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Anti-hyperbilirubinemic Potential of Aqueous Extract of *Mimosa pudica* Roots in Wistar Rats

Rupali A. Patil[1*], Pradhnya M. Ghate[2], Shubhangi H. Pawar[2], Manisha A. Tayde[3] and Suvarna A. Katti[2]
[1]K.K. Wagh College of Pharmacy, Hirabai Haridas Vidyanagari, Amrutdham, Panchavati, Nashik (Maharashtra), India.
[2]MGV's Pharmacy College, Mumbai-Agra Road, Nashik (Maharashtra), India.
[3]Shree Panchavati Education Society's, Institute of Pharmacy, Panchavati, Nasik (Maharashtra), India.

(Corresponding author: Rupali A. Patil)*
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ABSTRACT: Paracetamol and Phenylhydrazine (PHZ)-induced hyperbilirubinemia in rat is due to elevated bilirubin content causing damage to the liver. It is associated with hemolysis of RBC's which causes an over production of Bilirubin. Effect of aqueous extract of *Mimosa pudica* L. roots (AEMP) was investigated using paracetamol and PHZ-induced hyperbilirubinemia in Wistar rats. In both models, the common parameters estimated were serum Bilirubin, Hemoglobin (Hb), serum levels of liver biomarker enzymes *viz*., aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), various in vivo biochemical parameters like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and extent of lipid peroxidation (LPO) in the liver. Serum bilirubin and blood hemoglobin levels were measured on day 1, 5 and 10. AST, ALT and ALP levels measured on day 5 and 10. Paracetamol and PHZ exhibited significant increase in the level of bilirubin and LPO while levels of other parameters significantly decreased on 10th day. AEMP exhibited significant decrease in the levels of bilirubin and LPO and increase in the levels of other parameters on 10th day. Present study indicates that aqueous extract of *Mimosa pudica* root shows potential antihyperbilirubinemic activity associated with antioxidant activity in both the models indicating usefulness in various liver disorders.

Keywords: Bilirubin, Hyperbilirubinemia, Jaundice, *Mimosa pudica*, Paracetamol, Phenylhydrazine.

INTRODUCTION

Jaundice is a French term that means "yellow." Jaundice is characterized by yellow skin and eyes caused by bilirubin, a yellow pigment and a component of old red blood cells (RBCs). Bilirubin-containing bile is secreted by the liver. Bile travels via the lower digestive tract, and bilirubin is normally expelled from the body via the liver; however, in Jaundice, bilirubin levels in the body get elevated as excretion decreases (Dons and Soosairaj 2013).

Mimosa pudica L. (Family: Leguminosae), a tiny to medium-sized tree grown across India, is a multifunctional tree that serves as a vegetable, spice, source of cooking and cosmetic oil, and medicinal plant. Sensitive plant, Ajalikalika, Lajawanti, Lajjabate, Hadergitte, Kasirottam, and Manugumaramu are some other synonyms for it (Chauhan and Johnson 2009). *M. pudica* possesses many pharmacological activities such as antimicrobial, antioxidant, wound-healing (Singh *et al*., 2021), antioxidant (Prashar *et al*., 2020), antitumor (John *et al*., 2020), anti-parkinsonian (Duyu *et al*., 2020), hepatoprotective (Kowsalya and Sangeetha 2020). All components of the tree are said to have therapeutic characteristics and are used to cure biliousness, leprosy, vaginal and uterine ailments, inflammations (Kokane *et al*., 2009), cytotoxic (Vennila Preethi *et al*., 2022). It is utilised to regulate kapha and pitta. According to the Unani medical system, root is resolvant, alternate, and effective in disorders caused by blood impurities and bile, bilious fevers (Kowsalya and Sangeetha 2020), piles, jaundice, leprosy etc. It contains alkaloid, glycoside, flavonoid and tannins. It contains mimosine, an alkaloid, which has antiproliferative and apoptotic properties (Meenatchisundaram *et al*., 2009). Plants used traditionally for jaundice include *Cichorium intybus*, *Phyllanthus emblica* L, *Ziziphus jujuba* Mill, *Descurainia sophia* L, *Punica granatum* L (Gupta *et al*., 2014; Janghel *et al*., 2019; Jannat *et al*. 2019; Khedmat *et al*., 2021; Raghuvanshi *et al*., 2021).

Leaves extract contains adrenaline. Tannin content in roots is around 10 per cent. Derivatives of 4-o-(b-D-glucopyranosyl-6-sulphate) gallic acid are responsible for periodic leaf movement (Gandhiraja *et al*., 2009).

