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ORIGINAL ARTICLE

Antioxidant Effect of *Terminalia arjuna* Extract Against Acetaminophen-Induced Hepatotoxicity via the Regulation of Cytochrome P450 2E1, Phosphatidylinositol-3-Kinase/Protein Kinase B

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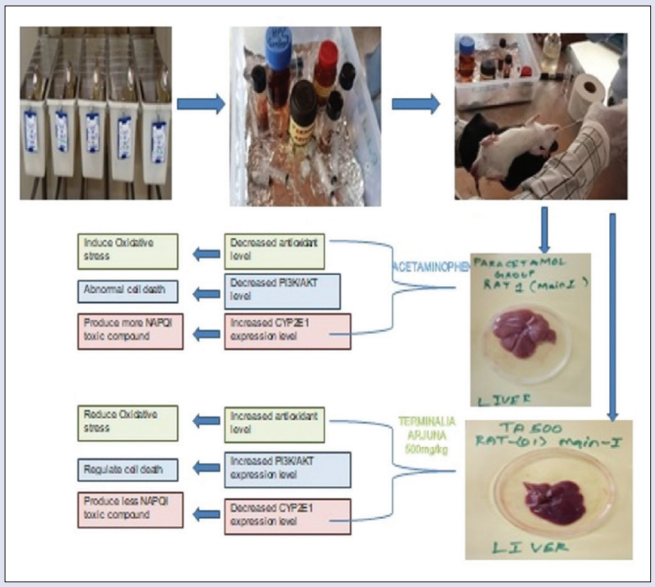
ABSTRACT

Aim: The present study explored the therapeutic in detail antioxidants and effect of aqueous *Terminalia arjuna* (TA) bark extract against acetaminophen (APAP) induced hepatotoxicity through studies on serum marker enzymes, phosphatidylinositol3kinase/protein kinase B (PI3K/AKT) pathway, CYP2E1 evaluations. Biochemical, antioxidant, cytochrome P450 2E1 (CYP2E1) enzyme, and PI3K/AKT cell signal enzymes were observed with the appropriate methods of study. **Materials and Methods:** The animals were divided into five groups (each having six animals): control group, Acetaminophen (APAP) toxic group, N-acetylcysteine (NAC) group, TA 250 mg/kg group, and TA 500 mg/kg group. APAP toxic dose of 750 mg/kg body weight was administered along with 0.5% of hydroxypropyl cellulose (vehicle) 24 h before sacrificing the animal. **Results:** The biochemical, antioxidant, Histopathological, CYP2E1 enzyme, PI3K, AKT protein expression analysis were shown increased antioxidant level, increased PI3K/AKT level, decreased liver function marker level and decreased CYP2E1 level in TA500 mg group compared with APAP toxic group ($P < 0.01$). The findings suggest that TA (500mg/kg) drug reduced Acetaminophen toxicity via antioxidant and molecular mechanisms. **Conclusion:** The present study concluded that TA 500 mg/kg high dose is more effective to restore the liver tissue through APAPinduced hepatotoxicity in Wistar albino rats.

Key words: Antiapoptotic, caspase, DNA damage, N-Acetyl-P-benzoquinone imine, oxidative stress

SUMMARY

- The aqueous extract of *Terminalia arjuna* (TA) 500 mg/kg reduced significantly the levels of liver enzyme markers such as alanine transaminase, aspartate transaminase, Alkaline phosphatase, and gamma glutamyl transferase (GGT) and increased the antioxidant levels of superoxide dismutase, catalase, glutathione, glutathione peroxidase, GGT, and glutathione-S-transferase which was reduced by acetaminophen (APAP)-induced oxidative stress
- Aqueous extract of TA 500 mg/kg significantly decreases the N-Acetyl-P-Benzoquinone Imine production by reducing cytochrome P450 2E1 expression which was increased by APAP-induced acute liver injury
- Aqueous extract of TA 500 mg/kg significantly increases the phosphatidylinositol-3-kinase/protein kinase B enzyme which was inhibited by APAP-induced abnormal cell death.



Abbreviations used: APAP: Acetaminophen; NAC: N-Acetylcysteine; PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; PIP_3: Phosphatidylinositol (3,4,5)-trisphosphate; PDK1: 3-phosphoinositide-dependent protein kinase-1; PDK2: Phosphoinositide-dependent kinase 2; BCL2: B-cell lymphoma 2; BAX: BCL2 Associated X; CYP2E1: Cytochrome P450 2E1; NAPQI: N-Acetyl-P-Benzoquinone Imine; PBS: Phosphate buffered saline; HRP: Horseradish peroxidase.

