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RESEARCH ARTICLE

SCREENING OF HEPATOPROTECTIVE POTENTIAL OF ETHANOLIC AND AQUEOUS EXTRACT OF *TERMINALIA ARJUNA* BARK AGAINST PARACETAMOL/CCL4 INDUCED LIVER DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

Screening hepatoprotective potential of ethanolic and aqueous extract of Terminalia arjuna bark against paracetamol/ CCl4 induced liver damage in wistar albino rats. Hepatotoxicity was induced by paracetamol/CCl4 and the biochemical parameters such as serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and serum alkaline phosphatase (sALP), serum bilirubin (SB) and histopathological changes in liver were studied along with silymarin as standard hepatoprotective agents. The phytochemical investigation of the extracts showed presence of alkaloids, glycosides, steroids and flavonoids, saponin, tanins. Pre-treatment of the rats with ethanol and aqueous extract prior to paracetamol/CCl4 administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB almost comparable to the silymarin. The hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animals. From the results it can be concluded the Terminalia arjuna possesses hepatoprotective effect against paracetamol/CCl4 -induced liver damage in wistar albino rats.

***Keywords**: Terminalia arjuna, Hepatoprotective, Paracetamol, CCl4, Silymarin.*

INTRODUCTION

Arjuna (*Terminalia arjuna*) is a widespread medicinal plant used in ayurvedic system of medicine to care for various ailments and is one of the active ingredients in numerous polyherbal hepatoprotective formulations now used in India. Its stem, bark, leaves possesses glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effects whereas glycosides are cardiotonic, therefore making *Terminalia arjuna* distinctive amongst currently used medicinal plants. *Terminalia arjuna* leaves, (Flavonoids) antimicrobial and antifungal activity. In the present study we have addressed and provides an overview of extraction, phytochemical analysis and TLC (Thin Layar Chromatography) analysis of *Terminalia arjuna* bark. (Nema *et al.*, 2012).

Experiments conducted with the bark of arjuna have been shown to possess hypolipidemic, hypocholesterolemic, hypotensive, antidiabetic and anti-infilammatory activities (Dwivedi, 2007). In addition the thick, white to pinkish gray bark has been shown to posses anticancer, antiulcer, antimutagenic and wound healing activities (Warrier *et al.*, 1996).

Recently there has been a large volume of work aimed at scientific validation of the efficacy of herbal drugs used in the traditional medicine. Modern medicine does not have suitable answers for many conditions such as liver disorders asthma, cardiovascular disorder etc.

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Liver is the vital organ of metabolism and excretion. About 20 000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 250 000 new cases each year. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and lutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Hurkadale *et al*., 2012).

In CCl4 induced hepatotoxicity model, cellular dehydration, hydropic changes, fatty changes, fatty changes with spread hepatocellular necrosis. Fatty vacuole, dilation of blood vessels and moderate hyperchromasia in nuclei with degenerative changes were observed (Bhandarkar MR *et al.*, 2004; Akindele AJ *et al.*, 2010).

The survey of literature reveals that the *Terminalia arjuna* bark are found to be used in the traditional system of medicine as a liver tonic. However, hepatoprotective activity of *Terminalia arjuna* bark has not been scientifically investigated. Therefore, in the present study hepatoprotective effect of methanol and aqueous extracts of Terminalia *arjuna* bark have been evaluated against paracetamol/CCl4 induced liver damage in the male Wistar albino rats.

MATERIAL AND METHODS

Plant material
The bark of *Terminalia arjuna* were collected in month of January-Faburary from local area of sagar District after authentication by Dr. Ptadeep Tiwari, Department of Botany, Dr. H. S. Gour University (Herbarium no. Bot. / 1723.

Drugs and chemicals
Paracetamol was obtained from Bro-shell Remedies, Sagar, Carbon Tetrachloride (CCl4) from Renchem Pvt. Ltd.,Silymarin was obtained from Micro Lab, Baddi, India. Standard kit of serum glutamate pyruvate transaminase (sGPT), serum glutamate oxaloacetate transaminase (sGOT) and alkaline phosphatase (sALP) were obtained from Beacon Diagnostic Pvt. Ltd., Navsari. All other reagents used for the experiments were of high analytical grade.

