment group was considered as normal control treated with normal saline 4ml/kg for 14-days.

Group-II: Diseased Control

Animals were administered with Paracetamol 3g/kg orally on the 1st day of the study.

Group-III: Positive Control Group

This group was treated with Silymarin 200mg/kg orally for 14-days.

Group-IV, V and VI

This group was treated with crude extract of *Moringa oleifera* with three different doses (125, 250 & 500mg/kg) orally for 14-days.

Group VII, VIII and IX

This group was treated with crude extract of *Curcuma longa* with three different doses (125, 250 & 500mg/kg) orally for 14-days.

All animals were maintained on overnight fasting, weighed, and then anesthetized with a mixture of xylazine and ketamine (1:10) at a dose of 0.2ml/100g. On the 0thday, the blood sample was taken by retro-orbital puncture. After 24 hours administration of paracetamol, the rats were again anaesthetized with a mixture of xylazine, and ketamine and blood sample were collected via retro-orbital. When hepatotoxicity is confirmed after LFTs, treatment of both the extracts was started [23]. After treatment of crude extracts for 14 days, the liver tissues were excised immediately after dissection, then washed with fresh normal saline and stored at-20ºC for histopathological examination. The doses used in this study were adopted from other studies with slight modification [24].

Liver Function Tests

The liver function tests (LFTs) including ALT, AST, ALP, and Bilirubin were analyzed by kit methods assigned by the manufacturer [22].

Histopathological Examination

A median lobe section was preserved in 10% formal saline. The remaining liver was quickly frozen in dry ice and then stored at-80°C for further evaluation. According to the standard procedure, microtome sections of 3-4 µm thickness were prepared and stained haematoxylin. Pathological findings of these sections were then examined (centilobular necrosis, lymphocytes, and fatty infiltration).

Statistical Analysis

The data were expressed as mean and statistical analysis was performed using one way ANOVA. It is used to compare between and within groups to measure differences between two or more groups [24].

RESULTS

Following intoxication with paracetamol (3g/kg) orally at the start of the study significant increase in the serum AST, ALT, ALP, and bilirubin as compared to the normal control served with normal saline (4ml/kg) orally for 14-days. All the treatment groups served with crude extracts of *Moringa oleifera* (MO.Cr) and *Curcuma longa* (Cl.Cr) showed significant reduction ($p<0.05$) when given (125, 250 and 500mg/kg) orally for 14-days. The positive control group was treated with standard silymarin (200mg/kg) orally for 14-days.

