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Modulatory Effects of *Alstonia scholaris* on Biochemical and Antioxidant Parameters in Experimentally Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Alstonia scholaris (Apocynaceae) is an important constituent of numerous ayurvedic preparations. The present study was aimed to evaluate the modulatory effects of *Alstonia scholaris* on biochemical and antioxidant parameters in acetaminophen (APAP) induced hepatotoxicity. Hepatic biomarkers viz. aspartate and alanine aminotransferases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Gamma Glutamyl Transferase (GGT), paraoxonase (PON1), arylesterase (AE), ceruloplasmin (CP), bilirubin, plasma proteins and oxidative stress biomarkers like Total Oxidant Status (TOS), Total Antioxidant Status (TAS), blood glutathione (GSH), total thiols (TTH), glucose-6-phosphate dehydrogenase (G6PDH), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Glutathione-S-Transferase (GST) activities were estimated to assess the extent of hepatic damage induced by APAP and the protection imparted against it by leaf extracts. Oral administration of APAP, hepatotoxicity indicated by increased ($p<0.05$) activities of AST, ALT, ALP, GGT, G6PDH, total bilirubin and TOS while decreased TAS, CAT, SOD, GPx, GST, TTH, GSH, PON1, AE, TTH, direct bilirubin, total plasma proteins and albumin in plasma was observed. The treatments with either aqueous or ethanolic leaf extract of *A. scholaris* restored the activities of ALT, GGT, ALP, LDH, PON1, AE, G6PDH, GPx, CAT, GSH, TTH, TAS and MDA in APAP induced hepatotoxicity. Hepatic and oxidative damage biomarkers indicated that treatments with aqueous or ethanolic leaf extract minimized APAP induced hepatic damage. High correlation coefficients between PON1/AE ratio and TTH levels and increased TAS, GSH levels suggests ethanolic extract to have higher hepatoprotective potential compared to aqueous extract of *A. scholaris*.

Key words: Total antioxidant status, hepatotoxicity, acetaminophen, *Alstonia scholaris*

INTRODUCTION

Alstonia scholaris L. (Apocynaceae) distributed throughout tropical and subtropical region of the world is widely used in traditional system of medicine in Asia, America and Africa (Dassanayake, 1982). Leaves of *A. scholaris* are rich source of flavonoidal glycosides, indole alkaloids, steroids and terpenoids (Desoky, 1999; Dung *et al*., 2001). The leaf extracts of *A. scholaris*

are constituents of various Ayurvedic preparations viz., Saptaparna satvadivati, Saptaparna ghanasara etc., due to their high therapeutic potential in respiratory disorders, fever, gastric complaints and hepatic disorders etc. (Chopra *et al*., 1956). The plant has anticancer (Jagetia and Baliga, 2006), anti-asthmatic (Shang *et al*., 2010a), antispasmodic (Shah *et al*., 2010), anti-inflammatory, analgesic (Shang *et al*., 2010b) and hepatoprotective (Kumar *et al*., 2012) activities which may be due to presence of different polyphenolic compounds (Shah *et al*., 2010; Subraya *et al*., 2012).

Drug induced hepatotoxicity is a major health issue concern for clinicians, drug regulatory agencies and pharmaceutical industry. Acetaminophen (APAP), a commonly used antipyretic drug, is a potent hepatotoxic when used more that of recommended doses (James *et al*., 2003). Reduced Total Antioxidant Status (TAS) and total thiols (TTH) levels are primarily responsible for APAP induced hepatotoxicity in mammals (Nelson, 1990). Overproduction of free radicals during metabolism which account for the dysfunction or death of hepatocytes and other liver cells contributes to the pathogenesis of acute or chronic liver diseases (Medina and Moreno-Otero, 2005; Mari *et al*., 2010). Liver is the primary source of plasma proteins, albumins, metabolizing enzymes viz., paraoxonase (PON1), arylesterase (AE), glutathione peroxidase (GPx), Glutathione-S-Transferase (GST) etc., which exhibit scavenging action on multiple compounds like arylesters, hydroperoxides and lactones produced during oxidative cellular damage (Kosaka *et al*., 2005). Activities of these enzymes and free availability of SH (sulfhydryl) group is essential for scavenging the excessive free radicals generated during the hepatic metabolism. Impaired liver functions during hepatotoxicity adversely affect the activities of scavenging enzymes and levels of plasma proteins responsible for maintaining antioxidant defense of mammalian body (Kilic *et al*., 2005).

