

HEPATOTOXICITY OF ACETAMINOPHEN TREATED BY *DELONIX REGIA* & *AEGLE MARMELOES*Anil Kumar*, Divya Sharma², Dipayan Purakayastha¹, Pravesh Sharma¹, Mohit Khandelwal¹¹Department of Pharmacy, Vivekananda Global University, Jaipur - 303012, Rajasthan, India.²SKIP, Jaipur - 303012, Rajasthan, India.**Article Info:** Received 18 April 2020; Accepted 30 May 2020**DOI:** <https://doi.org/10.32553/jbpr.v9i3.758>**Corresponding author:** Anil Kumar**Conflict of interest statement:** No conflict of interest**Abstract**

Acetaminophen is commonly known as Paracetamol. It is an antipyretic drug, analgesic. It first introduced in 1955 and it cause liver toxicity, which is big issue for Patients now days also. In this work *Delnix Regia* and *Aegle Marmeloës* both the plant collected from local area Jaipur and authenticify by Department of Botany, Rajasthan University, Jaipur. The authentication number is RUBL 211610 and RUBL 211611 and extracted the leaves content by soxhlation process and compare them, which one is more helpful as hepatoprotective because acetaminophen drug cause toxicity in liver and for evaluation purposes different types of test like SGOT, SGPT, Total bilirubine, Total protein, molisch test, wagner test, Hager test, Benedicts test etc performed. In this work we observed that after all evaluation that Delnex Regia is more heptoprotective as compare to *Aegle Marmeloës*

Key word: Acetaminophen, *Delnix Regia*, *Aegle Marmeloës*, Hepatotoxicity**Introduction**

Acetaminophen (APAP) commonly known as paracetamol (IUPAC name: N-(4-hydroxyphenyl) ethanamide). This medicine is commonly used for antipyretic purpose, pain reliever. Since 1955 this drug is available as single dose or with other drug combination, this drug introduced to the market by the McNeil Laboratories in UK. First hepatic failure case due to Acetaminophen comes in UK in 1966.

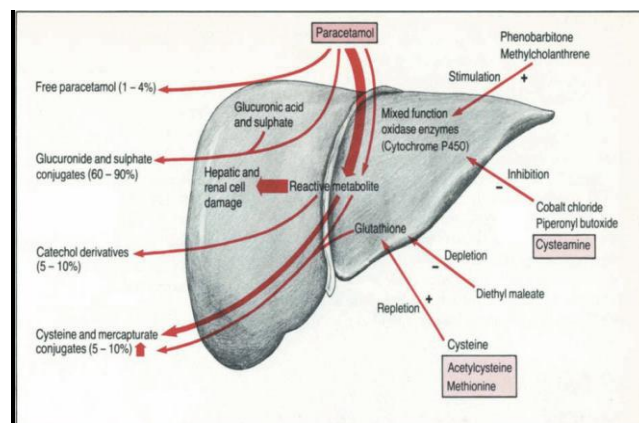
APAP absorbed rapidly in gastrointestinal tract with peak concentration achieved in 90 minutes, but if food is present in stomach then it may be delay the peak but not the absorption. APAP undergoes extensive hepatic metabolism. Half life of acetaminophen is around 2-2.5 hour. Acetaminophen is metabolized by glucuronidation and sulphate, almost 30% of acetaminophen excreted by urine from body.

Due to lack of anti-inflammatory component, Acetaminophen is out of the NSAIDs family in pharmacological category, it has been always discuss together with these drugs. The discussion on the mechanism of action of Acetaminophen should begin from the analysis of NSAIDs action. Acetaminophen cause liver toxicity in case of overdose of consumption. Every year lots of people died because of liver toxicity due to paracetamol toxicity.

Liver is an important organ which is center of the metabolism of nutrient and excretion of waste material

drugs in human body, and it also provide protection body from foreign material by detoxifying them. In case of hepatotoxicity, hepatotoxic chemical damage the liver cell by inducing the lipid peroxidation and oxidative damages. In modern days medicine there is hardly any drug available which can stimulate the liver function or regenerate the liver cells.

Natural medicine like medicinal plants is alternative and safe way for the treatment of the hepatotoxicity. Plan *Delonix Regia* also commonly known as Gulmohor, family leguminosea sub family fabaceae. This plant is cultivated in topical countries. This plant is useful in anti-depressant, anti-bacterial, anti-pyretic analgesic anti-rheumatic, in this work it used for hepatotoxicity due to acetaminophen poisoning.