1. Effects of Crude Extract of MO.Cr on Liver Function Tests

1.1. Effects of Crude Extract of MO.Cr on Alkaline Phosphatase (ALP)

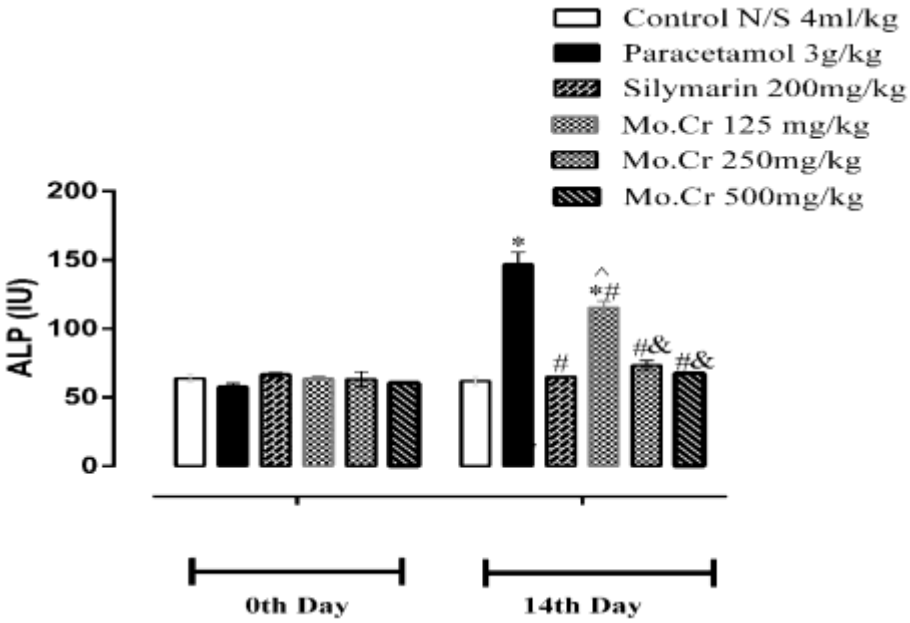

Fig.1.1 Serum

ALP of normal control, paracetamol, paracetamol + treatments groups.

The values mean ± SEM (n=6) in each group. Statistical analysis was done one way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all groups in the respective days. The results are considered significant (*) if $p < 0.05$. * Indicates $p < 0.05$ vs. Normal Control, # indicates $p < 0.05$ vs. Paracetamol, ^ indicates $p < 0.05$ vs. Paracetamol vs Silymarin and & indicates $p < 0.05$ vs. Paracetamol+Mo.Cr 125mg/kg.

ALP is evaluated by giving different doses of crude extract of MO.Cr. Toxicity caused by paracetamol was reduced according to dose.

1.2. Effects of Crude Extract of MO.Cr on Aspartate Transaminase (A

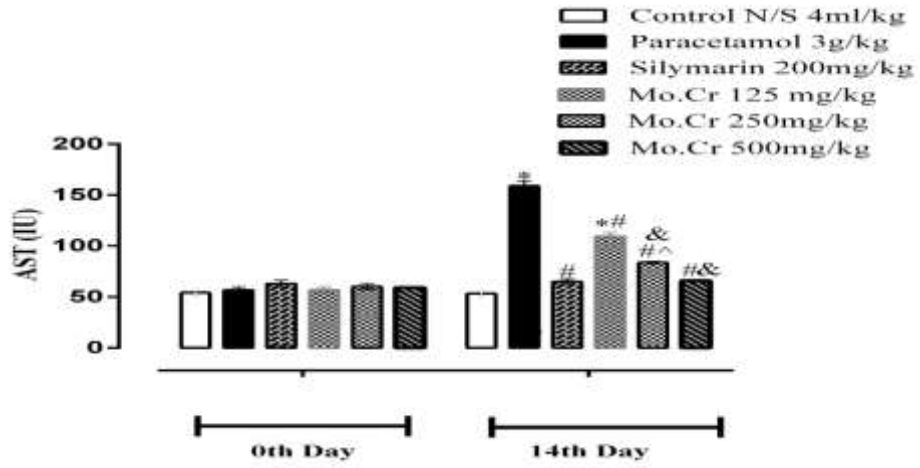

Fig.1.2 Serum AST of normal control, paracetamol, paracetamol + treatments groups.

The values mean ± SEM (n=6) in each group. Statistical analysis was done one way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all groups in the respective days. The results are considered significant (*) if p <0.05. * Indicates p < 0.05 vs. Normal Control, # indicates p < 0.05 vs. Paracetamol, ^ indicates p < 0.05 vs. Paracetamol vs Silymarin and & indicates p < 0.05 vs. Paracetamol+Mo.Cr 125mg/kg

AST is evaluated by giving different doses of crude extract of MO.Cr. Toxicity caused by paracetamol was reduced according to dose.

