Evaluation of cytoprotective effects of N-acetyl cysteine and silymarin on hepatic tissue against arsenic induced inflammation and oxidative stress in rats

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ABSTRACT

The present study was carried to evaluate the cytoprotective effects of N-acetylcysteine (NAC) and silymarin against sodium arsenate toxicity ($NaAsO_2$) induced oxidative stress in rats. Seventy-five male albino rats were divided into five main equal groups of 15rats each. Group I (control normal group): administered distilled water. Group II (sodium arsenate exposed group): received1/20th of LD_{50} of sodium arsenate orally (41mg/kg body weight/day) over a period of 8 weeks. Group III (sodium arsenate + NAC treated group): received sodium arsenate (41 mg/kg bodyweight) and treated daily with NAC (200mg/kg body weight /orally). Group IV (sodium arsenate + silymarin treated group): received sodium arsenate (41 mg/kg body weight) and treated daily with silymarin (200mg/kg body weight). Group V (sodium arsenate +NAC+ silymarin treated group): received sodium arsenate (41 mg/kg body weight) and treated daily with NAC (200mg/kg body weight) and silymarin (200 mg/kg body weight). The obtained results revealed that, a significant increase in serum liver markers enzymes (ALP, GGT, ALT and AST) activities, total bilirubin, IL-6, TNF-α and hepatic tissue L - MDA concentrations with marked decrease in serum total protein, albumin levels and antioxidant enzymes (SOD, CAT and GPx) activities in hepatic tissue were observed in sodium arsenate intoxicated rats. Also, various pathological changes in hepatic and renal tissues were observed in arsenate induced toxicity in rats. Interestingly, the severity of these alterations was reduced after administration of NAC and or silymarin that exhibited a significant change in all mentioned parameters with best results in combined group. It could be concluded that, NAC and silymarin have a protective antioxidant and anti-inflammatory effects and could be applicable as cytoprotective against oxidative stress of tissue damage mediated by Arsenic intoxication.

Key words: Arsenic, N-acetylcysteine, silymarin, oxidative stress, pro-inflammatory cytokines, Pathology

(http://www.bvmj.bu.edu.eg) (BVMJ-32(2): 224-238, 2017)

1. INTRODUCTION

Toxicity, aging and diseases are among the main causes of free radicals production inside the cell (El-Zayat and Amin, 2003). The main source of arsenic toxicity is the drinking of ground water contaminated with an inorganic form (Islam et al., 2009). This metal commonly found in two oxidation states trivalent arsenite and pentavalent arsenate. These inorganic arsenicals have been considered more toxic than organo arsenicals (Flora et al., 2007). Arsenic compounds are most readily absorbed from the gastrointestinal tract (Karmakar et al., 2011). The arsenicals can bind to sulfydryl groups of glycolysis and tri-carboxylic acid cycle enzymes inhibiting their pathways and the pentavalent arsenicals can interfere with the mitochondrial oxidative phosphorylation enzymes (Bhadauria et al., 2007). Generally, oxidative stress of arsenic is due to the production of free radicals like super oxide and hydrogen peroxide which were supposed to initiate lipid peroxidation (Hong et al., 2014). As has also been implicated, arsenic induced oxidants, such as superoxide anions and hydroxyl peroxide, are suggested to damage macromolecules in cells or can act as second messengers leading to alteration of gene expression and subsequent enhancement of cell proliferation (Jancsó and Hermesz, 2015). Liver is considered as the first target organ in arsenic metabolism where the element is subjected to methylation (Kannan et al., 2007). Cytotoxic and physiological dysfunctions in the liver, caused by arsenic toxicity, are associated with oxidative DNA damage, enhanced cell proliferation, altered DNA methylation, genomic instability and general hepto-toxicity (Yasmin et al., 2011). Kidney also has been considered as the second target organ for