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INTRODUCTION

Acetaminophen (APAP) is a largely preferred and easily available drug for fever and also used for pain relief. Prior research substantiates the belief that overdose of APAP has been proved to damage the liver. Overdose of APAP will damage the liver by the formation of N-Acetyl-P-benzoquinone imine (NAPQI) toxic substance and decrease the production of glutathione (GSH). Several studies agree that NAC is the main precursor of GSH and popularly being used against APAP toxic dose. However, it does not successfully rectify the damaged liver tissues. Therefore, many of the cases have gone in liver transplantation. The phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway is essential for control cell mechanism.[1] Cellular stimuli regulate this PI3K/AKT pathway.[2] Oxidatative stresses induced by APAP alter the PI3K/AKT signaling pathway and cause the uncontrolled apoptosis leading to severe liver cell death. Reactive oxygen species (ROS)/reactive nitrogen species have also been reported to cause various complications such as cardiac problems, cancer, arteriosclerosis, skin irritations, and inflammatory disorders.[3] Scientists are searching for better hepatoprotective components from herbal plants against drug induced organ toxicity, which can be found by phytochemical and antioxidant analysis. Many Indian medicinal plants are antiradical and cleavage protectors.[4] In *Terminalia arjuna* (TA) bark aqueous extract, major phytoconstituents are flavonoids (catechin, gallocatechin, epigallocatechin, quercetin, kaempferol, and luteolin), tannins (gallic acid, ellagic acid, casuarinin), triterpenoid (arjunic acid, arjunolic acid, terminolic acid), glycopyranosides, steroid glycoside, calcium, magnesium, zinc, and copper.[5-11] The TA has effective components and act as cardiotonic,[12] antioxidant,[13] antidiabetic,[14] anti-inflammation,[15] antiasthmatic,[16] liver disease,[17] antitumors,[18] and antihyperlipidimic.[19] Phenolic-rich component of TA has the ability to neutralize the accumulated free radicals.[20] The present study explored in detail the effect of TA on hepatotoxicity.

MATERIALS AND METHODS

Chemicals

Acetaminophen (99% purity), hydroxypropyl cellulose (HPC), BSA, DTNB, GSH, TBA, Epinephrine, and Trisodium citrate were obtained from Sigma Aldrich Chemical Company, USA. Nacetylcysteine (NAC) was purchased from Samarth life sciences Pvt Ltd. CDNB, NAD+, NADH, NADP+, Folin's Ciocalteau reagent were obtained from Sisco Research Laboratories, India. EDTA, ANSA, DNPH, TEMED were obtained from British Drug House Pvt. Ltd. Glaxo Division, Mumbai, India. Acids, bases, solvents, and salts used in the study were of analytical grade (AR) and were obtained from Glaxo Laboratories, SRL, Mumbai, India and Anilax chemicals, USA. Wagner's reagent, Salkowski reagent, lead acetate and ferric chloride, dimethyl sulfoxide were purchased from Randox chemicals Pvt Ltd. The GSH, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) standards were purchased from Sigma Aldrich, USA. The monoclonal antibody for P13K and AKT-308 protein were purchased from Thermo Fischer Scientific Company.

Instruments

- Soxhlet extractor - CG1374 Chemglass
- Vacuum evaporator - Royal scientific (Mini)
- Hot plate - New Lab Instruments
- Incubator - Hitech Instruments
- Western blotting - GE Amershan
- UV-V is Spectrophotometer - Shimadzu UV1800.

Methodology for aqueous extract preparation

The TA bark powder was purchased from Herbal Care and Cure Centre, Mylapore, Chennai - 600 004. 1 kg of the weighed plant bark powder was mixed with 2 L of hot boiled water. The sterile conical flask containing 250 ml mixture was plugged with sterile cotton and kept in shaking incubator with the 200 rpm for 24 h. The aqueous extract was filtered with muslin cloths repeatedly for three times. The filtrated extracts were dried under reduced pressure at 40°C on a rotary evaporator and stored in a refrigerator at 4°C. The percentage of yield is 17.9% [Figure 1].

Animals

Wistar Albino Male rats (180–280 g) were used in this study. The animals were housed in ten polypropylene cages (3 per cage) containing sterilized paddy husk as bedding material with pellet diet and water *ad libitum* and maintained in the Centre for Laboratory and Animal Research (CLAR, Saveetha University) under standard conditions. The bedding material of the cages was changed every day. Animals were maintained as per the approved guidelines of the "Committee for Control and Supervision of Experiments on Animals" (India), and the protocol was approved by the Institutional Animal Ethics Committee of Saveetha medical college (SU/CLAR/RD/005/2018 Dated December 10, 2018).

Experimental design

Acute toxicity study

The animals were divided into five groups (each of six animals):

- Group 1 – normal control and received 0.5% of HPC daily for 14 days
- Group 2 – APAP toxic group and received 0.5% of HPC daily for 14 days
- Group 3 – Administered with NAC (200 mg/kg) once daily for 14 days
- Group 4 – Administered with TA (250 mg/kg) once daily for 14 days
- Group 5 – Administered with TA (500 mg/kg) once daily for 14 days.