1.3. Effects of Crude Extract of MO.Cr on Alanine Transaminase (ALT)

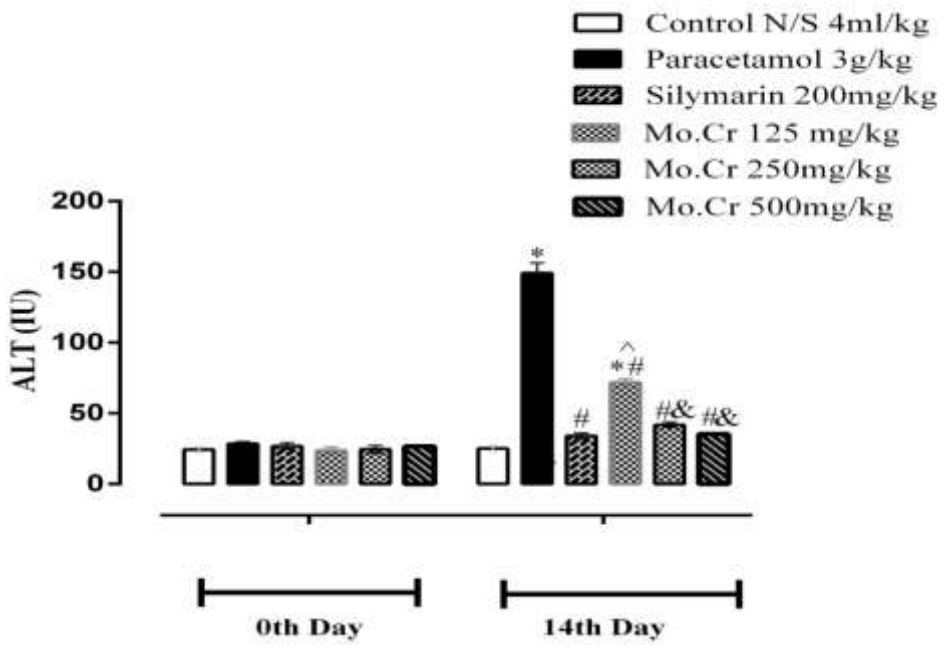

Fig.1.3 Serum ALT of normal control, paracetamol, paracetamol+treatments groups.

The values mean ± SEM (n=6) in each group. Statistical analysis was done one way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all groups in the respective days. The results are considered significant (*) if p < 0.05. * Indicates p < 0.05 vs. Normal Control, # indicates p < 0.05 vs. Paracetamol, ^ indicates p < 0.05 vs. Paracetamol vs Silymarin and & indicates p < 0.05 vs. Paracetamol+Mo.Cr 125mg/kg

ALT is evaluated by giving different doses of crude extract of MO.Cr. Toxicity caused by paracetamol was reduced according to dose.

1.4. Effects of Crude Extract of MO.Cr on Total Bilirubin (TB)

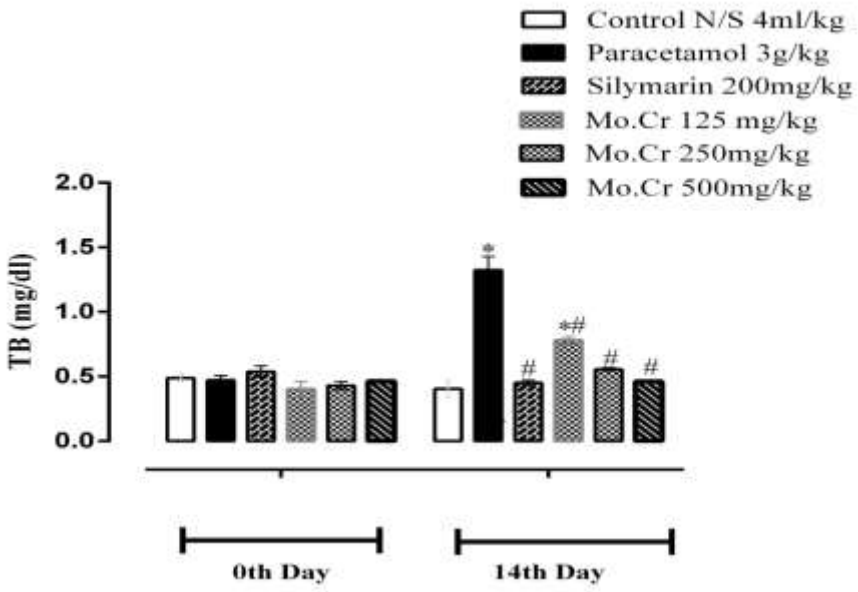

Fig.1.4 Serum TB of normal control, paracetamol, paracetamol + treatments groups.