MATERIAL AND METHODS

Animals. Adult Wistar rats (180-250 gm) of either sex, procured from Bharat Serum and Vaccines Ltd, Thane, were maintained under standard laboratory conditions. The protocol was approved by IAEC, MGV's Pharmacy College, Panchavati, Nashik (MGV/PC/CPCSEA/XXXI/2016/06).

Drugs and Chemicals. In the study, Paracetamol and Phenylhydrazine (Research lab), Silymarin (Silyibon), and Total Bilirubin kit (Beacon, Diagnostic) were used.

Preparation of extract. *M. pudica* roots obtained from a local nursery were authenticated by the Pharmacognosy Lab at M.G.V.'s Pharmacy College, Panchavati, Nashik (MGV/PC/PCGA/2016/11). The roots were thoroughly rinsed, crushed, and then boiled in distilled water to decrease the volume by up to 75%. Aqueous extract of *M. pudica* roots (AEMP) was filtered and refrigerated.

Phytochemical investigation. Phytochemical investigation of AEMP was performed according to the standard procedures (Trease and Evans 1996).

In vitro antioxidant study

Free radical scavenging assay. The potential for scavenging free radicals was assessed against an ethanolic solution of DPPH, a stable free radical. The degree of discoloration measured at 517nm shows scavenging activity of extract (Molyneux, 2004).

Reducing power assay. The more antioxidant chemicals that convert the oxidation form of iron, such as ferric chloride, to ferrous in the reducing power assays. Absorbance change was measured at 700nm. Scavenging activity was calculated as a percentage (Oyaizu, 1986).

Total Phenol content. The reaction between the Folin-Ciocalteau reagent and the phenolic compound produces a blue color complex that absorbs radiation that can be measured at 510nm (Bhalodia *et al*., 2011). The total phenol concentration was stated in Gallic acid equivalents (µg/ml).

Anti-hyperbilirubinemic activity

Paracetamol-induced hyperbilirubinemia. Group I Vehicle (Distilled water, 5 ml kg^{-1}; p.o), Group II: Paracetamol (2 mg kg^{-1}; p.o), Group III: Silymarin suspension (100 mg kg^{-1}, p.o) from 6^{th} day upto 10^{th} day. Group IV and V: AEMP (100 mg kg^{-1}, p.o) and AEMP (200 mg kg^{-1}; p.o) respectively from 6^{th} to 10^{th} day.

Paracetamol (2 mg kg^{-1}; p.o) for first 5 days was given to group II, III, IV and V to develop hyperbilirubinemia in rats (Usmani and Kushwaha 2010).

Phenylhydrazine (PHZ)-induced hyperbilirubinemia. Group I: Vehicle (Distilled water; 5ml kg^{-1}; p.o), Group II: PHZ (5 mg kg^{-1}; i.p), Group III: Silymarin suspension (100 mg kg^{-1}; p.o) from day 6 to 10. Group IV and V: AEMP (100 mg kg^{-1}; p.o) and AEMP (200 mg kg^{-1}; p.o) respectively from 6 to 10 days.

PHZ (5 mg kg^{-1}; i.p.) for first 5 days was given to group II, III, IV and V to develop hyperbilirubinemia in rats (Reddy, 2011).

Estimation of biochemical parameters. On day 1 and 5, the serum total bilirubin content was evaluated using Mod. Jendrassik and Grof's technique (Jendrassik and Grof 1938) to confirm hyperbilirubinemia in rats. The Sahli-hellige technique was used to determine haemoglobin levels (Kale and Kale 2006). On days 1, 5, and 10, blood was withdrawn through the retro-orbital route under anaesthesia to measure serum total bilirubin concentrations (IU/L) and blood haemoglobin levels (%). The serum levels of liver biomarker enzymes ALT and AST were measured using the Jendrassik and Grof technique (Jendrassik and Grof 1938), and the amount of alkaline phosphatase (ALP) was evaluated using the Reitman method (Reitman and Frankel 1957). ALT, AST and ALP were expressed in IU/L.

Estimation of in vivo antioxidant parameters. At the end of study, liver was isolated, perfused, homogenized and subjected to estimation of in vivo antioxidant parameters viz., superoxide dismutase (SOD) (Saggu *et al*., 1989), catalase (CAT) (Beers and Sizer 1952), reduced glutathione (GSH) (Ellman, 1959) and extent of lipid peroxidation (LPO) (Niehaus and Samuelsson 1968).