Groups 2, 3, 4, and 5 were administered with APAP (750 mg/kg) as a single dose on day 14th after 1 h of treatment drug. All the test drugs and APAP were administered orally. After 24 h of APAP feeding, the animals were anesthetized with isoflurane; blood was collected by retro-orbital puncture into heparinized vacutainers. The blood was centrifuged at 3500 rpm for 10 min. Serum was collected for liver biochemical test

Figure 1: *Terminalia arjuna* bark extract

and liver tissue was removed, weighed, stored at −80°C for further antioxidant and histological analysis.

Serum biochemical estimation

The activity of aspartate transaminase (AST) was assayed by the method of Mohur *et al.* 1975. The activity of alanine transaminase (ALT) was assayed by the method of Mohur *et al.* 1975. Alkaline phosphatize (ALP) was assayed by the method of King *et al.* 1965. Gamma glutamyl transferase (GGT) was estimated by the method of Indirani and Hill *et al.* 1977.[21]

Antioxidant level estimation

GSH was estimated as the total non-protein sulfhydryl groups by the method described by Moron *et al.* 1979.[22] MDA was estimated by Högberg *et al.* 1974.[23] CAT activity was assayed by the method of Sinha 1972.[24] SOD activity in tissue treated homogenate was assayed by the methodology of Misra and Fridovich.[25]

Hematoxylin and eosin analysis

The isolated fresh hepatic tissues were immediately stored in a buffered formalin (10%) for 24–48 h. The sequence methods of fixation, paraffin-embedding bath, tissue blocking, and tissue sectioning by rotary microtome were done for tissue processing. The mounted tissue slides were stained by hematoxylin and eosin stains. Alcohol, xylene were used in this procedure. The prepared slides were examined under the microscope to observe the pathological changes in the hepatic tissues.

Cytochrome P450 2E1 immunohistochemical staining procedure

Fresh liver tissue was stored into a liquid nitrogen container immediately after removal. Alcohol and xylene were used for tissue processing and staining tissue slides. Liver tissues were subjected to tissue processing, and tissue blocks were made. The tissue block was then sectioned in a microtome and duly transferred to a slide for staining. The mounted Slides were heated in citrate buffer for antigen retrieval and blocked endogenous peroxidase with 3% H_2O_2. Sections were incubated with rabbit polyclonal anti-CYP2E1 antibody (1:500) and biotin conjugated secondary antibody (1:2,000) in PBS for 20 min. Counterstaining with hematoxylin and mounting with glycerol were done before examining under a microscope.

Assay of Proteinkinase B (308) protein expression by western blotting

Protein samples (30 ug protein) were separated and transferred to polyvinylidene fluoride membranes with 10% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The membranes were washed with TBST buffer (10 mMTris-base, 0.15 M NaCl and 0.05% (w/v) Tween 20) for 10 min. Then, membrane was blocked by using 5% milk casein dissolved in phosphate buffered saline (PBS) to block nonspecific binding sites at room temperature for 1 h, followed by incubation with polyclonal anti-p-AKT 308 antibody and then incubated with horseradish peroxidase-labeled secondary antibody for 5 h at 37°C. The signal was read by 3,3diaminobenzidine tetrahydrochloride (DAB) using horseradish peroxidase (HRP) substrate the blots.

Assay of phosphatidylinositol-3-kinase protein expression by western blotting

Proteins (80 µg/lane) were separated and transferred to polyvinylidene fluoride membranes with 7.5%–12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, membrane blocked by 5% milk casein dissolved in PBS to block nonspecific binding sites at room temperature for 1 h, followed by incubation with polyclonal anti-P13K (1:1000) overnight at 4°C. On the 2nd day, the membranes were washed with PBS containing 0.1% Tween three times (10 min/wash) and then incubated with goat anti-mouse (1:1000) secondary antibodies for 1 h at 37°C. The signal was read by DAB as the HRP substrate and determined by scanning the blots.

Assay of P-AKT (308) gene expression by polymerase chain reaction

Total RNA was extracted from the homogenized liver tissue. Manufacturer's instructions proceeded for one step of reverse transcription polymerase chain reaction (RT-PCR) analysis. RT-PCR reactions contained total RNA with primer, RT-Taq Mix, and 25 µl of the reaction mix. A thermal cycle of cDNA synthesis, denaturation, and PCR amplification cycles was done. Electrophoresed agarose gel medium was used to analyze the prepared RT–PCR products and visualized by ethidium bromide. The used primer sequences are mentioned in Table 1.

Agarose gel electrophoresis

Gel tray contained 1.5% agarose, 1X TAE buffer, PCR components, and marker ladder. The PCR product sample ran at 50 V for 90 min and bands visualized. Gel proanalyzer software displayed the band intensity in digital images. The relative amount of each mRNA was normalized to the reference gene, β-actin mRNA.