Preparation of extracts
The fresh bark were cleaned, shade dried and then powdered. The dried coarse powder of the bark (300 g) was extracted.

Extraction methods
Successive solvent extraction 300 gm of shade dried stem bark powder of *Terminalia arjuna* (Roxb.) was extracted successively with various solvents in an increasing order of polarity viz. Petroleum ether (60-80^{0}C), Ethanol and Water. Each extract was concentrated to a reduced volume and allowed to dry. After drying the respective extracts were weighed and percentage extractive values were determined.

Extraction with Petroleum ether (defatting)
300 gm of weighed stem bark powder of *Terminalia arjuna* were packed in soxhlet apparatus and extracted with 600 ml of petroleum ether at 60 – 80^{0}C for 36 hrs. To ensure complete extraction few drops were collected from the thimble, which did not show the presence of any residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue (Shahriar et al., 2012).

Extraction with Ethanol
After defatting, the marc was dried in hot air oven at 50 ^{0}C, packed in soxhlet apparatus for further extracted with 95% ethanol at 50 -60 ^{0}C for 36 hrs. To ensure complete extraction few drops were collected from the thimble, which did not show the presence of any residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue (Morshed *et al*., 2011).

Extraction with Water
The marc was dried in hot air oven at 50 ^{0}C and macerated with water for 7 days with occasional shaking to get aqueous extract. The aqueous extract was concentrated by evaporating to dry resi (Manna *et al*., 2006).

Phytochemical test: The extracts were subjected to preliminary phytochemical investigations.

Suspensions of each extract 20% (ethanolic and aqueous) were prepared using Tween 20, Paracetamol suspension prepared by using Tween 80 and CCl4 - measured volume of CCL4 (1 part) was taken and added in (1 part) olive oil to get fine solution (1:1 in olive oil). and subjected for hepatoprotective activity against paracetamol/CCl4-induced liver damage.

EXPERIMENTAL MODELS

Healthy wistar albino rats of either sex, weighing 150-200 gms, were selected for study. The animals were acclimatized for seven days under laboratory conditions 25 ±2 ^{0}C 12h light and dark place. The animals were fed with commercially available rat pelleted diet (Amrut laboratories Pranava Agro Industries Ltd. Sangli). Water was allowed *ad libitum*.

EXPERIMENT

Albino rats (Wistar strain) of either sex (150-200) were used for this study. The animals were housed in the animal house of Scan Research Laboratory, Bhopal at a temperature of 24± 2 an^{0}C d at humidity of 50%, maintained on 12hr light/dark cycle and provided with food, water and libitum. The experimentation was conducted in accordance with the ethical rules on animal experimentation, approved by Animal Ethical Committee (Ethical Committee No. SIPS/2013/36).

Carbon tetrachloride induced hepatotoxicity model (Bhandarkar MR *et al.*, 2004; Akindele AJ *et al.*, 2010)

Forty-two rats of either both sexes were distributed into siven groups of six animals each.

Group-I (Normal control): received 20% Tween-20 (10 ml/kg, b.w, p.o.)

Group-II (Toxicant control): CCl_4 (1:1, 0.7 ml/kg, i.p. in olive oil).

Group-III (Standard): Silymarin (100mg/kg, b.w.,p.o.) + CCl_4 (1:1, 0.7 ml/kg, b.w., i.p. in olive oil)

Group-IV (Treated): Aqueous ***e***xtract (200mg/kg, b.w., p.o. in Tween-20) + CCl_4 (1:1, 0.7 ml/kg, b.w., i.p. in olive oil)

Group-V (Treated): Aqueous ***e***xtract (400mg/kg, b.w., p.o. in Tween-20) + CCl_4 (1:1, 0.7 ml/kg, b.w., i.p. in olive oil)