arsenic toxicity. Pentavalent arsenic and organic arsenic are rapidly and completely eliminated via kidney (Tiwari et al., 2015). It is the main pathway of this metalloid into the organism, where absorption takes place in the stomach and intestines, followed by release into the bloodstream. In chronic poisoning, arsenic is then converted by the liver to a less toxic form, from where it is eventually largely excreted in the urine. Only very high exposure can, in fact, lead to appreciable accumulation in the body. Minor alternative pathways of entry are known through inhalation and dermal exposure (Caroli et al., 1996). After absorption through lungs and the gastro-intestinal tract, 95 to 99 % of the arsenic is located in erythrocytes, bound to the globin of hemoglobin and is then transported to the other parts of the body. About 70% of the arsenic is excreted mainly through urine. Most arsenic absorbed into the body is converted by the liver to less toxic methylated form that is efficiently excreted in the urine. The rate of decrease of arsenic in the skin appears to be especially low compared with the rate for other organs (WHO). On absorption, arsenic is stored in liver, kidney, heart, and lungs. The lower amount of arsenic is observed in muscles and neuronal tissues. The accumulation of arsenic in these tissues is associated with many disorders including cancer, diabetes, hepatotoxicity, neurotoxicity, and cardiac dysfunction (Klaassen et al, 1996).

N -acetylcysteine (NAC) is a metabolite of the sulphur-containing amino acid cysteine. It has the molecular formula $HSCH_2CH(NHCOCH_3)CO_2H$ and formula weight 163.19. In humans it can be administered orally or by intravenous infusion and can also be inhaled using a nebuliser. Currently it is used as an antioxidant and a mucolytic agent. The therapeutic potential of NAC has been examined and is currently being further investigated across a range of illnesses as an antidote for specific toxins, as a bio-protective agent against oxidative stress and ischaemic injury, and as a treatment for certain mental and physical illnesses. In addition, it is also sometimes used as a dietary supplement. NAC forms the dimers N –acetylcystine and N, N –diacetylcystine, it covalently bonds to plasma proteins and can be deactylated to form cysteine. The latter is the rate-limiting precursor for the endogenous antioxidant glutathione. Supplementation with NAC has been demonstrated to increase available glutathione by up to 510% in a malnourished population. NAC can be used as chelating agent, mucolytic and anti-inflammatory (Seetal et al.,2013).

Also, silymarin (SM) an extract from the Silybum marianum (milk thistle) plant containing various flavonolignans (with silybin being the major one), has received a tremendous amount of attention over the last decade as a herbal remedy for liver treatment. Direct scavenging free radicals and chelating free Fe and Cu are mainly effective in the gut. Preventing free radical formations by inhibiting specific ROS-producing enzymes, or improving integrity of mitochondria in stress conditions, are of great importance. Maintaining an optimal redox balance in the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants, mainly via Nrf2 activation is probably the main driving force of antioxidant (AO) action of SM. Decreasing inflammatory responses by inhibiting NF-κB pathways is an emerging mechanism of SM protective effects in liver toxicity and various liver diseases. Activating vitagenes, responsible for synthesis of protective molecules, including heat shock proteins (HSPs), thioredoxin and sirtuins and providing additional protection in stress conditions (Surai, 2015).

Accordingly, the present study was designed to investigate the beneficial effects of NAC and or silymarin on biomarkers of oxidative stress and antioxidant enzymatic states in serum and hepatic tissue in addition to changes of pro-inflammatory cytokines in arsenate intoxicated rats.

2. MATERIALS AND METHODS

2.1. Experimental animals

Seventy-five white male albino rats of 12-16 weeks old and weighting 180 – 220 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad-libitum. All rats were acclimatized for minimum period of two weeks prior to the beginning of study.

2.2. Chemicals and drugs used

All chemicals were of analytical grade and obtained from standard commercial suppliers .The drug and chemicals used in the present study were:

Sodium arsenate: sodium arsenate was obtained by Chemie Lab as solid material with high purity as 98% pure that was dissolved in distilled water, freshly prepared and administered orally and daily at a dose level of 41 mg/kg body weight (1/20 of LD_{50}) as described by Science lab and Labchem.

N-acetyl cysteine: NAC was obtained as pack of three ampoules of15mg powder. NAC manufactured by Chemie Lab and given orally

and daily at a dose level 200mg/kg body weight (Henderson et al, 1985).
Silymarin: was purchased by help of Sigma Company from their own manufacture as apure raw powder which manufactured by sigma and administered daily and orally at adose of 200mg/kg body weight (Samah and Ibrahim, 2012).

2.3. Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into five groups (15 rats each) placed in individual cages and classified as follow: Group I (control normal group): Rats received no drugs, served as control non- treated for all experimental groups. Group II (sodium arsenate exposed group): Rats received sodium arsenate 1/20 of LD50 (41mg/kg body weight) orally and once per day over a period of 8 weeks. Group III (sodium arsenate + NAC treated group): Rats received sodium arsenate (41 mg/kg body weight) and treated daily with NAC (200 mg/kg body weight) over a period of 8 weeks. Group IV (sodium arsenate + silymarin treated group): Rats received sodium arsenate (41 mg/kg body weight) and treated orally with silymarin(200 mg/kg body weight/day) over a period of 8 weeks. Group V (sodium arsenate+ NAC+ silymarin): Rats received sodium arsenate (41 mg/kg body weight) and treated daily with NAC (200 mg/kg body weight) + silymarin (200 mg/kg body weight/orally) over a period of 8 weeks.