**Fig-1.2:** Liver toxicity

Acetaminophen, a commonly used analgesic and considered as safe therapeutic doses but overdose of Acetaminophen causes severe hepatotoxicity and necrosis in human body. Acetaminophen induced liver toxicity in animals has been used as an experimental model to test the potential hepatoprotective activity by several investigators. The present study is aimed to evaluate the hepatoprotective activity of methanol extract of *Delonix regia* and *Angle Marmelos* leaf.

Materials and Methods

Collection and identification and authentication of plant material:

Leaves of selected plant were collected and authenticated by Department of Botany, Rajasthan University, Jaipur. The authentication number is RUBL 211610 and RUBL 211611. The leaves were shade dried and powdered coarsely in a blender. The powdered plant materials were stored in air-tight container.

Preparation of Ethanolic extracts of *Delonix Regia* and *Aegle marmelos* leaves leaves :

Plant materials were collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant material was then shade dried and pulverized in a hand mill followed by sieving (sieve no. 40) to obtain coarse powder.

About 190 gm of dry powder were extracted with ethanol (40-60°C) for 48 hr in soxhlet extractor. The ethanolic extracts were filtered with Whatman filter paper, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extracts were weighed to obtain 28.2g and yield calculated and stored in a cool place.

Table 1: Percentage yield of *Delonix regia* and *Aegle marmelos* leaves with ethanol.

PLANT USED	PARTUSED	METHOD	COLOUR AND CONSISTENCY	YEILD
<i>Delonix Regia</i>	Leaves	Soxhletextraction with ethanol	Dark brown semi solid	28.2 gm
<i>Aegle</i>	Leaves	Soxhletextraction with ethanol	Brown semi solid	26.3 gm
<i>Marmelos</i>			solid	gm

Test for carbohydrates

Molisch's test

200 mg of extracts dissolved separately in 5ml of water and filtered. 2 ml of the above sample solution is placed in a test tube. Two drops Molisch reagent is added. The solution is then poured slowly into a tube containing 2 ml of concentrated sulphuric acid and observed.

Fehling's test

1ml of Fehling's solution A and 1ml of Fehling's solution B added to 100mg of extracts separately. They were heated on a boiling water bath for 5 min and observed.

Benedict's test

To the 150 mg of each extracts, 2ml of Barfoed's reagent was added. Then the mixture was heated on a boiling water bath for 5 min, cooled and observed

Test for alkaloids

To 250 mg of each extracts, 10 ml of dilute HCl was added, mixed and filtered. To the filtrate the following reagents were added and tested.

Wagner's test

2 ml of Wagner's reagent was added to the above filtrate solution and observed.

Hager's test

To the 2 ml of above filtrate solution, 2 ml of picric acid was added and observed

Test for glycosides

The extract was tested for the presence of

- Saponin glycosides
- cardiac glycosides
- Anthraquinone glycosides

Test for saponin glycosides

Foam test

To 200 mg of each extracts, 15 ml of distilled water added, shake it well and observed.

Test for cardiac glycosides

Legal's test

To 50 mg of each extracts, 1 ml of pyridine, 1 ml of Sodium nitro prusside solution were added and observed.

Keller-kiliani test

To 50 mg of each extracts, 2 ml of glacial acetic acid, 1 ml FeCl₃ solution were added, eated and then cooled. This was transferred to a test tube containing 2ml conc. H₂SO₄ and observed

Test for anthraquinone glycosides

Borntreger's test

To 200 mg of each extracts, dil. H₂SO₄ added and boiled. It was filtered and cooled. To the cold filtrate, 3 ml of benzene added and mixed. The benzene layer separated and to it, ammonia (2 ml) was added and ammonical layer observed.

Test for flavanoids

Lead acetate test

To the 100 mg of each extracts than lead acetate (5 ml) was added and observed.

Results

Table 2: Phytochemical screening of chemicals in ethanolic extracts of different plants.

S. No	PHYTOCHEMICALS	DELONIX REGIA	AEGLE MARMELOS
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Carbohydrates	+	+
4	Proteins	+	+
5	Saponins	-	+
6	Tannins	-	+
7	Anthraquinones	+	+
8	Glycosides	+	+