Group-VI (Treated): Ethanolic ***e***xtract (200mg/kg, b.w., p.o. in Tween-20) + CCl_4 (1:1, 0.7 ml/kg, b.w., i.p. in olive oil)

Group-VII (Treated): Ethanolic ***e***xtract (400mg/kg, p.o. in Tween-20) + CCl_4 (1:1, 0.7 ml/kg, i.p. in olive oil)

CCl_4 was given on alternate days for a period of 7 days (1st, 3rd and 5th day). Control, test and standard agents were administered for 7 successive days. On 8th days blood sample was collected by retro orbital puncturing method. Serum was separated by centrifuge it at 3000rpm at -20^{0}C or 15min and analyzed for SGOT, SGPT, AKP, TB using Erba Chem-7 using standard trasgesia kit.

Paracetamol–induced hepatotoxicity in rats (Wadekar RR *et al.*, 2008)

The animals were divided into siven groups, containing 6 rats in each group. Initial body weight was recorded.

Group I (***Normal control):*** received Tween-80 (1ml/kg, b.w., 1.5%) daily for 7 days.

Group II (**Toxicant):** Paracetamol (2gm/kg, b.w., p.o.) in Tween-80

Group III (**Standard):** Silymarin (100mg/kg, b.w., p.o.) + Paracetamol (2gm/kg, b.w., p.o.)

Group IV (**Treatment group):** Aqueous **e**xtract (200 mg/kg, b.w., p.o.)+ Papracetamol (2gm/kg, b.w., p.o.)

Group V (**Treatment group):** Aqueous **e**xtract (400 mg/kg, b.w., p.o.)+ Papracetamol (2gm/kg, p.o.)

Group VI (**Treatment group):** Ethanolic **e**xtract (200 mg/kg, b.w., p.o.)+ Papracetamol (2gm/kg, p.o.)

Group VII (**Treatment group):** Ethanolic **e**xtract (400 mg/kg, b.w., p.o.)+ Papracetamol (2gm/kg, p.o.)

Paracetamol was given once on 6th day only (once) while control, test and standard agents were administered daily for 7 successive days. On 8th days blood sample was collected by retro orbital puncturing method. Serum was separated by centrifuge it at 3000rpm at -20^{0}C or 15min and analyzed for SGOT,SGPT,AKP,TB using Erba Chem-7 using standard trasgesia kit.

ENZYME ASSAYS

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed in serum using standard kits from Merck using colorimetric method7, 8, 9,10. The results were expressed as units/litre (U/L).

PROTEIN ESTIMATION

The level of total protein was estimated in serum of experimental animals by biuret method11. Standard kit was obtained from Span diagnostics

STATISTICAL ANALYSIS

The results were expressed as mean SEM and percentages protection where appropriate. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett' t-test, using graph pad prism5.0. P values >0.05 were considered significant.

*Indicates P>0.05, **indicates P>0.001 and ***indicates P>0.0001

Effect of extracts of *Terminalia arjuna* bark on serum parameters of CCl_4 treated rats

Groups	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB (Mg/l)
Group-I Normal	41.47±3.72***	54.14±4.68***	64.22± 4.49***	0.94±0.06***
Group-II CCl_4 Toxicant	142.8±3.79	127.6±3.78	164.7±7.03	3.37±0.08
Group-III AE(200mg/kg) + CCl_4	137.2±6.50^{ns}	118.7±3.94^{ns}	154.6±5.89^{ns}	3.14±0.07^{ns}
Group-IV AE(400mg/kg)+ CCl_4	118.2±7.63*	107.34±3.2**	141.3±7.39*	2.95±0.06**
Group-V, ETOH (200mg/kg)+ CCl_4	95.24±5.04***	110.36±2.77*	134.7±5.22**	3.06±0.08*
Group-VI ETOH (400mg/kg)+ CCl_4	76.91±3.45***	98.97±2.56***	102.4±4.18***	2.01±0.06***
Group-VII Silymarin(100mg/kg) + CCl4	61.9±4.57***	75.81±4.59***	89.19±4.12***	1.75±0.05***