The use of pharmaceutics based on ethno-botanicals is increasing globally (WHO., 1996); hence, the interest of scientific community on research involving herbal formulations to maximize utilization of natural resources is increased (Neil and Laurie, 2000; Tilburt and Kaptchuk, 2008). Various studies have shown the enhanced expression or activities of PON1, AE, TTH and TAS by supplementation of different drugs and natural dietary products provides protection against oxidative stress induced cellular damage in various disorders in mammals (Costa *et al*., 2003; Aviram and Rosenblat, 2005; Kiyici *et al*., 2010; Holmgren and Sengupta, 2010). Thus determination of TAS, TTH, Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) levels will help in correlating the extent of hepatic damage i.e., lipid peroxidation (MDA, malondialdehyde levels) with oxidative stress index. Therefore, the present study was aimed to evaluate the modulatory effect of *Alstonia scholaris* on biochemical and antioxidant parameters in acetaminophen (APAP) induced hepatotoxicity in wistar rats.

MATERIALS AND METHODS

Collection and preparation of extracts: The leaves of *Alstonia scholaris* L. were collected from University Campus, R S Pura, Jammu (India). Plant sample was taxonomic identified by Taxonomist, Department of Botany, University of Jammu (AU-2874). Sufficient fresh leaves were collected and air-dried in shade (temperature not exceeding 40°C) for 3-4 weeks. Air dried leaves were pre-crushed and later pulverized into fine powder using electric blender. Aqueous extract was prepared by soaking dry powder in 1:10 ratio in distilled water for 72 h with intermittent shaking. After 72 h of soaking, the content was filtered through filter papers (0.45 μm) and filtrate was concentrated under reduced pressure using rotatory evaporator (temp 50-55°C, 10-15 rpm).

Ethanolic extract was prepared by using ethyl alcohol in extract container of soxhlet apparatus according to standard method (Harborne, 1998). The dried aqueous and ethanolic leaf extracts of *A. scholaris* were stored in air tight container. The extracts were reconstituted in 0.1% Carboxy Methyl Cellulose (CMC) for oral gavage in wistar rats.

Induction of hepatotoxicity in experimental animals: Standard protocol for induction of hepatotoxicity with APAP was used (Visen *et al*., 1993). Wistar rats of either sex (150-200 g) were procured from Indian Institute of Integrative Medicine, Jammu. Animals were provided standard pelleted ration and drinking water *ad libitum* under standard managemental conditions. Prior to start of experiment, rats were acclimatized in the laboratory conditions for a period of 3 weeks. The experimental protocol was approved by Institutional Animal Ethics Committee (FVSc/C-11/2456-68). Forty two rats were randomly divided into 7 groups with 6 rats in each. The control (Group I) received only distilled water for 7 days, Group II received vehicle (0.1%, CMC) 1 mL followed by a single oral dose (3 g kg^{-1} b.wt.) of APAP (Sigma-Aldrich, USA) on the 5th day of the administration. Group III was fed with standard drug silymarin (100 mg kg^{-1}, b.wt.) (Sigma-Aldrich, USA) orally daily for 7 days and received APAP at same dose rate on 5th day of administration. Group IV and V received aqueous or ethanolic extract (300 mg kg^{-1} b.wt.) of *A. scholaris* orally daily for 7 days, respectively. Group VI and VII received aqueous and ethanolic extract treatments similar to group IV and V, respectively, in addition APAP was administered on the 5th day. The dose of plant extract was determined on the basis of reported toxic dose and different pharmacological activities of the plant (Jagetia and Baliga, 2006; Subraya *et al*., 2012). After 7 days treatment, blood samples were collected from retro-orbital fossa in a sterilized tube containing heparin as anticoagulant. The blood samples were centrifuged at 4000 rpm for 10 min for separation of plasma and collected in storage vials for the estimation of blood biochemical and antioxidant parameters.