The values mean ± SEM (n=6) in each group. Statistical analysis was done one way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all groups in the respective days. The results are considered significant (*) if $p < 0.05$. * Indicates $p < 0.05$ vs. Normal Control, # indicates $p < 0.05$ vs. Paracetamol.

TB is evaluated by giving different doses of crude extract of MO.Cr. Toxicity caused by paracetamol was reduced according to dose.

ALP and AST are evaluated by giving different doses of crude extract of Cl.Cr. Toxicity caused by paracetamol was reduced according to dose.

Groups	ALP (IU)		AST (IU)	
	0th	14th	0th	14th
Control (4ml/kg)	62.50±2.54	63.33±3.16	54.33±2.57	53.50±2.99
Paracetamol (3g/kg)	57.17±2.31	154.0±7.77*	57.00±2.64	158.8±3.77*
PCM+ Silymarin 200mg/kg	60.17±2.58	64.33±1.35#	61.67±3.40	64.50±1.47#
PCM+ Cl.Cr 125mg/kg	61.67±3.08	115.2±4.21*#^	57.50±2.02	107.7±3.54*#^
PCM+ Cl.Cr 250mg/kg	63.17±5.55	72.00±4.22#&	61.69±3.63	82.50±0.76*#^&
PCM+ Cl.Cr 500mg/kg	60.17±1.83	66.83±0.80#&	58.67±1.94	65.00±0.93*#&$

Table.1 Effect of Cl.Cr Treatment on ALP and AST in Paracetamol-induced liver damage

*Notes: (n=6) Wistar Albino rats per group, each value expressed as Mean±SEM. One-way analysis of variance (ANOVA) was applied for statistical analysis followed by Bonferroni post hoc test for all groups in respective days. * p < 0.05 versus Control; # p < 0.05 versus Paracetamol; ^p < 0.05 versus PCM+Silymarin, & p < 0.05 versus PCM+Cl.Cr 125, $p < 0.05 versus PCM+Cl.Cr 250 Abbreviations: PCM=Paracetamol*

ALT and TB are evaluated by giving different doses of crude extract of Cl.Cr. Toxicity caused by paracetamol was reduced according to dose.

Groups	ALT (IU)		TB (mg/dl)	
	0th	14th	0th	14th
Control (4ml/kg)	24.00±2.55	24.83±3.04	0.48±0.03	0.40±0.06
Paracetamol (3g/kg)	28.00±1.88	161.2±7.19*	0.49±0.04	1.32±0.10*
PCM+ Silymarin 200mg/kg	27.67±2.12	31.67±0.96#	0.51±0.03	0.45±0.01#
PCM+ Cl.Cr 125mg/kg	24.33±2.77	70.00±2.00*#^	0.37±2.05	0.73±0.03*#
PCM+ Cl.Cr 250mg/kg	24.83±2.37	40.17±1.55#&	0.48±0.03	0.52±0.01#
PCM+ Cl.Cr 500mg/kg	27.83±0.83	33.67±1.08#&	0.47±0.02	0.41±0.06#&

Table.2 Effect of Cl.Cr Treatment on ALT and TB in Paracetamol-induced liver damage

*Notes: (n=6) Wistar Albino rats per group, each value expressed as Mean±SEM. One-way analysis of variance (ANOVA) was applied for statistical analysis followed by Bonferroni post hoc test for all groups in respective days. *p < 0.05 versus Control; #p < 0.05 versus Paracetamol; ^p < 0.05 versus PCM+Silymarin, &p < 0.05 versus PCM+Cl.Cr 125. Abbreviations: PCM=Paracetamol*