Statistical analysis: Animals were randomly divided into five groups, five animals in each group. Data was analysed by One-Way ANOVA followed by Dunnett's test. *P<0.05 compared to Vehicle group was considered as significant. #P<0.05 compared to Paracetamol or PHZ group was considered as significant.

RESULTS AND DISCUSSION

Phytochemical screening. Phytochemical analysis of AEMP revealed the presence of cardiac glycosides, flavonoids, alkaloids, steroids, tannins and phenolic compounds.

In vitro antioxidant study

Free radical scavenging activity. In DPPH assay, the % scavenging activity increased with the increase in concentration of AEMP. The IC_{50} value was found to be 999 µg/ml.

Reducing power assay. AEMP exhibited increased % scavenging activity with increase in concentration. The IC_{50} value was found to be 155 µg/ml.

Total phenolic content. Total Phenolic content of AEMP was found to be 155µg/ml in terms of gallic acid equivalents.

Anti-hyperbilirubinemic activity

Paracetamol-induced hyperbilirubinemia. Animals treated with Paracetamol exhibited significant decrease in serum bilirubin (Fig. 1) and LPO levels while significant increase in blood haemoglobin (Fig. 2), serum AST, ALT, ALP and liver SOD, CAT and GSH levels (Table 1) was observed at the end of 10 days. Administration of Paracetamol shows significantly

reduced Hb level on 5th day compared to vehicle treated group which was reversed by treatment with AEMP. AEMP administration showed significant rise in Hb level on 10th day than paracetamol treated animals. Animals treated with AEMP showed significant increase in serum total bilirubin content, AST, ALT and ALP level on 5th day than vehicle treated group. AEMP exhibited significant decrease in serum total bilirubin level, and AST, ALT and ALP levels on 10th day compared to vehicle treated group.

Phenylhydrazine induced hyperbilirubinemia. Phenylhydrazine treatment for 5 Days exhibited significant decrease in serum bilirubin (Fig. 3) and LPO levels while levels of haemoglobin (Fig. 4), serum AST, ALT, ALP, and liver SOD, CAT and GSH levels (Table 2) were significantly elevated.

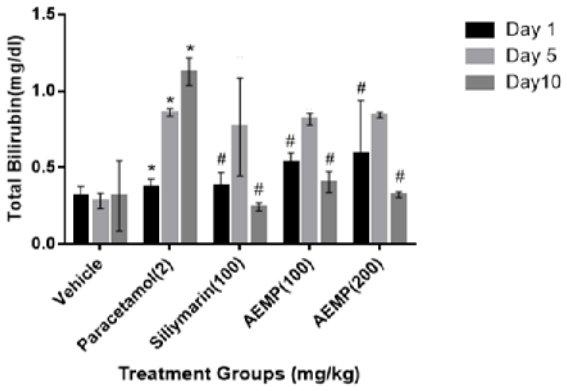

Fig. 1. Effect of AEMP on Total bilirubin levels in paracetamol induced hyperbilirubinemia in rats.

Table 1: Effect of AEMP on biochemical parameters in Paracetamol-induced hyperbilirubinemia in Wistar rats.

Groups (mg/kg) Parameters		Vehicle	Paracetamol (2)	Silymarin (100)	AEMP (100)	AEMP (200)
AST (IU/L) (Day)	1	33.33±0.88	37±1.15*	36.67±0.88	33±1.15	38.33±0.88
	5	34±0.577	195.3±1.45*	185±1.15	192.7±1.45	198±1.1.5
	10	37.67±1.45	207±1.15*	86±0.577#	114±2.08#	95.33±1.45#
ALT (IU/L) (Day)	1	84±0.577	86±0.577*	90±0.577	85.67±0.66	83±1.15
	5	86±0.577	269.7±11.35*	247.7±1.76	253±1.15	248.7±2.02
	10	84.67±0.88	301±2.08*	119±3.05#	207.7±1.45#	142.7±1.45#
ALP (IU/L) (Day)	1	57.67±1.45	66±1*	64.33±1.76#	68.67±0.88#	65.67±1.20#
	5	66.67±0.88	127±1.15*	137.7±1.45	129±0.577	137±1.45
	10	66.67±0.88	122.3±1.45*	105±0.577#	112±1.45#	114.3±3.48#
SOD (U/mg)		4.70±0.16	0.7±0.1*	3.7±0.7#	2.1±0.133#	2.67±0.166#
CAT (U/mg)		681±101.4	176.5±48.1*	613.7±135.2#	380±44.4#	527.6±85.3#
GSH (µg/gm)		0.528±0.03	0.279±0.019*	0.437±0.06#	0.299±0.008#	0.324±0.027#
LPO (nmoles/mg)		64.8±11.8	113.9±6.83*	71.3±11.32#	88.3±1.80#	83.4±2.94#