Statistical analysis

Results for all the parameters analyzed were expressed as the mean ± standard error of the mean. The statistical analysis of data was conducted with Statistical Package for Social Sciences software version 24 (IBM Corp, Armonk, NY). Comparisons among groups that were more than two were performed using one-way analysis of variance followed by Dunnett's *t*-test.

RESULTS

Terminalia arjuna effects on liver biochemical markers

Aspartate transaminase and alanine transaminase level estimation

The levels of AST and ALT were significantly reduced in TA 500 mg/kg group as compared to the APAP group [Table 2]. In the high dose of TA500 mg/kg group, levels were significantly decreased than NAC standard drug group. However, the NAC group was shown a decreased AST level than low dose TA 250 mg/kg group. The result of this test suggests that high dose of TA extract has more effect than low dose.

Table 1: AKT and β-actin primer sequences

Genes	Forward primer	Reverse primer
Akt	5′-GCTGGACGATAGCTTGGA-3′	5′-GATGACAGATAGCTGGTG-3′
β-actin	5′-GTGGGGCGCCCCAGGCACCA-3′	5′-CTCCTTAAGTCACGCACGATTTC-3′

Alkaline phosphatize and gamma glutamyl transferase level estimation

TA 500 mg/kg group was shown significant decreased level of ALP, GGT than APAP and NAC groups [Table 2]. In the GGT test, NAC group showed significantly decreased level than TA250 mg/kg group. This result also proved that a high dose of TA (500 mg/kg) has more effect than a low dose of TA (250 mg/kg).

Terminalia arjuna effect on antioxidants

Effect of glutathione, superoxide dismutase, catalyze estimation

The antioxidant levels were significantly elevated in TA 500 mg/kg (Group V) compared to other groups [Table 3]. NAC group was significantly elevated the antioxidant levels than TA 250 mg/kg group. TA 500 mg/kg group helps to detoxify the more toxic compound of NAPQI produced by APAP through elevated GSH.

Effect of lipid peroxidize on experimental groups

The decreased levels of MDA were seen in the TA high dose group when compared with other treatment groups [Table 3]. The reduced level of MDA can prevent centrilobular necrosis in liver tissue.

Terminalia arjuna effect on histopathological changes in liver tissues

The recovery of hepatic cords with normal nuclei was appearing in TA500 mg/kg group liver tissue [Figure 2]. In Figure 2a, normal cords of hepatocytes which are bounded by an intact endothelium is shown. In Figure 2b, APAP-treated rats show extensive centrilobular necrosis (large arrows), hydropic degeneration (small arrows), severe hemorrhage (asterisks) with congestion of sinusoidal spaces, and destruction of central vein (cv) karyorrhexis of nuclei (arrowheads). In Figure 2c, NAC-treated rats show mild glycogen depletion (large arrow) and sinusoidal congestion (small arrow) was evident slightly on the hepatic lobule. In Figure 2d, TA (500 mg/kg)-treated rats show less sinusoidal congestion (small arrows), recovery of damaged hepatocytes and normal hepatic nuclei (large arrow).

Table 2: Biochemical parameters

Variables	Serum AST (IU/L)	Serum ALT (IU/L)	Serum ALP (IU/L)	Serum GGT (IU/L)
Control	67.43±12.038	36.01±1.61	41.51±0.86	18.36±1.31
APAP***	128.75±9.91	92.9±6.64	60.86±4.64	123.5±1.33
NAC^{c}	119.9±2.37	92.16±3.83	57.13±2.11	86.03±5.96
TA 250 mg$^{\#\#\#}$	121.98±4.70	59.61±7.19	52.16±1.95	88.4±7.85
TA 500 mg$^{@@@}$	85.3±2.18	38.25±2.14	48.23±1.68	64.78±3.47

Biochemical parameters' data are expressed as mean±standard error of mean (n=6/Group). ***P<0.001 statistically significant as compared with control Group rats; $^{c,\#\#\#,@@@}P$<0.001 statistically significant as compared with Acetaminophen Group rats. AST: Aspartate transaminase; ALT: Alanine transaminase, ALP: Alkaline phosphatize, GGT: Gamma glutamyl transferase; NAC: n-Acetylcysteine; TA: *Terminalia arjuna*; APAP: Acetaminophen

Terminalia arjuna effect on immunohistochemical changes in the liver tissue

Figure 3a contral group showed normal liver section. But immunoreactivity of CYP2E1 was highly expressed in liver damaged cells which exhibited positive reaction ((arrow mark indicates dark brown color) in APAP induced group [Figure 3b]; However, very mild CYP2E1 protein expression was detected in TA500 mg/kg treated rats [Figure 3d] as compared to the NACtreated group [Figure 3c]. CYP2E1 expression levels were showed in the Figure 4.

Western blotting analysis of phosphatidylinositol-3-kinase and protein kinase B enzyme

Effect of Terminalia arjuna on the phosphatidylinositol-3-kinase

PI3K protein expression was significantly elevated in TA 500 mg/kg group in comparison with other treatment groups. NAC also significantly elevated the PI3K protein expression [Figures 5 and 6].