Histopathological Examination

The liver of disease control rats showed liver structure, histopathological changes including severe fatty denegation of inflammatory cells and hepatocytes infiltration as well as increase in the number of hepatic cells, most of the blood sinusoids appeared narrow and obliterated (Fig. 2A). Treatment with Silymarin and Paracetamol showing improvement in liver histology and hepatic cells have recovered (Fig. 2B). While treatment with *Moringa oleifera* and *Curcuma longa* with paracetamol shows improvement in liver histology, most areas of the hepatic cells appear to have recovered, hepatocytes well preserved and there is no area of necrosis (Fig. 2C, 2D).

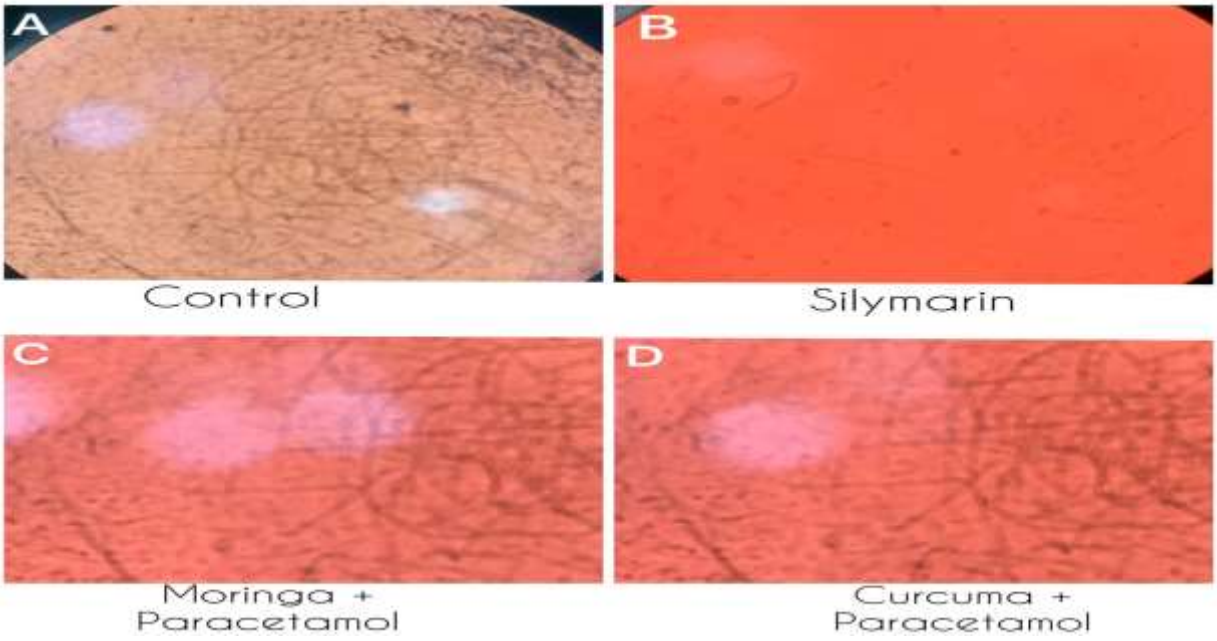

Fig2: Photomicrograph of liver sections (A): diseased control rats showing vascular degeneration, (B): Silymarin group showing improvements in hepatic cells, (C&D): Moringa+Paracetamol and Curcuma+Paracetamol showing recovered hepatic necrosis.

DISCUSSION

Liver is largest organ and is involved in metabolic processes, also responsible for the removal of waste products and xenobiotics [3]. Paracetamol is used as an anti-pyretic and analgesic drug. As this drug has beneficial effects, it is also the major cause of liver breakdown and drug-induced liver disease not only in human beings but also in the experimental animals [5].