2.4. Blood samples

Blood samples were collected by ocular vein puncture from all animal groups two times after (4 and 8 weeks) along the duration of experiment in dry, clean tubes and allowed to clot for30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pipette and received in dry sterile tubes, then kept in a deepfreeze at -20^{o}C for subsequent biochemical analysis. All sera were analyzed for the following parameters: total bilirubin, total protein, albumin, AST, ALT, ALP, γ-GT, TNF-α, and IL-6.

2.5. Liver tissue specimens for biochemical analysis

Liver specimen was taken two times from each groups of rats after had been sacrificed at four and eight weeks from the onset of the experiment. The specimen were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analyses. Briefly, hepatic tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant were used for the determination of the following parameters: Catalase (CAT), superoxide dismutase(SOD), Glutathione peroxidase (GPX) and L-Malondialdhyde (L-MDA).

2.6. Tissue specimens (Liver and kidney) for histopathological examination

Liver and kidney specimen were preserved in 10% buffered neutral formalin. The fixed tissue were rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax. 5µm thick sections were cut and stained with hematoxylin and eosin (H&E) and then the tissues were examined microscopically according to (Banchroft et al, 1996).

2.7. Biochemical analysis

Serum ALT and ASTwere determined by the method of Bergmeyer et al (1986), ALP, γ-GT, total bilirubin, Total protein, albumin, IL-6 and TNF-α were determined according to the method described by Tietz et al, (1983), Szasz (1969), Young (1995), Burtis et al,(2012), Doumas et al, (1971), Chan and Perlstein, (1987) and Beyaert and Fiers, (1998), respectively. Moreover, hepatic tissue L-MDA, SOD, CAT and GPx were determined according to the method described by Mesbah et al, (2004), Kakkar et al., (1984), Luck, (1974) and Gross et al (1967), respectively.

2.8. Statistical analysis

The results were expressed as mean ± SE and statistical significance was evaluated by ANOVA using SPSS (version10.0) program followed by the post hoc test,least significant difference (LSD). Values were considered statistically significant when $P<0.05$.

3. RESULTS

3.1. Protective effect of NAC and or silymarinon some serum and hepatic tissue parameters in sodium arsenate in toxicated rats (four weeks)

The obtained data in table (1) revealed that, a significant decrease in serum total protein and albumin concentrations and hepatic tissue SOD, CAT and GPx activities with significant increase in serum total bilirubin, Hepatic markers enzymes

(ALT, AST, ALP, GGT) activities, TNF-α, IL-6 and hepatic tissue MDA concentrations were observed in sodium arsenate intoxicated rats after four weeks of experiment when compared with normal control group. Treatment with NAC to arsenic exposed rats showedsignificant decrease in serum TNF-α, IL-6, ALT, AST, and liver MDA level.. However, a non-significant increase in serum total proteins, albumin with significant increase in hepatic tissue CAT, GPX and SOD activities were observed in NAC treated rats as compared with sodium arsenite exposed group. Also, four weeks silymarin treatment to arsenic intoxicated rats showed significant decrease in hepatic tissue MDA and in serum total bilirubin, AST, ALT, ALP, GGT, IL-6 and TNF-α with a non significant increase in serum total proteins concentration. Moreover, a significant increase in serum albumin and hepatic tissue CAT, GPX and SOD activities were observed when compared with sodium arsenate exposed group. Four weeks administration of NAC along with silymarin to arsenic exposed rats showed significant decrease in hepatic tissue MDA and in serum total bilirubin, AST, ALT, ALP, GGT, IL-6 and TNF-α. However, hepatic tissue CAT, GPX and SOD activities and serum total proteins and albumin concentrations were significantly increased when compared with sodium arsenate exposed group.