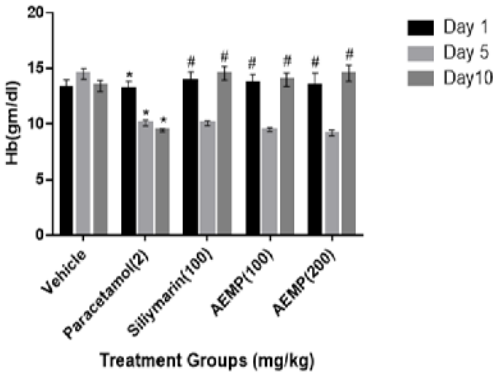

Fig. 2. Effect of AEMP on Haemoglobin level in paracetamol induced hyperbilirubinemia in rats.

Administration of PHZ caused significant decrease in Hb level on 5th day compared to vehicle treated group. AEMP administration showed significant increase in Hb level compared to PHZ treated animals on 10th day. Animals treated with AEMP showed significant increase in serum total bilirubin level, AST, ALT and ALP level on 5th day compared to vehicle treated group. AEMP exhibited significant decrease levels of serum total bilirubin, and AST, ALT and ALP content on 10th day as compared to vehicle treated group.

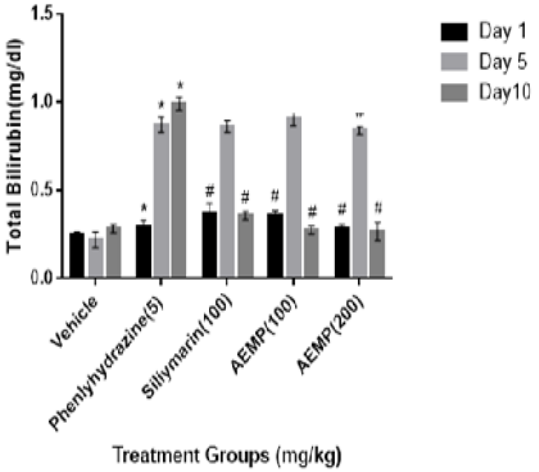

Fig. 3. Effect of AEMP on Bilirubin level in Phenylhydrazine induced hyperbilirubinemia in rats.

Table 2: Effect of AEMP on biochemical parameters in Phenylhydrazine-induced hyperbilirubinemia in Wistar rats.

Groups (mg/kg) Parameters		Vehicle	Phenylhydrazine (5)	Silymarin (100)	AEMP (100)	AEMP (200)
AST (IU/L) (Day)	5	51.67±1.45	298.2±8.41*	287.3±9.38	275±4.48	297±8.14
	10	60±1.15	298.3±3.48*	58.33±2.90#	127±3.21#	124.3±2.60#
ALT (IU/L) (Day)	5	47.67±2.02	276±3.21*	292±4.35	288.7±3.52	293±4.16
	10	45±1.73	284±2.64*	57±4.04#	101.3±4.48#	121.3±1.45#
ALP (IU/L) (Day)	5	68.67±2.02	276±3.21*	292±4.35	288.7±3.52	293±4.16
	10	63±2.30	143±1.73*	68±2.30#	106±3.21#	97±2.08#
SOD (U/mg)		2±0.814	0.5±0.204*	1.15±0.385#	1.07±0.13#	1.26±0.318#
CAT (U/mg)		463.7±161.2	124±28.97*	443.6±72.45#	179.5±48.14#	259.7±44.38#
GSH (µg/gm)		0.387±0.073	0.297±0.015*	0.353±0.032#	0.309±0.012#	0.344±0.013#
LPO (nmoles/mg)		55.63±11.33	107.6±7.14*	61.43±3.91#	97.23±1.33#	64.37±5.96#

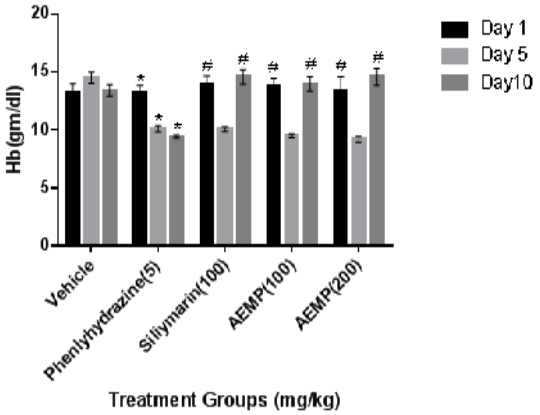

Fig. 4. Effect of AEMP on Haemoglobin in Phenylhydrazine induced hyperbilirubinemia in rats.