In this study, a single dose of paracetamol was used to elevate liver enzymes such as ALP, AST, ALT, and Serum bilirubin. The results have shown reduction after giving extract of *Moringa oleifera* and *Curcuma longa* by preventing all the changes seen in the Paracetamol group.

Aspartate aminotransferase (AST) is an enzyme that is released from the cytosol and mitochondria of liver cells, cardiac muscle, pancreas, skeletal muscle, brain, leucocytes, kidney, and erythrocytes. AST has less specificity and sensitivity for the assessment of liver functions. Acute and chronic liver diseases were specified with the increased level of AST in serum. Increased level of AST indicates the existence of non-alcoholic fatty liver disease (NAFLD) [25].

Alanine transaminase (ALT) is an enzyme and is found in the cytosol of liver cells. ALT leaks into serum when there is a disturbance in liver cells. Following conditions has increased level of ALT; toxic heptoses, muscular dystrophy, and in metal poisoning [26]. In alcoholic hepatitis, the level of AST to ALT increases, and the increased level shows abnormality caused by alcohol in the abnormal LFT's. There is also an increase in the level of ALT in; autoimmune liver disease, chronic hepatitis B and C, Wilson's disease, non-alcoholic fatty liver disease, haemochromatosis, celiac disease and antitrypsin deficiency [25].

Raised level of ALP occurs mostly in these cases; osteoporosis, sclerosing cholangitis, and primary biliary cirrhosis. It triggers the release of ALP into the systemic circulation. It also increase the reactions that can cause necrosis, lipid peroxidation, over expression of CYP2E1 and reduce level of GSH, that leads to breakdown of RBCs and the enzymes leakage into systemic circulation [26].

Bilirubin is the major indicator of the severity of necrosis. Raised level of bilirubin leads to

jaundice and cause damage to the liver cells [27]. The mechanism of liver injury produced by the doxorubicin is still unclear. It might be due to the production of lipid peroxidase that may produce reactive oxygen species within the body. There is greater antioxidant potential in plants which have flavonoids & phenolic contents and this is because of the antioxidant activity which is essential to scavenge free radicals formed within the body [28].

Results of this study indicate that hepatotoxicity caused by paracetamol was recovered by giving extract of *Moringa oleifera* and *Curcuma longa*. Treatment was given in dose dependent manner. Three different doses of both plants (MO.Cr & Cl.Cr) were used (125mg/kg, 250mg/kg, & 500mg/kg) to check the effect of extract on three different doses. Treatment with 125mg/kg of *Moringa oleifera* and *Curcuma longa* results in small reduction of liver enzymes, dose of 250mg/kg has better reduction than 125mg/kg, and 500mg/kg has a significant reduction in the levels of liver enzymes and suggests that these pharmacological effects of *Moringa oleifera* and *Curcuma longa* are dose dependent. The study found that hepatotoxicity was raised in diseased control rats and the rats treated with MO.Cr and Cl.Cr extracts at different doses (250mg and 500mg) reduce hepatotoxicity [10].

Both of the plants are compared and MO.Cr has better restoration of liver enzymes, and this showed that MO.Cr has better hepatoprotective effect. Previous studies reported that the use of roots and flowers of *Moringa oleifera* and underground stems of *Curcuma longa* have prevented Paracetamol induced hepatotoxicity. Present study has demonstrated that, ethanolic administration of the leaves of MO and rhizomes of CL protects the release of the liver enzymes into the bloodstream [24, 29].

Our results suggest that the protective effect of *Moringa* and *Curcuma* preserves the structural integrity of hepatocytes. The results have also shown that the dose dependent manner of *Moringa* and *Curcuma* are equally effective with Silymarin in restoration of liver enzymes after induction of Paracetamol [23].

Silymarin is a radical scavenger and is a hepatoprotective drug which causes decrease in the elevated liver enzymes ALT, ALP, AST, and serum bilirubin in various drug induced hepatotoxicity. Silymarin was used as standard drug for comparing the hepatoprotective effect of various plant extracts due to its hepatoprotective effect [24].