3.2. Protective effect of NAC and or silymarinon some serum and hepatic tissue parameters in sodium arsenate in toxicated rats (eight weeks)

The obtained results presented in table (2) revealed that, arsenate intoxicated rats for eight weeks showed significant decrease in serum total protein and albumin, hepatic tissue CAT, GPx and SOD with significant increase in liver tissue MDA and serum total bilirubin levels, liver markers enzymes(AST, ALT, ALP, GGT) activities, IL-6 and TNF-α concentrations when compared with control normal group. On the other hand, treatment with NAC in arsenic intoxicated rats for eight weeks showed significant decrease in serum total bilirubin, AST, ALT, GGT, IL-6, TNF-α and hepatic tissue MDA with a non significant decrease in serum ALP activity. While, a significant increase in hepatic tissueCAT, GPX, SOD and a non significant increase in serum total protein and albumin levels were observed as compared with sodium arsenate exposed rats. Also, eight weeks silymarin treatment to arsenic intoxicated rats showed significant decrease in serum total bilirubin, AST, ALT, ALP, GGT, IL-6,TNF-α and hepatic tissue MDA with significant increase in serum total protein, albumin and CAT,

Table (1): Protective effect of NAC and or silymarin on some serum and hepatic tissue parameters in sodium arsenate intoxicated rats (four weeks)

parameters	Group I	Group II	Group III	Group IV	Group V
Serum:					
Total bilirubin(mg/dl)	0.61± 0.07^{b}	0.98± 0.07^{b}	0.62±0.08^{b}	0.57± 0.08^{b}	0.70± 0.06^{b}
Total protein (gm/dl)	6.94±0.28^{a}	4.87±0.31^{b}	5.00±0.03^{b}	5.27±0.33^{b}	6.34±0.28^{a}
albumin (gm/dl)	4.26 ± o.25^{a}	2.95±0.07^{c}	3.31±0.08^{c}	3.44±0.22^{bc}	3.96±0.24^{ab}
AST (U/L).	86.96±12.10^{c}	324.76±25.99^{a}	300.26±32.27^{a}	177.70±17.05^{b}	104.80±3.23^{c}
ALT (U/L).	69.91±2.07^{b}	245.97±30.35^{a}	232.04±27.08^{a}	117.36±9.57^{b}	68.89±9.58^{b}
ALP (U/L)	152.97±20.68^{d}	369.02±9.63^{a}	288.11±6.99^{b}	232.81±27.84^{c}	190.25±11.74^{cd}
GGT (U/L)	34.37±8.33^{d}	93.63±11.33^{a}	78.64±7.58^{ba}	64.22±6.8^{cb}	45.19±3.74^{cd}
IL-6 (pg/ml)	100.98±24.31^{c}	349.17±19.68^{a}	277.83±30.55^{a}	199.64±23.16^{b}	147.47±11.44^{bc}
TNF (pg/ml)	13.76±1.62^{d}	52.76±7.06^{a}	41.19±3.03^{ab}	33.02±2.97^{cb}	26.36±1.26^{c}
Liver tissue:					
CAT (mmol/g.tissue)	72.23± 6.58^{a}	19.35±4.11^{c}	36.44±5.09^{b}	44.41±6.54^{b}	61.40±1.71^{a}
GPx(ng/g.tissue)	34.83±3.53^{a}	14.20±1.75^{c}	21.49±1.32^{b}	23.15±1.89^{b}	28.14±1.49^{ba}
SOD(u/g.tissu)	40.15±5.91^{a}	15.3±1.07^{d}	24.59±1.18^{dc}	26.82±2.71^{cb}	35.29±2.00^{ba}
MDA(mmol/g.tissue)	38.32±5.83^{c}	177.25±9.19^{a}	106.32±7.43^{b}	124.94±1.88^{b}	33.49±6.99^{c}

Data represented as (Mean±S.E) S.E=Standard error. Mean values with different superscript letters in the same row are significantly different at (P≤0.05). Group I (control normal), Group II (sodium arsenate), Group III (sodium arsenate + NAC), Group IV (sodium arsenate + silymarin), Group V (sodium arsenate +NAC+ silymarin).