(-) Indicates Absence

(+) Indicates Presence

Hepatotoxicity is clear that when Acetaminophen used to induce liver toxicity there is a substantial increase in enzyme activity of SGOT, SGPT, SALP and Serum Bilirubin. Any decrease in the activity of above enzymes would indicate reversed of induced liver toxicity. The results of serum biochemical parameters in pre-treatment of *Delonix Regia* and *Aegle marmelos* with respect to induction of Hepatotoxicity using Acetaminophen are shown in tables. Ethanolic extract of *Aegle marmelos* has reduced the elevated levels of SGOT, SGPT, SALP and Serum Bilirubin to lesser extent compared with ethanolic extract of *Delonix Regia*. The ethanolic extract of *Delonix regia* has reduced the increased SGOT levels from 126.00 IU/L to 52.80 IU/L, SGPT levels from 138.89 IU/L to 80.00 IU/L, SALP levels from 90.62 K.A. units to 39.24 K.A. units and serum bilirubin levels from 12.00 mg/dl to 5.90 mg/dl. The ethanolic extract of *Aegle marmelos* has reduced the increased SGOT levels from 126 IU/L to 62.00 IU/L, SGPT levels from 138.00 IU/L to 103 IU/L, SALP levels from 90.62 K.A. units to 46 K.A. units and Serum bilirubin levels from 12 mg/dl to 6.00 mg/dl. While the standard drug Silymarin has reduced increased SGOT levels from 126.00 IU/L to 43.44 IU/L, SGPT levels from 138.00 IU/L to 67.02 IU/L, SALP levels from 90.62 K.A. units to 33.00 K.A. units and Serum bilirubin levels from 12 mg/dl to 5.40 mg/dl. The enzymatic levels of SGOT, SGPT, SALP and serum bilirubin are indicated in the Table. The level of total protein depleted in the group treated with Acetaminophen (toxin control) and were significantly decreased ($P < 0.05$) from 6.90 g/dl to 5.14 g/dl when compared with the normal control group.

The groups that received the pre-treatment of *Delonix regia* and *Aegle marmelos* at dose levels of 400 mg/kg body weight significantly controlled the change in the biochemical parameters. The extracts at dose levels of 400 mg/kg exhibited significant increases ($P < 0.05$) in the serum total protein level as compared to toxin control group. The total protein and GSH levels from liver homogenate in plant extracts treated groups elevated, but total protein level was not significant. However, pretreatment with *Delonix Regia* and *Aegle marmelos* significantly recovered the paracetamol induced Hepatotoxicity in I high dose (400mg/kg) group ($P < 0.01$, $P < 0.05$ respectively). The catalase and GPx activity increased of both the extracts; *Delonix Regia* exhibited good activity ($P < 0.01$, $P < 0.05$ respectively). GPx activity of *Delonix Regia* group was similar to that of standard drug treated group.

Table 3: Effect of ethanolic extracts of *Delonix regia* and *Aegle marmelos* on SGPT and SGOT in Acetaminophen induced acute liver damage in rats.

GROUP PS	Treatment	SGPT (U/L)	SGOT (U/L)
GROUP P 1	Normal control (DMSO 5%)	44.66±1.006 **b	36.60±0.170 **b
GROUP P 2	Paracetamol control (500mg/kg)	138.80±0.67 0***a	126.00±0.065 5***a
GROUP P 3	Ethanolic extract of <i>Delonix Regia</i> (400mg/kg, p.o.) + Paracetamol (500mg/kg)	80.26±0.400 **b	52.80±0.801 **b
GROUP P 4	Ethanolic extract of <i>Aegle marmelos</i> (400mg/kg, p.o.) + Paracetamol (500mg/kg)	103.00±1.70 7**b	60.00±0.640 **b
GROUP P 5	Silymarin (25mg/kg oral) + Paracetamol (500mg/kg)	67.02±0.801 *b	43.44±0.520 b

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ and ns-non-significant (a-comparison with control group,) (b-comparison with Acetaminophen control group)

Table 4: Effect of ethanolic extracts of *Delonix regia* and *Aegle marmelos* on ALP and Total Bilirubin in Acetaminophen induced acute liver damage in rats.

GROUPS	Treatment	ALP (U/L)	Total Bilirubin (mg/dl)
GROUP 1	Normal control (DMSO 5%)	24.60±1.660**b	3.180±0.561**b
GROUP 2	Acetaminophen control (500mg/kg)	90.62±0.201**a	12.00±0.0546**a
GROUP 3	Ethanolic extract of <i>Delonix regia</i> (400mg/kg, p.o.) + Paracetamol (500mg/kg)	39.24±0.311**b	5.90±0.241**b
GROUP 4	Ethanolic extract of <i>Aegle marmelos</i> (400mg/kg, p.o.) + Acetaminophen (500mg/kg)	46.00±1.616**b	6.00±0.400**b
GROUP 5	Silymarin (25mg/kg oral) + Acetaminophen (500mg/kg)	33.00±0.900*b	5.44±0.500***b

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ and ns-non-significant (a-comparison with

control group,) (b-comparison with Acetaminophen control group).