In a study, a comparison has been made between the hepatoprotective effects of *Moringa oleifera* and *Curcuma longa* to evaluate the more powerful hepatoprotective drug. Results of both plants confirmed that both drugs significantly reduce the levels of ALT, ALP, AST, and Serum bilirubin, but the values of these parameters showed that *Moringa* has better hepatoprotective effect in recovering the level of ALT, ALP, AST, and Serum bilirubin, than *Curcuma longa.*

Different studies have been conducted to evaluate the hepatoprotective effect of many plants like Moringa oleifera and Curcuma longa, but no comparison has been made to check which plant has better hepatoprotective effect. That's why this study was performed to evaluate the comparison of hepatoprotective effect of Moringa oleifera and Curcuma longa which was not performed before this study.

CONCLUSION

Toxicity induced by Paracetamol was recovered by giving treatment of *Moringa oleifera* leaves extract and *Curcuma longa* rhizomes extract which reduces the raised level of ALT, AST, ALP, and Serum bilirubin because both plants has antioxidant activity and therefore both plant can produce hepatoprotective effect but the hepatoprotective effect of *Moringa oleifera* has better capability to restore raised level of liver enzymes that's why it should be preferred to treat hepatotoxicity.

NOVALITY OF STUDY

LIMITATIONS

1. Ethical considerations: Animal experimentation requires strict adherence to ethical guidelines, including the humane treatment of animals, proper housing, and minimizing pain and distress.

2. Interpretations of results: There are many factors that affected the outcome of the results such as age, sex, health condition, and duration & frequency of administration plant extract.

3. Extrapolation to human studies: It can be difficult to extrapolate the results of animal studies to humans, further studies may be required to confirm the potential hepatoprotective effects of Moringa oleifera and Curcuma longa in humans.

REFERENCES

1. Atta, A.H., et al., *Hepatoprotective and antioxidant effects of methanol extractof Moringa oleifera leaves in rats.* Wulfenia, 2017. **24**(3): p. 249-268.
2. Sohaib, a.u., et al., *Evaluation of In Vitro Anti-arthritic and Antioxidant Activities of Extracts of Cotula Anthemoides l.*
3. Asgari-Kafrani, A., M. Fazilati, and H. Nazem, *Hepatoprotective and antioxidant activity of aerial parts of Moringa oleifera in prevention of non-alcoholic fatty liver disease in Wistar rats.* South African Journal of Botany, 2020. **129**: p. 82-90.
4. Parmar, S.R., P.H. Vashrambhai, and K. Kalia, *Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats.* J Herbal Med Toxicol, 2010. **4**(2): p. 101-106.
5. Tittarelli, R., et al., *Hepatotoxicity of paracetamol and related fatalities.* Eur Rev Med Pharmacol Sci, 2017. **21**(1 Suppl): p. 95-101.
6. Arshad, z., et al., *Evaluation of Anxiolytic Activity of Ethanolic Extract of Cuminum cyminum in Rodents.*
7. Muzumbukilwa, W.T., et al., *Mapping the evidence of hepatoprotective properties of Moringa oleifera from sub-Saharan African countries: a systematic review protocol.* Syst Rev, 2019. **8**(1): p. 197.
8. Hameed, r., et al., *Evaluation of Cytotoxicity and Anti-viral Activity of Moxidectin against Influenza Virus h9.* Evaluation, 2022. **29**(04).
9. Ferner, R.E., J.W. Dear, and D.N. Bateman, *Management of paracetamol poisoning.* Bmj, 2011. **342**.
10. Muzumbukilwa, W.T., M. Nlooto, and P.M.O. Owira, *Hepatoprotective effects of Moringa oleifera Lam (Moringaceae) leaf extracts in streptozotocin-induced diabetes in rats.* Journal of functional foods, 2019. **57**: p. 75-82.
11. Nasr, S., et al., *Phytochemical, antioxidant and hepatoprotective effects of different fractions of Moringa oleifera leaves methanol extract against liver injury in animal model.* Asian Pacific Journal of Tropical Medicine, 2018. **11**(7).
12. Farid, A.S. and A.M. Hegazy, *Ameliorative effects of Moringa oleifera leaf extract on levofloxacin-induced hepatic toxicity in rats.* Drug Chem Toxicol, 2020. **43**(6): p. 616-622.
13. Islam, R. and M.J. Alam, 2019.
14. Vergara-Jimenez, M., M.M. Almatrafi, and M.L. Fernandez, *Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease.* Antioxidants (Basel), 2017. **6**(4).
15. Fakurazi, S., S.A. Sharifudin, and P. Arulselvan, *Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature.* Molecules, 2012. **17**(7): p. 8334-50.
16. Singh, I., et al., *Hepatoprotective activity of aqueous extract of curcuma longa in ethanol induced hepatotoxicity in albino wistar rats.* Int J Phytopharmacol, 2012. **3**(3): p. 226-233.