Values are means ± S.E.M, n=6. Compared to toxicant (one way ANOVA followed by Dunnett t-test). P<0.05 considerd as ns. *indicates P>0.05, **indicates P>0.001and ***indicates P>

Effects of extracts of *Terminalia arjuna* bark and standard compounds of *Terminalia arjuna* on serum parameters of Paracetamol treated rats

Groups	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB (Mg/dl)
Group-I Normal	38.04±2.21***	47.47±4.13***	70.94±2.77***	0.88±0.06***
Group-II Paracetamol (Toxicant)	114.4±4.34	119.3±4.31	173.1±4.49	2.78±0.18
Group-III AE(200mg/kg)+ Paracetamol	104.1±3.64^{ns}	112.3±3.81^{ns}	161.3±4.48^{ns}	2.40±0.12^{ns}
Group-IV AE(400mg/kg)+ Paracetamol	97.35±5.36*	107.3±5.31^{ns}	151.2±3.92**	2.17±0.13*
Group-V, ETOH (200mg/kg)+ Paracetamol	95.21±3.99*	96.6±3.97**	126.4±3.15***	2.02±0.06**
Group-VI ETOH (400mg/kg)+ Paracetamol	62.02±6.59***	79.1±3.01***	97.02±5.04***	1.58±0.14***
Group-VII Silymarin(100mg/kg) + Paracetamol	48.57±2.76***	65.77±2.6***	83.72±4.10***	1.45±0.13***

Values are means ± S.E.M, n=6. Compared to toxicant (one way ANOVA followed by Dunnett t-test). P<0.05 considerd as ns. *indicates P>0.05, **indicates P>0.001and ***indicates P>0.0001

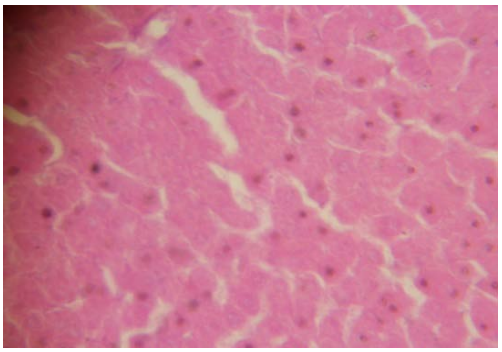
HISTOPATHOLOGICAL STUDIES

One animal from the each group was utilized for this purpose. The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin fixed liver samples were stained with haematoxylin-eosin for photomicroscopic observations of the liver histopathological architecture.

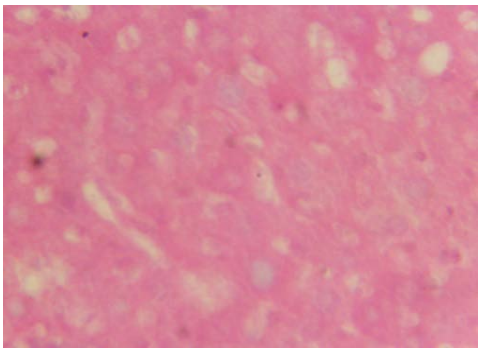
RESULTS

Preliminary phytochemical investigation revealed the presence of alkaloids, glycosides, triterpenoids, phenolic compounds, tannins, steroids and flavonoids in ethanolic extract and glycosides, triterpenoids, phenolic compounds, tannins, steroids and flavonoids in aqueous extract.

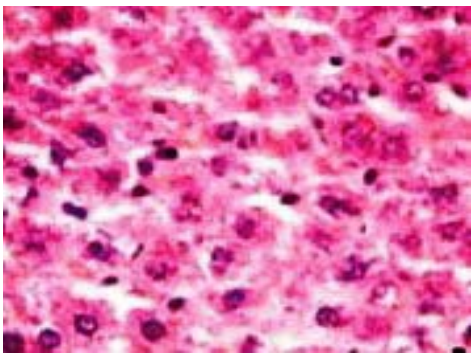
Administration of paracetamol (2gm/kg, b.w., i.p.)/CCl4 (0.7ml/kg, b.w.) induced a marked increase in the serum hepatic enzyme levels, sGOT, sGPT, sALP and SB as compared to normal controls indicating liver damage (centrilobular necrosis). Pre-treatment of the rats with ethanol and aqueous extract prior to paracetamol/CCl4 administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB almost comparable to the silymarin.