Effect of Terminalia arjuna on protein kinase B

APAP group showed a decreased level of protein expression, and P-AKT level was increased in TA 500 mg/kg group than the NAC group [Figures 7 and 8]. P-AKT plays a key role in apoptosis regulation by stimulating B-cell lymphoma 2 (Bcl2) family protein. The increased level can activate antiapoptotic Bcl2 to inhibit abnormal cell death.

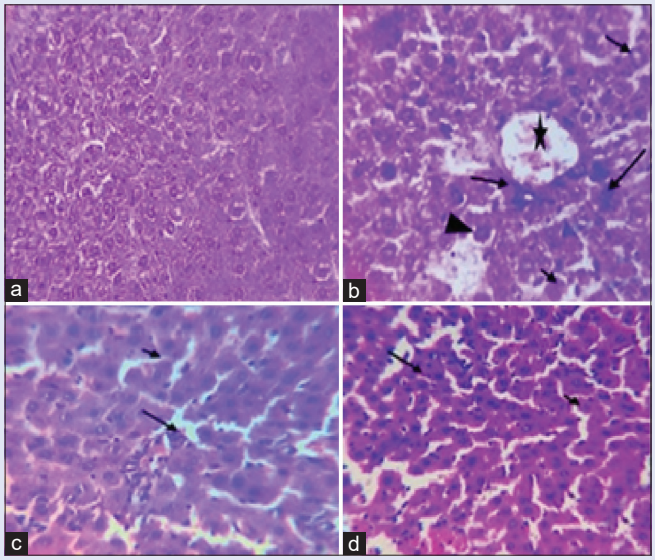

Figure 2: H and E stained liver tissue. Control group (a), acetaminophen group (b), N-acetylcysteine group (c), *Terminalia arjuna* 500 mg/kg (d). Results were expressed as mean ± standard error of the mean

Table 3: Antioxidant parameters

Variables	MDA (mol/l)	GSH (mmol/min/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Control	56.81±0.83	26.95±2.78	60.98±0.70	0.19±0.0041
APAP***	123.36±6.60	14.6±1.21	31.87±0.53	0.13±0.009
NAC^{c}	81.11±5.60	18.73±0.49	46.9±0.80	0.151±0.002
TA250 mg$^{\#\#\#}$	77.13±5.027	18.81±1.48	43.96±5.22	0.15±0.010
TA500 mg$^{@@@}$	64.6±2.93	25.56±0.31	52.81±1.36	0.172±0.003

Antioxidant parameters' data are expressed as mean±standard error of mean. ***P<0.001 statistically significant as compared with control rats; $^{c,\#\#\#,@@@}P$<0.001 statistically significant as compared with APAP rats. NAC: n-acetylcysteine; TA: *Terminalia arjuna*; MDA: Malondialdehyde; GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; APAP: Acetaminophen

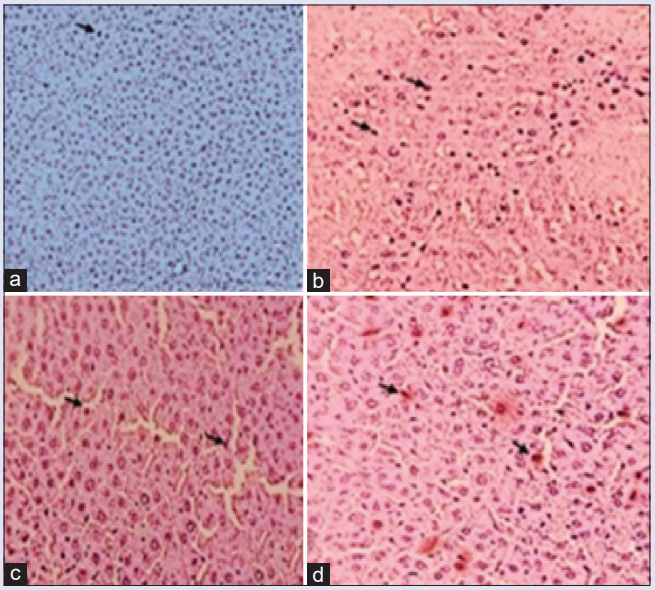

Figure 3: Immunohistochemical analysis of cytochrome P450 2E1 expression in liver. Control group (a), acetaminophen group (b), Nacetylcysteine group (c), and *Terminalia arjuna* 500 mg/kg (d). Results were expressed as mean ± standard error of the mean (n=6 rats/group)