Assaying of hepatic biomarkers: The PON1 and AE activities were determined by spectrophotometric methods using paraoxon (Sigma-Aldrich, USA) and phenyl acetate (Sigma-Aldrich, USA) as substrate, respectively (Burlina *et al*., 1977; Furlong *et al*., 1988). The activity of G6PDH was assessed based upon the ability of enzyme to catalyze the conversion of Glucose-6-phosphate and $NADP^+$ to 6-phosphogluconolactone and NADPH (reduced nicotinamide adenine dinucleotide phosphate) as per the method described by Deutsch (1978). Other hepatic biomarkers like activities of aspartate and alanine aminotransferase (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Gamma Glutamyl Transferase (GGT) and levels of plasma proteins, albumin and bilirubin profiles were determined by standard kits (Transasia Bio-Medicals Ltd., India) using Chemistry Analyzer (CHEM-7, ERBA, Mannheim).

Assaying of antioxidant parameters: Total Antioxidant Status (TAS) was determined spectrophotometrically by using 2,2-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) according to the method described (Re *et al*., 1999); final TAS values were expressed as mM of ascorbic acid equivalents in the plasma. Similarly, TOS level in plasma was measured using a novel automated method developed by Erel (2005) and results expressed in terms of μmol H_2O_2 per liter. The percent ratio of TOS to TAS level was Oxidative Stress Index (OSI) (Aycicek *et al*., 2006). The enzymatic parameters viz., catalase (CAT) and glutathione peroxidase (GPx) were determined as described by Aebi (1983) and Hafeman *et al*. (1974), respectively, the activities of

superoxide dismutase (SOD) and Glutathione-S-Transferase (GST) were determined as per the method described by Marklund and Marklund (1974) and Habig *et al*. (1974), respectively. Total thiol (TTH) and malondialdehyde (MDA) levels were determined in plasma as per the method of Motchnik *et al*. (1994) and (Shafiq-ur-Rehman, 1984), respectively. The levels of reduced blood glutathione (GSH) and plasma ceruloplasmin (CP) were determined as per the standard methods (Beutler, 1975; Sunderman and Nomoto, 1970).

Statistical analysis: Data was presented in Mean±Standard error. The results were evaluated by analysis of variance (ANOVA) in Completely Randomized Design (CRD) using the Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS

Significantly ($p<0.05$) increased activities of AST, ALT, GGT, ALP and LDH in plasma of APAP administered rats indicated acute hepatic damage (Table 1). The administration of silymarin along with APAP lowered ($p<0.05$) the activities of AST, ALT and GGT as compared to APAP alone. The oral administration of aqueous or ethanolic extract didn't alter ($p>0.05$) the activities of ALT and GGT; however, significantly ($p<0.05$) elevated AST activity was observed with aqueous treatment. Treatment with aqueous and ethanolic extracts significantly ($p<0.05$) depressed the activities of AST, ALT, GGT, ALP and LDH by APAP administration.

Table 2 depicted the changes in total, direct, indirect bilirubin, total plasma proteins and albumin in different groups of animals treated with either aqueous or ethanolic leaf extract of *A. scholaris* in plasma of APAP induced hepatotoxic rats. Single oral administration of APAP significantly ($p<0.05$) reduced levels of direct bilirubin, total plasma proteins and albumin and increased ($p<0.05$) total and indirect bilirubin as compared to control group. Pre-treatment with silymarin or aqueous or ethanolic extracts in APAP treated animals restored all these parameters except direct bilirubin.