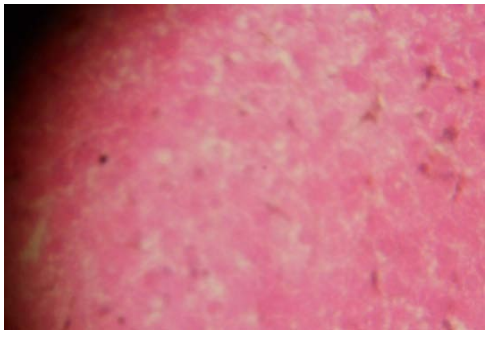
(A).Normal control



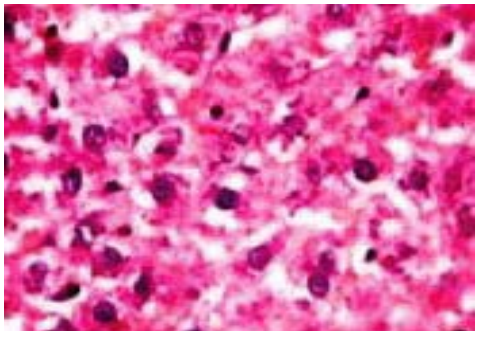
(B). Negative control (PCM)



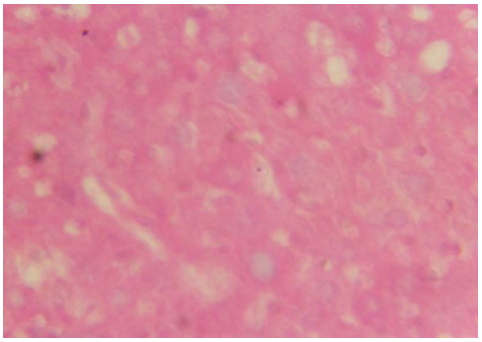
(C). Negative control (CCl4)



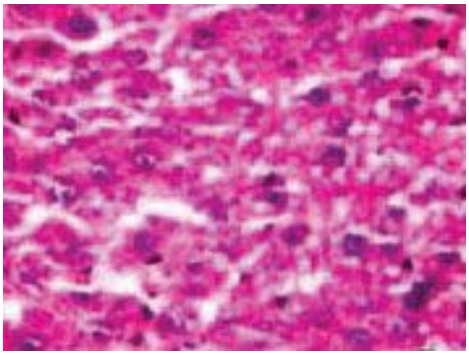
(D). Silymarin treated + (PCM)



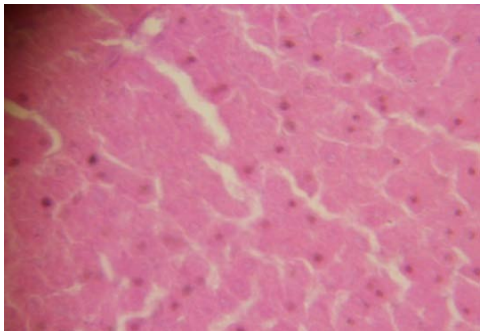
(E). Silymarin treated + (CCl4)



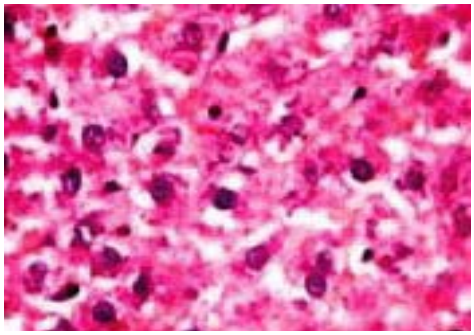
(F). Aqueous extract 400mg/kg + (PCM)



(G). Aqueous extract 400mg/kg + (CCl4)



(H). Ethanol extract 400mg/kg + (PCM)



(I). Ethanolic extract 400mg/kg + (CCl4)

The hepatoprotective effect of *Terminalia arjuna* bark was confirmed by histopathological examination of the liver tissue of control and treated animals.