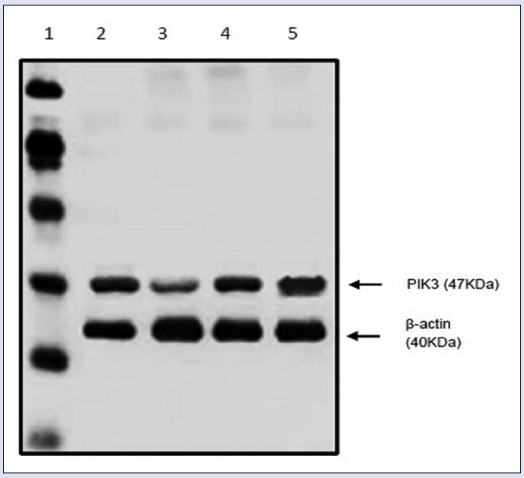

Figure 5: Phosphatidylinositol-3-kinase protein expression in western blot. Lane 1 – Marker lane; Lane 2 – Normal control; Lane 3 – Acetaminophen Lane 4 – N-acetylcysteine; Lane 5 – *Terminalia arjuna* 500 mg/kg

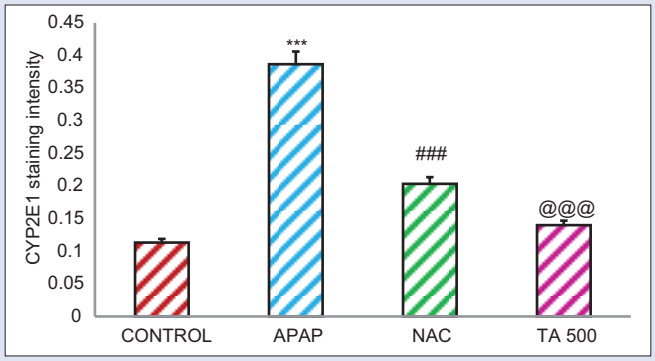

Figure 4: This graph showed the cytochrome P450 2E1 expression levels of experimental rats. Results were expressed as mean ± standard error of the mean. ***P < 0.001 statistically significant as compared with Control rats; ###,@@@P < 0.001 statistically significant as compared with acetaminophen rats

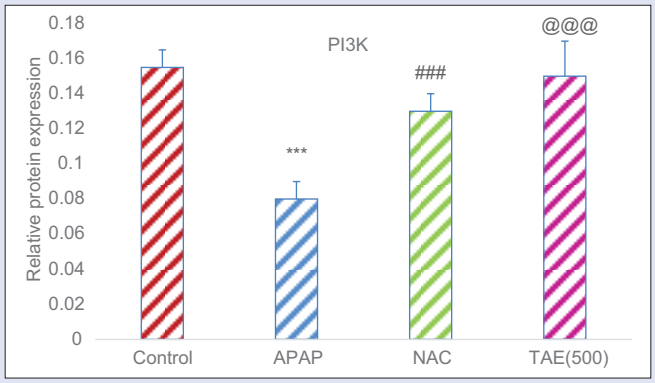

Figure 6: Quantitative data for phosphatidylinositol-3-kinase protein expression. Data values were expressed in intensity units, and the graph represents mean ± standard error of the mean. Data were obtained by computerized analysis of the western blots. ***P < 0.001 statistically significant as compared with control group rats; ###,@@@P < 0.001 statistically significant as compared with acetaminophen group rats

Effect of Terminalia arjuna on gene expression of protein kinase B enzyme

P-AKT gene expression was inhibited in an APAP -induced group compared with the normal control group [Figures 9 and 10]. In TA500 mg/kg treated group of P-AKT gene was significantly increased expression as compared with other groups. The APAP group showed moderate AKT gene expression. The control and TA500 mg/kg groups showed elevated gene expressions which showed a controlled cell death process.

DISCUSSION

ALT, AST, and ALP are important liver enzymes for studying hepatocellular necrosis.[26] AST and ALP levels are altered in other organ disorders also.[27] Hence, ALT is the most specific liver marker than other enzymes. The study proved that pretreatment with high dose of TA significantly reduced the levels of liver biochemical markers, and the enzyme levels were almost reverted to the control group. APAP mainly depletes the GSH levels and produces more of a toxic compound of NAPQI, which can increase the level of lipid peroxidation and damage the DNA. GSH level changes can determine the detoxification capacity on toxic compounds. SOD enzyme protects the cells from oxidative damage by superoxide radicals.[28,29] Oxidative stress marker MDA was reduced and elevated antioxidants levels were found in the TA high dose group than APAP group. This can prevent oxidative stress by interacting with scavenging free radicals and terminate the chain reaction.[30] The previous study also has shown that TA exhibited antioxidant effect on the alloxan induced liver and kidney of rats.[31] The data showed the poor effect of the TA low-dose (250 mg/kg) group than the TA high-dose (500 mg/kg) group in the biochemical and antioxidant analyses. Therefore, the low-dose TA group was not included in further studies.