Table 1: Effects of aqueous and ethanolic leaf extracts of *A. scholaris* treatments on hepatic biomarkers viz. AST, ALT, GGT, ALP and LDH activities in plasma of APAP induced hepatotoxic rats

Groups	AST (IU L^{-1})	ALT (IU L^{-1})	GGT (U L^{-1})	ALP (U L^{-1})	LDH (U L^{-1})
I. Control	92.73±6.20^{a}	72.28±4.90^{a}	3.96±0.38^{a}	283.18±18.02^{ab}	239.90±6.39^{a}
II. APAP	334.43±21.73^{d}	213.73±10.78^{b}	8.03±0.38^{b}	408.82±26.19^{d}	618.30±51.81^{c}
III. Silymarin+APAP	89.07±4.93^{a}	54.81±2.19^{a}	4.43±0.47^{a}	267.88±10.67^{c}	267.22±25.09^{c}
IV. Aqueous ext.	147.33±7.36^{c}	71.09±4.93^{a}	3.53±0.47^{a}	313.78±27.72^{b}	395.32±32.24^{b}
V. Ethanolic ext.	105.09±4.92^{ab}	64.43±4.02^{a}	3.86±0.40^{a}	222.08±25.27^{a}	376.48±24.26^{b}
VI. Aqueous ext.+APAP	118.76±10.26^{abc}	67.01±2.52^{a}	3.30±0.31^{a}	334.70±19.24^{bc}	243.35±11.43^{a}
VII. Ethanolic ext.+APAP	128.58±4.01^{bc}	72.67±5.12^{a}	3.82±0.29^{a}	323.50±20.95^{bc}	216.82±13.32^{a}

Values are given as Mean±SE, (n = 6). Values having different superscripts (^{a,b,c}) in a column are significantly different ($p<0.05$), AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyltransferase, ALP: Phosphatase, LDH: Lactate dehydrogenase, APAP: Acetaminophen

Table 2: Effects of aqueous and ethanolic leaf extracts of *A. scholaris* treatments on plasma proteins and bilirubin profiles in plasma of hepatotoxic rats

Groups	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})	Indirect bilirubin (mg dL^{-1})	Total proteins (g dL^{-1})	Albumin (g dL^{-1})
I. Control	0.458±0.04^{a}	0.130±0.009^{d}	0.328±0.04^{ab}	8.10±0.18^{c}	3.28±0.15^{b}
II. APAP	0.787±0.06^{b}	0.083±0.003^{b}	0.703±0.06^{d}	6.15±0.10^{a}	2.50±0.16^{a}
III. Silymarin+APAP	0.573±0.04^{a}	0.112±0.006^{c}	0.572±0.04^{c}	7.18±0.28^{c}	4.05±0.23^{c}
IV. Aqueous ext.	0.405±0.03^{a}	0.127±0.007^{cd}	0.278±0.03^{a}	6.64±0.16^{b}	3.46±0.08^{b}
V. Ethanolic ext.	0.443±0.04^{a}	0.062±0.005^{a}	0.382±0.04^{ab}	7.56±0.32^{c}	3.33±0.16^{b}
VI. Aqueous ext.+APAP	0.525±0.06^{a}	0.082±0.003^{b}	0.443±0.06^{bc}	6.79±0.18^{b}	3.01±0.09^{b}
VII. Ethanolic ext.+APAP	0.517±0.03^{a}	0.087±0.006^{b}	0.430±0.03^{bc}	7.58±0.17^{c}	3.06±0.07^{b}

Values are given as Mean±SE, (n = 6). Values having different superscripts ($^{a, b, c}$) in a column are significantly different ($p<0.05$)

Table 3: Effects of aqueous and ethanolic leaf extracts of *A. scholaris* treatments on antioxidant parameters viz., TAS, TOS, OSI, TTH and GSH in plasma of hepatotoxic rats