The liver sections of normal control rats (Photograph A) showed normal architecture of liver. Hepatic cells were seen with preserved cytoplasm, prominent nucleus and central vein and arranged in the form of hepatic chords. Sinusoids were also and normal. Hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein were observed.

The liver of rats treated with Paracetamol (Photograph B) showed centrilobular necrosis characterized by degenerated nuclei, cell vacuolation and karyolysis. Cell lyses was visible around the interlobular vein. Necrosis of hepatocytes was obserbed in the centrilobular region and these changes were also obserbed in areas other than the centrilobular region. The normal architecture of liver was completely lost. High degree of damage is characterized by vacuolization, degenerated nuclei and fatty changes.

In rats treated with CCl4 (Photograph C), the normal architecture of liver was completely lost. High degree of damage is charactertzed by cell vacualization, degenerated nuclei and fatty changes. Necrosis of hepatocytes was observed in the centrilobular region and these changes were also observed in areas other than the centrilobular region. Wide spaces were seen at some sinusoids. Nacrosis of liver, ballooning, degeneration, inflammation of lymphocytes, damage of parenchymal cells, steatosis, hydropic degeneration of liver tissue. Prominent damage of central lobular region appeared in the liver. Cellular dehydration, hydropic changes, fatty changes, fatty changes with spread hepatocellular necrosis. Fatty vacuole, dilation of blood vessels and moderate hyperchromasia in nuclei with degenerative changes were observed.

The liver of rats treated with Silymarin and intoxicated with Paracetamol/CCl4 (Photograph D-E), there is a little damage to the centrilobular vein which causes the spread of blood in sinusoids in vicinity. Because of CCl_4 few cells around the centrilobular vein are damaged. At a dose of 100 mg/kg, b.w., silymarin showed almost normal architecture of liver. the hepatocytes were arranged redially in the form of hepatic chords. The cells are normal with their cytoplasm. Sinusoids were also clear and normal which shows strong hepatoprotection and recovery of Silymarin. Very little necrosis or degeneration. Normal hepatocytes, dilation of blood vessel with perivescular edema were observed.

The liver section of rats treated with aqueous extract at a dose of 400 mg/kg, b.w. (Photograph F-G) showed that the nuclei were not very clear as in normal hepatocytes but as compared to the hepatotoxins group, the number of hepatocytes with normal nucleus were much more. Pyknotic nucleus and vacuolation were observed to be low as compared to toxin group. There seems to be an appreciable recovery. Restored the histopathological abnormality induced by Paracetamol/CCl_4.

The effect of mixture of combination is more or less like Silymarin and Paracetamol/CCl_4 treated group. There is a little more damage in CLV (centrilobular vein), there by more area of the globule is seen filled with blood. Interestingly more nucleoli with smaller cells and white blood cells are present around the vein which can be clear indication of first regeneration of hepatocytes cells.

The histopathological pattern of liver of rats treated with ethanolic extract of *Terminalia arjuna* at a dose of 400mg/kg, b.w. (Photograph H-I) showed liver damage which is lesser comparable with toxin group but extent of liver damage is less than toxin group, which can be characterized by fewer necrosis zones and less spaces at the sinusoids than toxin group. Lesser hepatocellular damage except areas of focal degeneration and sinusoidal dilation, slight dilation in central vein were observed.

4. DISCUSSION

In the present study aqueous and ethanolic extracts of the *Terminalia arjuna* bark were tested for their hepatoprotective activity in albino wistar rats against CCl_4 and Paracetamol induced hepatotoxicity models. The degree of protection was measured using biochemical parameters like SGOT, SGPT, SALP Total Bilirubin and histopathological study.

The liver damage produced by CCl_4 and Paracetamol is characterized by elevated levels of SGOT, SGPT, SALP, Total Bilirubin and decreasing level of total protein level and damage to the normal architecture of liver.