Table (2): Protective effect of NAC and or silymarin on some serum and hepatic tissue parameters in sodium arsenate intoxicated rats (eight weeks)

parameters	Group I	Group II	Group III	Group IV	Group V
Serum:					
Total bilirubin (mg/dl)	0.55±0.06^{b}	0.91±0.02^{a}	0.55±0.06^{b}	0.53±0.04^{b}	0.51±0.05^{b}
Total protein (gm/dl)	7.23±0.38^{a}	4.96±0.24^{b}	5.09±0.22^{b}	6.81±0.25^{a}	6.80±0.21^{a}
albumin (gm/dl)	4.53±0.23^{a}	3.14±0.17^{b}	3.33±0.33^{b}	4.34±0.16^{a}	4.35±0.29^{a}
AST (U/L).	96.97±10.78^{b}	222.16±13.89^{a}	151.54±29.45^{b}	94.05±13.89^{b}	98.84±9.13^{b}
ALT (U/L).	75.48±10.63^{b}	167.02±24.67^{a}	98.22±8.33^{b}	74.87±13.81^{b}	79.18±11.44^{b}
ALP (U/L)	179.92±17.67^{cb}	286.31±10.17^{a}	249.72±17.46^{a}	198.80±13.16^{b}	139.16±6.57^{c}
GGT (U/L)	46.67±6.39^{b}	85.61±8.09^{a}	66.49±9.70^{ba}	50.14±10.11^{b}	48.91±5.12^{b}
IL-6 (pg/ml)	84.61±16.97^{d}	301.39±220.75^{a}	220.42±21.50^{b}	140.63±15.64^{dc}	157.08±24.49^{cb}
TNF (pg/ml)	13.90±1.48^{c}	47.03±3.60^{a}	37.28±5.94^{ba}	25.14±5.09^{cb}	20.59±2.45^{c}
Liver tissue:					
CAT (mmol/g.tissue)	56.46±10.08^{a}	19.35±4.11^{c}	31.79±4.39^{cb}	47.44±3.84^{ba}	59.98±2.40^{a}
GPx(ng/g.tissue)	31.35±2.27^{a}	13.21±0.87^{c}	17.54±0.10^{b}	18.07±0.32^{b}	27.63±1.51^{a}
SOD(u/g.tissu)	37.28±6.46^{a}	12.86±1.51^{d}	17.59±1.20^{dc}	24.62±1.43^{cb}	33.45±1.87^{ba}
MDA(mmol/g.tissue)	29.62±6.78^{d}	177.25±9.19^{a}	123. 87±7.56^{b}	68.07±17.35^{c}	42.43±9.60^{dc}

Data represented as (Mean±S.E) S.E=Standard error. Mean values with different superscript letters in the same row are significantly different at (P≤0.05). Group I (control normal), Group II (sodium arsenate), Group III (sodium arsenate + NAC), Group IV (sodium arsenate + silymarin), Group V (sodium arsenate +NAC+ silymarin)

Group I: control normal. Microscopical examination of the kidneys and liver of this group showed normal histological structure) as well as normal histological feature was also seen in the liver. Group II: Rats were given sodium arsenate (41mg/Kg.b.wt/day) in drinking water for eight weeks. The liver of ratsgiven sodium arsenate in drinking water for eight weeks showed severe congestion and dilatation of the central vein and portal blood vessels(Fig. A)the hepatocytes suffering from degenerative changes in the form of vacuolar degeneration as the hepatocytes showing the presence of faint eosinophilic single or multiple vacuoles in the cytoplasm. Group III: Rats were given sodium arsenate (41mg/Kg.b.wt/day) orally together with NAC (200mg/Kg.b.wt/day) for eight weeks. The liver of ratsgiven sodium arsenate in combination with NAC for eight weeks showed severe congestion of the portal blood vessels with degeneration of hepatocytes in the hepatic parenchyma in the form of vacuolar and hydropic degeneration (Fig. B).Moreover ,the bile duct showing mild thickening of their wall with mild degree of hyperplasia. Group IV: Rats were given sodium arsenate (41mg/Kg.b.wt/day) orally together with silymarin (200mg/Kg.b.wt/day) for eight weeks. Liver of rats treated with sod. Arsenate and silymarin showed congestion of central vein and portal blood vessels, the hepatocytes were suffering from degenerative changes in the form of vacuolar and hydropic degeneration together with presence of minute fat vacuoles in some of the affected hepatocytes (Fig. C). Group V: Rats were given sodium arsenate (41mg/Kg.b.wt/day) orally together with NAC (200mg/Kg. b. wt/day) and silymarin (200 mg/Kg.b.wt/day)for eight weeks. Liver of rats treated with sod. Arsenate together with NAC and silymarin showed congestion and dilatation of the central vein with mild degenerative changes in the form of hydropic degeneration (Fig.D)**.** GPX and SOD in hepatic tissue. Moreover, eight weeks treatment of NAC along with silymarin to arsenic exposed rats showed significant decrease in serum total bilirubin, liver markers enzymes, pro-inflammatory cytokines (IL-6 and TNF-α) and hepatic tissue MDA with significant increase in serum total protein, albumin and hepatic tissue antioxidant enzymes (CAT , GPX and SOD) activities as compared with sodium arsenate exposed rats.