Groups	TAS (mM)	TOS*	OSI	TTH (mM)	GSH (μM)
I. Control	0.967±0.069^{b}	3.60±0.30^{b}	0.388±0.054^{b}	0.110±0.007^{bc}	4.86±0.21^{c}
II. APAP	0.538±0.061^{a}	8.62±0.41^{a}	1.602±0.057^{d}	0.080±0.006^{a}	2.10±0.09^{a}
III. Silymarin+APAP	1.092±0.046^{bc}	4.76±0.53^{c}	0.432±0.037^{b}	0.124±0.011^{c}	3.71±0.20^{b}
IV. Aqueous ext.	1.189±0.036^{c}	4.05±0.21^{bc}	0.344±0.029^{ab}	0.116±0.004^{c}	5.44±0.42^{cd}
V. Ethanolic ext.	1.114±0.023^{bc}	3.94±0.21^{b}	0.354±0.012^{ab}	0.119±0.009^{c}	2.84±0.29^{a}
VI. Aqueous ext.+APAP	1.021±0.075^{b}	1.13±0.10^{a}	0.904±0.008^{c}	0.090±0.006^{ab}	4.93±0.40^{c}
VII. Ethanolic ext.+APAP	1.215±0.030^{c}	3.56±0.15^{b}	0.295±0.018^{ab}	0.128±0.004^{c}	6.15±0.30^{d}

Values are given as Mean±SE, (n = 6). Values having different superscripts ($^{a, b, c}$) in a column are significantly different ($p<0.05$). *Values of TOS was presented in μmol H_2O_2 per liter, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, TTH: Total thiol, GSH: Blood glutathione

Table 4: Effects of aqueous and ethanolic leaf extracts of *A. scholaris* treatments on activities of CAT, SOD, GPx, GST and plasma CP level in hepatotoxic rats

Groups	CAT	SOD	GPx	GST	CP
I. Control	37.22±4.58^{b}	36.97±0.48^{b}	2.20±0.20^{d}	0.237±0.015^{c}	0.155±0.010^{c}
II. APAP	19.67±2.04^{a}	27.90±0.98^{a}	1.38±0.16^{abc}	0.045±0.005^{a}	0.109±0.005^{b}
III. Silymarin+APAP	35.69±3.84^{b}	33.97±2.36^{b}	1.70±0.20^{bcd}	0.205±0.017^{c}	0.164±0.013^{c}
IV. Aqueous ext.	34.98±3.99^{b}	24.47±1.35^{a}	0.94±0.21^{a}	0.061±0.027^{a}	0.132±0.013^{bc}
V. Ethanolic ext.	36.85±3.40^{b}	25.12±0.50^{a}	1.64±0.14^{bcd}	0.100±0.023^{a}	0.202±0.015^{d}
VI. Aqueous ext.+APAP	36.90±3.58^{b}	27.35±1.01^{a}	1.22±0.18^{ab}	0.061±0.011^{ab}	0.070±0.014^{a}
VII. Ethanolic ext.+APAP	35.83±1.64^{b}	34.36±2.05^{b}	1.86±0.20^{cd}	0.126±0.017^{b}	0.107±0.013^{b}

Values are given as mean±SE, (n = 6), Values having different superscripts ($^{a, b, c}$) in a column are significantly different ($p<0.05$). Values of CAT: Catalase are expressed in μmol H_2O_2 decomposed min^{-1} mg Hb^{-1}, Values of SOD: Superoxide dismutase and Gpx: Glutathione peroxidase are expressed in $Unit^{-1}$ mg Hb^{-1}, Values of GST: Glutathione s transferase are expressed in μmol of CDNB conjugate $formed^{-1}$ min^{-1} mg Hb^{-1}, Values of plasma CP: Ceruloplasmin are expressed in g L^{-1}

Significantly ($p<0.05$) decreased plasma levels of TAS, TTH and GSH and increased ($p<0.05$) levels of TOS and OSI were observed on APAP administration compared to control animals (Table 3). Pre-treatment of silymarin along with APAP restored the levels of TAS and TTH whereas levels of TOS and OSI declined ($p<0.05$) as compared to APAP treated group. The treatments of aqueous leaf extract of *A. scholaris* along with APAP maintained TTH and GSH levels while TOS and OSI levels lowered ($p<0.05$) from APAP exposed group. Similarly, ethanolic extract treatment with APAP increased ($p<0.05$) the levels of TAS and GSH as compared to control and APAP treated group.