CCl_4 hepatotoxicity caused gross necrosis of centribular hepatocytes characterized by nuclear pyknosis, space formation at sinusoids and vacuolated cells. In the liver of rats treated with ethanolic extract of *Terminalia arjuna*, it was revealed that the nuclei were not very clear as in normal hepatocytes but as compared to hepatotoxins group, the number of hepatocytes with normal nucleus were much more. Pyknotic nucleus and vaculation were observed to be low as compared to toxin group. There seems to be an appreciable recovery of liver functioning. The extract restored the histopathological abnormality induced by CCl_4.

The recovery due to aqueous extract of *Terminalia arjuna* from liver damage induced by CCl_4 was more than that of CCl_4 induced liver damage. Ethanolic extract of *Terminalia arjuna* at a dose of 400mg/kg, b.w., or Silymarin 100mg/kg, b.w. showed a significant recovery from CCl_4 – induced liver damage–as evidenced from normal hepatocytes with well defined nuclei. Vacuolization and fatty degeneration and fatty damage were remarkably prevented by the treatment with ethanolic extract of *Terminalia arjuna* and Silymarin. Interestingly more nuclei with smaller cells and white blood cells are present around the vein which is a clear indication of first regeneration of hepatocytes cells.

Results of biochemical estimations and histopathological study demonstrate, that ethanolic extract of *Terminalia arjuna* is effective against hepatotoxicity induced by CCl_4 and Paracetamol.

Ethanolic extract of *Terminalia arjuna* is found to be more effective than its aqueous extract in diminishing the liver damage and maintain the cellular integrity. The protective effect of ethanolic extract of *Terminalia arjuna* is almost comparable to that of Silymarin.

The hepatoprotective effect of ethanolic extract of *Terminalia arjuna* might be due to presence of active constituents include alkaloids, glycosides, tannins, cardenolide, triterpenoid saponins(arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone,luteolin), gallic acid, ellagic acid, oligomericproanthocyanidins (OPCs), calcium, magnesium, zinc, and copper.

REFERENCES

1. Abidemi JA*, Kenneth OE, Chidozie CA, Olufunmilayo OA. Hepatoprotective and *in vivo* antioxidant effects of *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae). *Journal of Ethnopharmacology* 2010; (129): 46–52.

2. Dwivedi s. *Terminalia arjuna* Wt. And Arn. a useful drug for cardiovascular disorders. *J. Ethnopharmacol.* 2007; 2(2): 114-116.

3. Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD, Khedkar AS. Hepatoprotective activity of *Amorphophallus paeonifolius* tubers against Paracetamol induced liver damage in rats. *Asian Pacific Journal of Tropical Biomedicine* 2012; (s2): 38-42.

4. Manna P, Sinha M, Sil PC. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *Bio Med Central Journal*. 2006; 6: 33.

5. Morshed MA, Uddin A, Rahman A, Hassan T. In-vitro antimicrobial and cytotoxicity screening of *Terminalia arjuna* ethanolic extract. *International Journal of Biosciences* 2011; 1(2):31-38.

6. Nema R., Jain P, Khare S, Pradhan A, Gupta A. Evalution of antimicrobial and antifungal activity of *Terminalia arjuna* leaves. *Research Journal of Medicine and Clinical Sciences* 2012; p. 63-65.

7. Shahriar M, Akhter S, Hossain I. Evaluation of in-vitro antioxidant activity of bark extracts of *Terminalia arjuna. Journal of Medicinal Plants Research* 2012; 6(39):5286-98.

8. Wadekar RR, Supale RS, Tiwari KM, Patil KS, Jalapure SS. Screening of root of *Baliospermum montanum* for hepatoprotective activity against Paracetamol induced liver damage in albino rats. *International Journal of green pharmacy* 2008; 2(4): 220-223.

9. Warrier PK, Nambiar VPK, and Ramankutty C. *IndianMedicinal Plants -A Compandium of 500 species*. 1996; 1(5): 253-257.

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