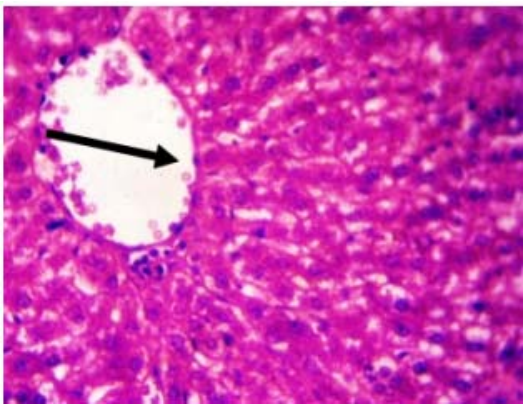

Figure (A): Liver of rats treated with sodium arsenate (41mg/ kg.b.wt./day) orally for eight weeks showed severe congestion and dilatation of the central vein. H&E x 400

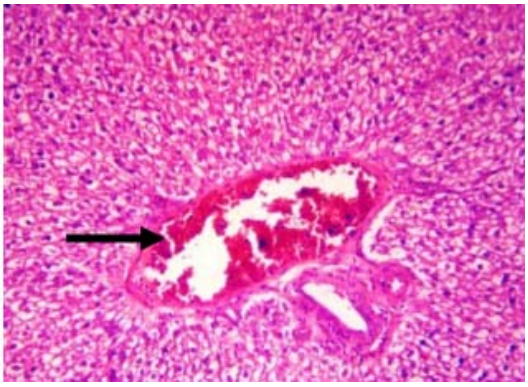

Figure (B): Liver of rats received sodium arsenate (41mg/kg.b.wt/day) orally together with NAC (200mg/kg.b.wt/day)for eight weeks showing severe congestion of the portal blood vessels with degeneration of hepatocytes.H&Ex 200

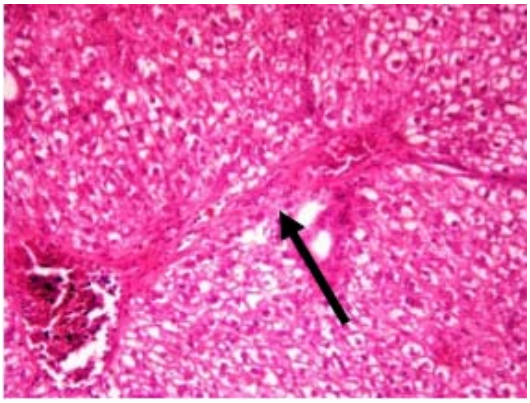

Figure (C): Liver of rats received sodium arsenate (41mg/ kg.b.wt./day) orally together with silymarin (200 mg/ kg.b.wt./day) for eight weeks showed mild proliferation of fibrous connective tissue in the portal area. H&E x 200

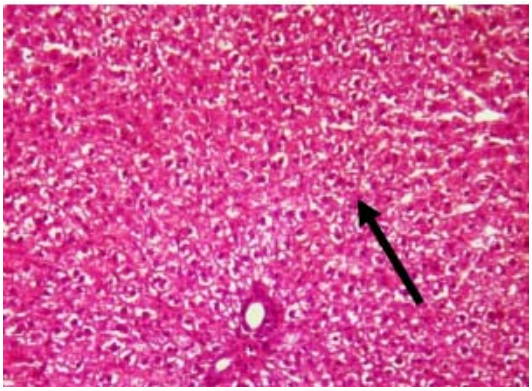

Figure (D): Liver of rats received sodium arsenate (41mg/ kg.b.wt./day) orally together with silymarin (200 mg/ kg.b.wt./day) and NAC (200 mg/ kg.b.wt./day) for eight weeks showing mild preductal mononuclear cellular infiltration.H&E x 200