Activities of CAT, SOD, GPx, GST and CP were reduced ($p<0.05$) on treatment with APAP in rats as compared to control animals. Treatment with silymarin following APAP administration maintained the activities of CAT, SOD, GPx, GST and CP. Treatments with aqueous leaf extract along with APAP failed to restore the activities of SOD, GPx, GST and CP as compared to control animals; the ethanolic extract restored the levels of CAT, SOD, GPx and GST excluding GST and CP (Table 4).

Table 5 presents the alterations in the activities of PON1, AE, PON1/AE ratio, G6PDH and MDA levels in rats treated with APAP alone or along with silymarin or aqueous or ethanolic extract of *A. scholaris*. The APAP administration significantly ($p<0.05$) reduced PON1 (183.41%) and AE (49.59%) activities while increased ($p<0.05$) G6PDH (64.98%) and MDA (299.56%) levels as compared to control rats. Treatment with silymarin with APAP significantly ($p<0.05$) increased the PON1 (64.3%) and AE (22.9%) activities compared to group II and restored MDA levels. Treatments of aqueous leaf extract with APAP increased PON1 (54.27%) and AE (208.69%) and decreased MDA (43.68%) levels as compared to APAP exposed group. The ethanolic extract treatment along with APAP increased PON1 (59.43%) and AE (132.28%) activities and decreased MDA (57.43%) levels compared to APAP exposed group. Treatment with either of aqueous and ethanolic extract with APAP restored G6PDH activities.

Table 5: Effects of aqueous and ethanolic leaf extracts of A. scholaris treatments on activities of PON1, AE, PON1/AE ratio, G6PDH and MDA in hepatotoxic rats

Groups	PON1 (U L^{-1})	AE (U mL^{-1})	PON1/AE ratio	G6PDH (U L^{-1})	MDA levels
I. Control	25.17±1.14^{c}	27.94±2.29^{b}	0.93±0.09^{c}	2885.87±171.04^{ab}	0.61±0.10^{a}
II. APAP	8.88±0.81^{a}	16.68±1.10^{a}	0.47±0.04^{ab}	4761.30±384.84^{c}	2.64±0.18^{c}
III. Silymarin+APAP	24.90±1.40^{c}	24.22±1.52^{b}	1.05±0.09^{c}	2703.73±258.52^{ab}	0.83±0.09^{a}
IV. Aqueous ext.	25.52±1.87^{c}	41.99±2.05^{c}	0.46±0.05^{ab}	2400.17±135.83^{ab}	0.97±0.18^{b}
V. Ethanolic ext.	24.19±2.35^{c}	52.56±1.78^{c}	0.62±0.06^{b}	4245.83±364.28^{c}	1.08±0.12^{b}
VI. Aqueous ext.+APAP	19.43±0.64^{bc}	57.66±1.67^{c}	0.34±0.01^{a}	2247.08±85.68^{a}	1.34±0.09^{b}
VII. Ethanolic ext.+APAP	21.96±1.15^{bc}	43.49±3.46^{c}	0.52±0.05^{ab}	3206.343±18.75^{b}	1.07±0.09^{b}

Values are given as Mean±SE, (n = 6). Values having different superscripts (a, b, c) in a column are significantly different ($p<0.05$). Values of MDA (malondialdehyde) levels are expressed in nmoles MDA produced mg of Hb^{-1} h^{-1}, PONI: Paraoxonase, AE: Arylesterase, G6PDH: Glucose-6-phosphate dehydrogenase, MDA: Malondialdehyde levels

There was negative correlation ($r = -0.919$, $p<0.05$) of PON1 with ALT in APAP exposed (group II) group whereas positive correlation ($r = 0.888$, $p<0.05$) between AST and AE in silymarin treated group was observed. There was negative correlation ($r = -0.822$, $p<0.05$) of AE with GGT in aqueous extract treated animals. The correlation coefficient between PON1/AE ratio and total thiols level was significantly negative ($r = -0.899$, $p<0.05$) in APAP administered group.