Conclusion:

Acetaminophen has hepatotoxicity issue and in this investigation *Delonix Regia* and *Aegle marmelos* plant leaves taken and extract through the Soxhlet process and by different types of test. In this work we find out that *Delonix Regia* is more effective as Hepatoprotective compare to *Aegle Marmelos*.

Discussion

In the present study; ethanolic extracts of aerial parts of *Delonix Regia* and *Aegle Marmelos* leaves were extracted for the hepatoprotective activity using hepatotoxicity induced by Acetaminophen in rat model and find out the therapeutically better efficacious extract. The shade-dried powder of seeds extracted in a Soxhlet extractor by exhaustive extraction using ethanol as solvent and it gives 17 % yield of extract.

In the present study, leaves of *Delonix Regia* and *Aegle marmelos* were evaluate for its pharmacognostical, phytochemical and pharmacological aspects. Acute toxicity testing of both extracts were determine as per OECD guidelines and its ambenment time to time. LD50 of both test extracts were found to be 2000 mg/kg. The degree of hepatotoxicity development can be known by elevated levels of SGOT, SGPT, SALP and Serum Bilirubin enzymes which is attributed due to its covalent bonding N-acetyl-p benzoquinoneimine. Oxidation product of Acetaminophen to sulphhydryl group of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver, causes hepatotoxicity. Both extracts were extracted and phytochemical screening done for hepatoprotective activity in albino rats (wistar strains). Hepatotoxicity is clear that when paracetamol was used to induce liver toxicity there is a substantial increase in enzyme activity

of SGOT, SGPT, SALP and Serum Bilirubin. Any decrease in the activity of above enzymes would indicate reversed of induced liver toxicity.

References

1. Aashish Pandit, Tarun Sachdeva and Pallavi Bafna Drug-Induced Hepatotoxicity Journal of Applied Pharmaceutical Science 02 (05); 2012: 233-243.
2. Aly M. El-Sayed¹, Shahira M. Ezzat², Maha M. Salama³ and Amany A. Sleem. "Hepatoprotective and cytotoxic activities of *Delonix regia* flower extracts. Article in Pharmacognosy Journal · January 2011 Impact Factor: 1.26 · DOI: 10.5530/pj.2011.19.10.
3. Ali M. Indigenous traditional drugs: In Textbook of Pharmacognosy. 1st ed. Delhi: CBS Publishers and Distributors; 1994. pp. 312–3.
4. Biswadev B, Subhashree R, Somya G, Mahuya SG. "Hepatoprotective and immunomodulatory properties of *Tinosporacordifolia* in CCl₄ intoxicated mature albino rats". The journal of toxicological sciences 2002;27:139-46.
5. Bisset N. In: "Herbal Drugs and Pharmaceuticals. CRC Press": London; 1994. p. 121-3.
6. BT Kavitha, SD Shruthi, S Padmalatha Rai, YL Ramachandra, Phytochemical analysis and hepatoprotective properties of *Tinosporacordifolia* against carbon tetrachloride induced hepatic damage in rats" Journal of Basic and Clinical Pharmacy; 2011.
7. C. Girish, B.C. Koner, S. Jayanthi, K.R. Rao, B. Rajesh† & S.C. Pradhan. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian J Med Res 129, May 2009, pp 569-578.
8. Chaudhari N. B., Chittam K. P., Patil V. R., Hepatoprotective Activity of *Cassia fistula* Seeds against Paracetamol-Induced Hepatic Injury in rats, Arch Pharm Science & Research Vol 1 No 2, October 2009, 218-221
9. Chung RT, Stravitz RT, Fontana RJ, et al. Pathogenesis of liver injury in acute liver failure. Gastroenterology. 2012;143:e1-e7.
10. CPCSEA guidelines for laboratory animal facility. Committee for the purpose of control and supervision of experiments on animals. Indian Journal Pharmacol. 2003;35:257–74.
11. Dossing M, Sonne J. Drug induced hepatic disorders: Incidence, management and avoidance. Drug Safety. 1993;9:441–9.
12. Dixit N, Baboota S, Kohli K, Ahmad S, Ali J. Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. Indian J Pharmacol 2007;39:172-9
13. Extraction and extractives. In: Remington's Pharmaceutical sciences. 14th ed. Easton Pennsylvania: Mack Publishing Company; 1965. pp. 1578–93.