4. DISCUSSION

Liver is considered as the first target organ in arsenic metabolism where the element is subjected to methylation (Kannan et al., 2007). Cytotoxic and physiological dysfunctions in the liver, caused by arsenic toxicity, are associated with oxidative DNA damage, enhanced cell proliferation, altered DNA methylation, genomic instability and general heptotoxicity (Yasmin et al., 2011). Kidney also has been considered as the second target organ for arsenic toxicity. Pentavalent arsenic and organic arsenic are rapidly and completely eliminated via kidney (Tiwari et al.,2015).The obtained results exhibited significant increase in serum liver markers enzymes ALT, AST, ALP and GGT activities and total bilirubin concentration in sodium arsenate intoxicated rats. While serum total protein and albumin concentrations were significantly decreased.These results are nearly similar to those described by Muthumani and Prabu, (2012) who reported that, oral administration of arsenic caused abnormal liver function in rats and the activities of serum liver specific markers enzymes, such as ALT, AST, ALP, lactate dehydrogenase and GGT and the level of total bilirubin were significantly increased in arsenic-treated rats when compared with control rats. Moreover, Owumi et al., (2013) showed that, sodium arsenite treatment in rat's significantly elevated serum ALT, AST, ALP and GGT activities. Recently, Ola and Akinrinde, (2016) reported that, rats treatment with sodium arsenite caused an increase in serum ALT, AST, ALP and GGT activities. Who added that, the values of serum total protein, albumin, and globulin levels

were reduced in rats exposed to sodium arsenite.Infact, the major site of arsenic metabolism is liver and thus arsenic exposure causes liver disease in exposed humans (Das et al., 2012). Also, liver is an important target organ for arsenic toxicity and its importance as an organ for arsenic biotransformation is well established in its enzymatic reactions (Muthumani and Prabu, 2012). In vivo, the exact cellular mechanisms by which arsenic produces hepatotoxicity are still unclear, but the advancement of research over the past decade demonstrated that oxidative stress is the key contributor in arsenic-induced hepatic injury as it is known to produce ROS, namely superoxide (O^{2-}), peroxyl radicals (ROO^{-}), hydrogen peroxide (H_2O_2), and hydroxyl (^{-}OH) (Liu et al., 2001). Moreover, exposure to arsenic was reported to depress the antioxidant defense system (Sharma et al., 2007) leading to cellular macromolecules oxidative damage including proteins, DNA, and lipids that cause damage of cell membrane (Das et al., 2012).

Treatment with n-acetyl-cysteine and silymarin individually or in combination in sodium arsenate intoxicated rats significantly reduced elevated serum ALT, AST, ALP and GGT activities and total bilirubin level and markedly increased the values of serum total protein and albumin concentrations after 8 weeks of experiment. These findings are in harmony with results of other studies concerning the protective role of NAC and silymarin in liver pathology against carbamazepine-induced hepatotoxicity. Maheswari et al., (2014) reported that, NAC treated rats significantly reduced the values of serum ALT, AST, ALP, bilirubin and liver weight and increased the values of serum total protein and albumin concentrations and body weight. The author attributed such effects to the property of NAC as antioxidant and it exerts hepato-protection and reduces lipid peroxidation due to its antioxidant activity. Also, Kaya et al., (2008) reported that, a significant elevation in serum AST and ALT activities were observed after treatment with the immunosuppressive agent cyclosporine A in rats. Who concluded that, NAC treatment prevented the toxic side effects induced by cyclosporine A through the antioxidant and radical scavenging effects of NAC? Regarding to the hepatoprotective role of silymarin, the obtained results are nearly similar to Sabiu et al., (2015) who reported that, circulating activity of serum ALT, AST and ALP and total bilirubin level were significantly reduced while, total protein concentration showed a significant increase after administration of silymarin with vitamin C, in case of hepatic oxidative insults in Wistar rats. Also, Kabiri et al., (2013) study the effect of silymarin on liver injury induced by thioacetamide in rats and found that, silymarin administration significantly reduced the activity of serum ALT, AST and ALP and total bilirubin level. Furthermore, Elshiaty (2011) reported that, silymarin administration significantly protect against hepatotoxicity induced by acetaminophen. This protective role was manifested by a significant decrease in activity of serum ALT, AST and ALP with marked increase in serum albumin concentration. Recently, Kelany and Abdallah, (2016) reported that, silymarin has protective effects against ketoprofen-induced hepatotoxicity in rats.