17. Sohaib, a.u., et al., *Dose Dependent Platelet Aggregation and Blood Coagulation Activity of Prosopis cineraria.*
18. *Prosopis Cineraria Druce (Jand) – A Review of Its Ethnomedicinal, Phytochemical and Pharmacological Properties: Ahmed Umer Sohaib, Atta-ur-Rehman, Roheena Sohail.* International Journal of Pharmacy and Integrated Health Sciences, 2021. **2**(1).
19. Yadav, D., et al., *Turmeric (Curcuma longa L.): A promising spice for phytochemical and pharmacological activities.* International Journal of Green Pharmacy (IJGP), 2013. **7**(2).
20. Alkhudhayri, D.A., et al., *Moringa peregrina leaf extracts produce anti-obesity, hypoglycemic, anti-hyperlipidemic, and hepatoprotective effects on high-fat diet fed rats.* Saudi J Biol Sci, 2021. **28**(6): p. 3333-3342.
21. Mošovská, S., et al., *Antioxidant properties of curcuminoids isolated from Curcuma longa L.* Acta Chimica Slovaca, 2016. **9**(2): p. 130-135.
22. Fakurazi, S., I. Hairuszah, and U. Nanthini, *Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level.* Food Chem Toxicol, 2008. **46**(8): p. 2611-5.
23. El-bakry, K., et al., *Hepatoprotective effect of Moringa oleifera leaves extract against carbon tetrachloride-induced liver damage in rats.* World J Pharm Pharm Sci, 2016. **5**(5): p. 76-89.
24. Fakurazi, S., I. Hairuszah, and U. Nanthini, *Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level.* Food and chemical toxicology, 2008. **46**(8): p. 2611-2615.
25. Limdi, J. and G. Hyde, *Evaluation of abnormal liver function tests.* Postgraduate medical journal, 2003. **79**(932): p. 307-312.
26. Adeyemi, O., et al., *Toxicological evaluation of the effect of water contaminated with lead, phenol and benzene on liver, kidney and colon of Albino rats.* Food and chemical toxicology, 2009. **47**(4): p. 885-887.
27. Barshes, N.R., et al., *Support for the acutely failing liver: a comprehensive review of historic and contemporary strategies.* Journal of the American College of Surgeons, 2005. **201**(3): p. 458-476.
28. Jestadi, D.B., et al., *Effects of short term exposure of atrazine on the liver and kidney of normal and diabetic rats.* Journal of toxicology, 2014. **2014**.
29. Kalantari, H., L. Khorsandi, and M. Taherimobarakeh, *The protective effect of the Curcuma longa extract on Acetaminophen–induced hepatotoxicity in mice.* Jundishapur Journal of Natural pharmaceutical products, 2007. **2**(1).