DISCUSSION

Liver, an important visceral organ of body synthesizes major plasma proteins and is responsible for metabolism of the endogenous and exogenous molecules to facilitate their excretion besides homeostasis of internal environment (Nelson, 1990). Acetaminophen is a dose dependent hepatotoxicant that can cause severe acute hepatocellular injury (James *et al*., 2003). Increased activities of GGT, AST, ALT and ALP on APAP administration in present study indicate acute hepatic injury leading to leakage of cytoplasmic contents into circulation due to loss of hepatocytes membrane integrity (Sallie *et al*., 1991). Rapid increase in activity of GGT is indicative of acute hepatocellular damage. The AST is present in high concentration in liver and to a lesser extent to cardiac, skeletal muscle, kidney, brain, pancreas, lung etc. unlike AST. The enzyme ALT is mainly present in liver cells and is a relatively specific indicator of hepatocellular damage (Sallie *et al*., 1991; Forbes *et al*., 2002). Similarly, reduction in total plasma proteins, albumin, direct bilirubin and increased total and indirect bilirubin concentrations indicate the reduced functional activity of hepatocytes to produce bile or plasma proteins or metabolize bilirubin via glucuronide conjugation (Zunszain *et al*., 2008). Treatments with either aqueous or ethanolic leaf extract of *A. scholaris* improved the hepatic damage induced by APAP administration as shown by restoration of enzymatic activities and levels of plasma proteins and bilirubin profile towards normalization thereby improving hepatic functions.

Reduced plasma levels of TAS, TTH, GSH and increased TOS, OSI in APAP administered rats indicate increased oxidants production and reduced radicals scavenging potential. Reduced scavenging potential or TAS may be attributed to decreased plasma levels of TTH and GSH, which either directly scavenges the radicals generated or indirectly used as a substrate by GSH dependent enzymes for exclusion of peroxides generated during cellular damage. Significant reduction in TTH and GSH in present study may be either due to increased utilization of SH group or decreased production or recycling during APAP induced hepatotoxicity (Kyle *et al*., 1990). Excess production of N-acetyl-p-benzo-quinoneimine (NAPQI) during overdosing of APAP or reduced level of thiol during stress facilitate interaction of NAPQI with other macromolecules especially cysteine (-SH group) of important cellular proteins and membrane lipids (Nelson, 1990; Sallie *et al*., 1991). Most of these alterations begin from mitochondrial proteins due to excessive oxidative reactions

leading to loss of energy production and disruption of ionic transport across the cell membrane; further, interactions with transporter proteins or membrane lipids alter the membrane permeability leading to leakage of cellular enzymes and distort the cellular architecture causing hepatotoxicity (Nelson, 1990).

Reduction in activities of SOD, CAT, GPx, GST and CP were observed in APAP administered rats reducing the cellular antioxidant defense. The declined activities of GST and GPx may be due to reduced level of GSH after APAP administration. Various studies had demonstrated that erythrocyte GPx activity significantly decreased in patients with chronic liver disease (Czuczejko *et al.,* 2003). Pre and post treatments of aqueous or ethanolic extract of *A. scholaris* with APAP restored the activities of these enzymatic components of antioxidant system. Similar observations were reported for fruits, bark and leaf of *A. scholaris* in hepatotoxicity induced by various chemicals in wistar rats (Shankar *et al*., 2012; Kumar *et al*., 2012). However, treatments with aqueous and ethanolic extract of *A. scholaris* with APAP failed to restore the activities of CP in plasma.