DISCUSSION

Phytochemical investigation of aqueous extract of *M. pudica* roots has revealed the presence of alkaloids, cardiac glycosides, tannins and phenols, flavonoids' and steroids.

Previous studies revealed that Paracetamol and Phenylhydrazine-induced hyperbilirubinemia in rat showed a significant increase in the bilirubin level due to occurrence of damage to the liver which leads to hemolysis of RBC's which causes an over production of Bilirubin compound (Arthur *et al*., 2012).

Silymarin, a unique flavonoid complex has hepatoprotective property due to its cell membrane stabilizing property (Fraschini *et al*., 2002). *M. pudica* roots also shows antioxidant activity[3] and traditionally is used in treatment of jaundice (Britt and Burkhart 1997).

Paracetamol causes acute hepatocyte necrosis due to the production of N-acetyl-p-benzoquinoneimine (NAPQI) and the saturation of the paracetamol sulphate and glucoronide pathways. Serum total bilirubin, ALT, and AST levels in rats treated with paracetamol increased significantly (Hemamalini *et al*., 2012).

Animals treated with paracetamol exhibited significant increase in serum total bilirubin on 5th day compared to vehicle treated animals indicating development of hyperbilirubinemia. Serum levels of liver biomarker enzymes AST, ALT and ALP were decreased on 5th day compared to vehicle group indicating liver dysfunction. AEMP treatment showed significant decrease in serum total bilirubin level on 10th day as compared to paracetamol treated group.

As compared to the vehicle group, administration of paracetamol caused a significant drop in Hb level on the fifth day. In comparison to paracetamol-treated animals, AEMP treatment produced a significant rise in Hb level on the 10th day. Paracetamol causes a significant decrease in RBC count, which might signal that matured RBC were destroyed. This might also imply that paracetamol suppresses the release of erythropoietin from the kidneys. Ikpi and Nku reported similar findings in rats treated with *Dennettia tripetala* extract (Ikpi and Nku 2008).

Paracetamol causes a significant fall in Hb concentration, implying a decrease in the oxygen-carrying capacity of blood and the amount of oxygen given to the tissues. Adedapo *et al*. (2007) reported similar results in rats treated with *A. cordifolia* and *S. virosa* extracts.

Acute paracetamol overdoses have been documented to induce potentially toxic liver damage, and its toxicity is the leading cause of acute liver failure in the Western world. It has been observed that large doses of paracetamol (> 2000 mg day^{-1}) increase the risk of upper gastrointestinal problems such as stomach haemorrhage (Garcia and Hernández-Díaz 2001). Similar report was given by Zhang *et al.* (2015) to study the effect of *Agaricus brasilienisis* extract to Phenylhydrazine-induced neonatal jaundice in rats.

Phenylhydrazine induced hemolysis causes induction of liver haemeoxygenase, which in turn causes increase in the bilirubin level in serum. PHZ is known to decrease Hemoglobin levels (Unami *et al*., 1996). In the present study, animals treated with PHZ exhibited a significant increase in serum total bilirubin level, AST, ALT and ALP level on 5th day compared to vehicle group. AEMP showed significant decrease in serum total bilirubin level on 10th day as compared to Phenylhydrazine treated group. Administration of Paracetamol caused significant decrease in Hb level on 5th day as compared to vehicle group. AEMP

administration showed significant increase in Hb level compared to paracetamol treated animals on 10th day. PHZ treated animals showed significant decrease in levels of liver SOD, CAT and GSH and increase in extent of lipid peroxidation indicating involvement of oxidative stress. AEMP treatment significantly reversed these changes indicating antioxidant activity as observed in in-vitro models. Patil and Makwana also reported protective effect of *C. procera* in hyperbilirubinemia induced by paracetamol and PHZ due to antioxidant property (Patil and Makwana 2015). Nawaz *et al*. (2022) proved role of leaf extract of *Phyllanthus emblica* L. in management of hyperbilirubinemia.

CONCLUSION

From the above study it can be concluded that aqueous extract of roots of *Mimosa pudica* possesses an anti-hyperbilirubinemic activity which may be attributed to the antioxidant activity and alkaloids indicating its usefulness in treatment of liver disorders.

FUTURE SCOPE

Fractionation and isolation of compound responsible for hepatoprotective activity can be further studied.

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Conflict of Interest. None.

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