The acute liver injury is caused due to excessive intake of APAP which promotes CYP450 level.[32,33] In the process of oxidation, minor fraction of APAP is converted to toxic metabolite NAPQI by CYP450. The isoforms of CYP such as CYP2E1, CYP1A2, CYP3A4, and CYP2D6

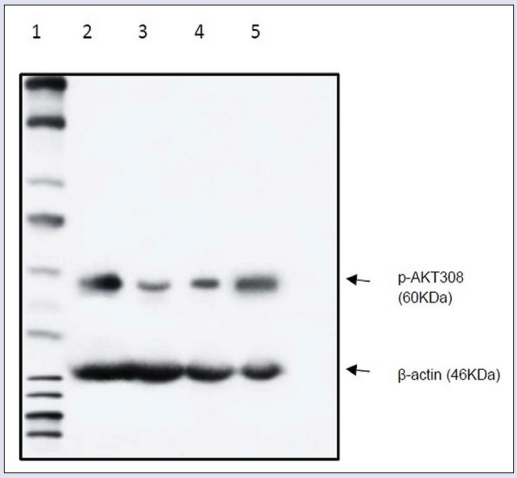

Figure 7: P-AKT protein expression in western blot. Lane 1 – Marker lane; Lane 2 – Normal control; Lane 3 – Acetaminophen; Lane 4 – N-acetylcysteine; Lane 5 – *Terminalia arjuna* (500mg/kg)

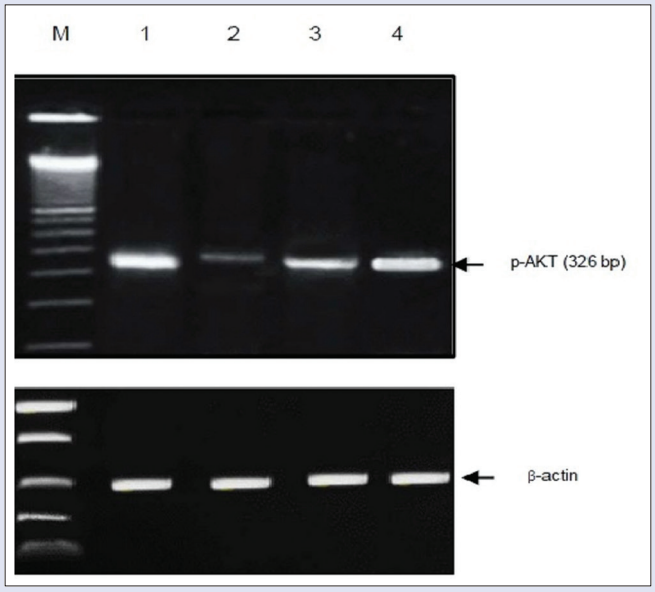

Figure 9: Protein kinase B gene expression in polymerase chain reaction. Lane 1- Marker lane; Lane 2 – Normal control group; Lane 3 – acetaminophen group; Lane 4 – N-acetylcysteine group; Lane 5 – *Terminalia arjuna* (500 mg/kg) group

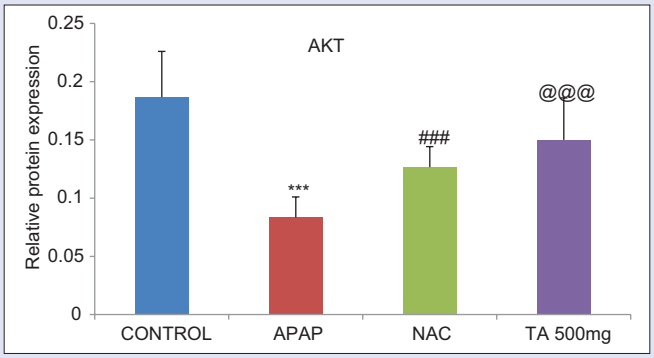

Figure 8: Quantitative data for P-AKT 308 protein expression. Data were obtained by computerized analysis of the Western blots and results were expressed as mean ± standard error of the mean. ***$P < 0.001$ statistically significant as compared with control group rats; ###,@@@$P < 0.001$ statistically significant as compared with acetaminophen group rats

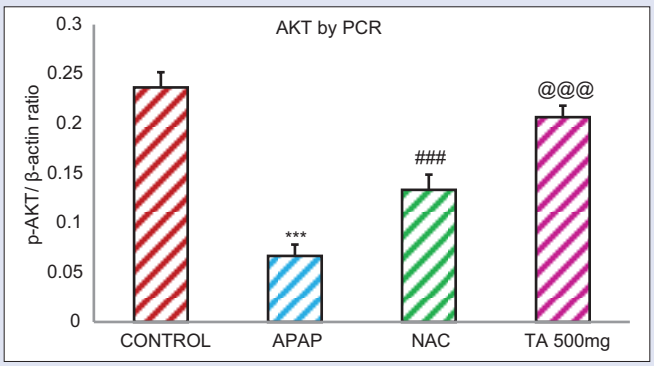

Figure 10: Protein kinase B gene expression in polymerase chain reaction. Results were expressed as mean ± standard error of the mean. ***$P < 0.001$ statistically significant as compared with control rats; ###,@@@$P < 0.001$ statistically significant as compared with acetaminophen rats