In the present study, APAP administration increased the activity of G6PDH, a rate limiting enzyme of hexose monophosphate pathway responsible for the production of NADPH. Significant increased activity of G6PDH following APAP administration may be the adoptive mechanism to increased supply of reductant (NADPH) to maintain the activity of glutathione reductase and CAT which are required for the synthesis of GSH and scavenging of H_2O_2, respectively from the medium (Mari *et al*., 2010). Polyphenolic compounds present in the plant extracts may increase the synthesis of total thiols by modulating the activity of γ-glutamylcysteine ligase (Di Monte *et al*., 1984; Myhrstad *et al*., 2002) or decrease the production of NAPQI by inhibiting the activities of CYP3A4 enzymes (Ho *et al*., 2001; Pal and Mitra, 2006). The potential of plant components to inhibit the aromatase activity of cytochrome 450 favoring liver regeneration is another interesting factor in their hepatoprotective effect (Al-Qarawi *et al*., 2004). Similarly, various phytochemicals from different plants have shown hepatoprotective potential in experimental models (Rusu *et al*., 2005).

Arylesters, hydroperoxides and lactones produced during oxidative cellular damage are scavenged by the action of PON1 and AE (Kosaka *et al*., 2005). Reduction in the activities of PON1 and AE during APAP induced hepatic damage in present study may be due to reduced liver ability to synthesize high density lipoprotein associated PON1 and AE proteins (Ferre *et al*., 2002; Kilic *et al*., 2005). Studies have also suggested that during hepatotoxicity, due to depletion of sulfhydryl (-SH) group, the rise in concentration of NAPQI would make it to interact with unique free sulfhydryl group (Cys 284) of PON1 protein causing inhibition of PON1 activities. Restoration of plasma PON1 and AE activities in present study with aqueous and ethanolic leaf extracts of *A. scholaris* may be due to presence of polyphenolic compounds (flavonoids and tannin etc.) in the extracts (Shah *et al*., 2010). Although there is some evidence that polyphenols may induce PON1 in cultured human hepatoma cells (Gouedard *et al*., 2004), the data regarding PON1 induction *in vivo* is sparse. Enhanced plasma PON1 activity after treatment with aqueous or ethanolic extract in hepatotoxicity may be due to increased synthesis of enzymes in liver or prevented inactivation by free radicals (Deakin *et al*., 2002; Costa *et al*., 2003).

Antioxidative effect of green tea rich in flavonoids prevents progression of atherogenesis by preserving PON1 activity (Tas *et al*., 2011). Various studies have also shown enhancement of plasma PON1 activity by dietary supplementation of pomegranate juice (Aviram *et al*., 2004), grape seed extracts (Kiyici *et al*., 2010) and vitamin E (Tsakiris *et al*., 2009). Herbal preparations have

recently attracted much attention as alternative medicine for treatment and prevention of wide variety of disorders primarily induced by xenobiotics. Various phytochemical products claim to have hepatoprotective potential, although their exact mechanism behind this protection is uncertain (Zhang *et al*., 2013). Treatment with aqueous and ethanolic extract restored the functional activities of PON1 and AE during hepatotoxicity but failed to restore the PON1/AE ratio, the latter was restored by silymarin. Significant increased activity of AE after exclusive treatment of either of the extracts or in APAP induced hepatotoxicity was primarily responsible for alterations in PON1/AE ratio.

Significant reduction in TAS and increased TOS and OSI as observed in present study may be due to reduced levels of different enzymatic and non-enzymatic components of antioxidant system leading to oxidative damage as indicated by increased MDA levels in APAP administered rats. Pre and post treatment with aqueous or ethanolic extract of *A. scholaris* in APAP administered rats restored the activities of enzymatic and non-enzymatic components of antioxidant system which in turn be reduced MDA levels (Shankar *et al*., 2012; Kumar *et al*., 2012).

CONCLUSION

Treatments with either aqueous or ethanolic extract of *A. scholaris* restored the levels of TAS, TTH, GSH and significantly reduced MDA levels in APAP induced hepatotoxicity in wistar rats. Hepatic and oxidative damage biomarkers indicated that treatments with aqueous or ethanolic leaf extract minimized APAP induced hepatic damage. High correlation coefficients between PON1/AE ratio and TTH levels and increased TAS and GSH suggested the ethanolic extract to have higher hepatoprotective potential compared to aqueous extract of *A. scholaris*.

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