are concerned with drug metabolism.[34] The hemoprotein contains CYP which has the ability to detoxify the harmful chemicals.[35] CYP families are associated with 70%–80% of harmful chemical substance detoxification in liver.[36,37] Free radicals were suspected to release at the time of xenobiotics' detoxification. Those radicals are bound with lipid macromolecules to form the lipid peroxidation around the CV.[38] Hence, CYP enzyme expression changes are obligatory to find the hepatic toxicity level in the tissue. Flavonoid group of flavonol and tannin (gallic acid) are known to have antioxidant potential to decrease ROS generation and mitochondrial membrane dissipation.[39,40] Flavonoids and tannins are potent CYP inhibitors to control the production of a toxic compound.[41,42] In the immunohistochemistry study, the attenuated CYP2E1level in TA high-dose group indicating the pretreated TA has effectively alleviated the production of NAPQI toxic metabolite. PI3K/AKT is associated with cell proliferation, differentiation, and apoptosis.[43] In a healthy liver, the high-affinity cell surface receptor tyrosine kinase phosphorylates the PI3K after binding with the extracellular ligand. The activated PI3K phosphorylates the phosphatidylinositol 4,5-bisphosphate into active phosphatidylinositol 3,4,5-triphosphate (PIP_3). AKT is a key protein responsible for cell growth, survival, migration, and cell glucose metabolism.[44] AKT gets activated by 3phosphoinositide-dependent kinase1 phosphorylate at Thr308 along with bound PIP_3, PH domain, and phosphoinositide-dependent kinase 2 like multicellular proteins phosphorylate at Ser473 to form fully activated AKT. Then, AKT can downstream the different proteins like mTORC for protein synthesis, BCL2-Associated X, Bcl-2, caspase-9 for apoptosis and GSK-3 for glucose metabolism.[45] In our finding, APAP attenuates regular protein expression of PI3K/AKT. The suppressed protein expression of PI3K/AKT can increase the level of caspase-9 by activating the proapoptotic Bcl-2 proteins. The active caspase-9 has the ability to cleave the executioner caspases-3 and caspase-7 to promote abnormal apoptotic cell death. The pretreated TA high-dose group has shown elevated the levels of PI3K/AKT protein expression and suppressed the level of caspase-9 then the APAP group. Therefore, the data suggesting that pretreated TA highdose has been proven to regulate the PI3K/AKT and inhibiting caspase9 mediated apoptosis pathway by regulating Bcl2 family to inhibit the uncontrol activation of caspase9. Therefore, inhibited initiator caspase-9 unable to activate the executioner tcaspase3 and caspase-7. Our results

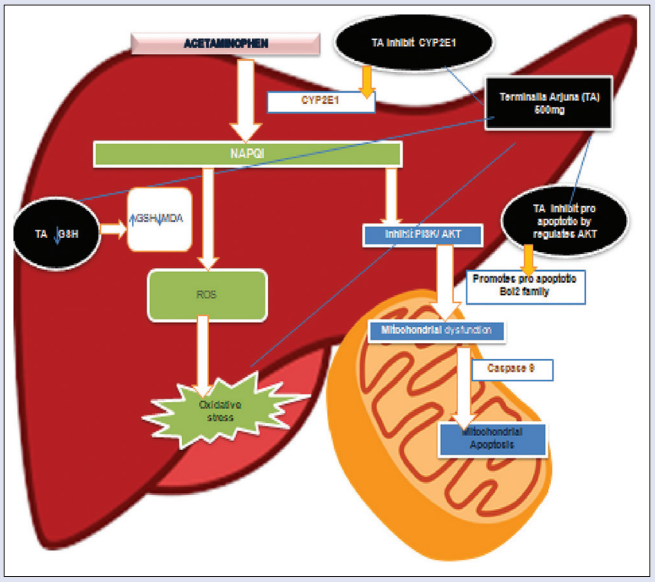

Figure 11: The *Terminalia arjuna* restoration of liver tissue against acetaminophen-induced hepatotoxicity

showed that TA 500 mg/kg significantly increase the expression of PI3K and AKT, which in turn control the mitochondrial apoptosis pathway by inhibiting the caspase-9 [Figure 11].

CONCLUSION

The experimental data explored that TA bark 500 mg/kg reduced APAP toxicity and promotes the antioxidant levels. The biochemical studies of liver enzyme levels were decreased by TA high dose (500 mg/kg). CYP2E1 and PI3K, AKT protein expressions were controlled by TA high dose (500 mg/kg). Hence, the TA 500 mg/kg drug could be a suitable alternative substance for NAC against APAP induced hepatotoxicity.

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Conflicts of interest

There are no conflicts of interest.

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