Treatment with sodium arsenate in rats exhibited significant increase in serum IL-6 level when compared with control normal group. Similarly, Aras et al., (2015) reported that, the induced oxidative and inflammatory macromolecular damage in Wistar rats by sodium arsenate which associated with significant increase in serum IL-6 concentration. Verma et al., (2010) found that, sodium arsenate significantly increased serum IL-6 level. IL-6 is a cytokine not only involved in inflammation and infection responses but also in the regulation of metabolic, regenerative, and neural processes (Scheller et al., 2011). Chronic arsenic exposure has been linked to an increased risk of vascular diseases. One of the molecular mechanisms through which arsenic causes injuries to blood vessels is the expression of some related genes involved in the animal endothelial cells including IL-6. The mRNA levels of IL-6 were significantly increased by arsenic (Wang et al., 2012). Also, chronic exposure to arsenic causes cutaneous diseases such as hyperkeratosis and skin cancer. Sodium arsenite increase IL-6 production in human epidermal keratinocyte cells (Sumi et al., 2016).

Concerning the protective role of NAC and silymarin in arsenic intoxicated rats the obtained results revealed that, administration of n-acetyl-cysteine and silymarin individually or in combination in sodium arsenate intoxicated rats significantly reduced elevated serum IL-6 concentration. These results are nearly similar with the findings of Aldbass et al., (2013) who reported that, NAC administration reduced the cellular production of pro-inflammatory mediators as IL-6 in propionic acid induced brain intoxication in rats. Also, Saleh., (2015) demonstrated that, daily oral administration of NAC significantly reverse the increased expression of pro inflammatory cytokines of IL-6 and tumor necrosis factor-α in the rat cerebral cortex. Moreover, Tseng et al., (2015) observed that, supplementation with NAC reduces ROS production and the released of pro-

inflammatory cytokines including IL-6 and TNF-α. Furthermore, Alkaladi and Abdelazim, (2012) studied the protective role of silymarin in liver carcinogenesis induced in rats. Who reported that, circulating IL-6 was significantly lowered in silymarin treated groups. Silymarin post-treatments were effectively able to relieve most of the above imbalances that induced rat liver damage including reverse of the increase in circulating IL-6 levels (Abdel-Moneim et al., 2015). The obtained results showed significant increase in serum TNF-α level in sodium arsenite exposed rats. Similarly, Adil et al., (2015) found that, there was significant up-regulation in hepatic TNF-α mRNA expression in sodium arsenate treated rats as compared to normal rats. Moreover, Ramanathan et al., (2005) reported that, a significant increase in the level of TNF-α was observed in leucocyte, liver and kidney of arsenic intoxicated rats. TNF-α is an important pro-inflammatory chemokines and cytokines that play a key role in adaptive and innate immunity. Due to its pro-inflammatory characteristic it involves in recruitment and activation of inflammatory cells at the injury sites (Ernandez and Mayadas, 2009). It is well described that ROS is produced by cytokines and elevated level of ROS by the metabolism of arsenic may be responsible for the activation of TNF-α synthesis (Schreck et al., 1991). It has been already documented that ROS generation is the consequences of arsenic exposure that leads to tissue fibrosis (Guha, 2008). TNF-α play a foremost role in the actuation of hepatic stellate cells (HSCs), predominant cells impart in the creation of collagen type-I in the liver and involve in remodeling of the extracellular matrix and histological hepatic fibrosis (Friedman, 2008).

Treatment with n-acetyl-cysteine and silymarin separately or in combination in sodium arsenate intoxicated rats significantly reduced elevated TNF-α level. Similarly, Wang et al., (2011) shown that, NAC can decrease the TNF- alpha in liver tissue and the expression of the nuclear factor-kappa B, thus improve liver function. Also, Toklu et al., (2008) demonstrated that, rising in serum TNF-α level was significantly abolished with NAC and silymarin treatment against sepsis-induced oxidative damage in lung and brain tissues. Silymarin is a polyphenolic flavonoid extracted from the milk thistle that has a strong antioxidant activity and exhibits cytoprotective, anti-inflammatory, and anticarcinogenic effects (Matsuda et al., 2005) and is used clinically to treat chronic inflammatory liver disease and hepatic cirrhosis. Kim et al., (2012) demonstrates the possible protective effect of silymarin against diet induced non-alcoholic steatohepatitis (NASH) by disturbing the role of the inflammatory cytokine, TNF-α, and suppressing the activation of HSCs. Moreover, Sherif and Al-Gayyar (2013) suggested that, treatment with silymarin markedly decrease the elevated TNF-α. Also, Nouf et al., (2015) reported that, oral treatment of the intoxicated rats with silymarin markedly ameliorated liver TNF-α level. Likewise, Younis et al., (2016) showed that, the administration of silymarin resulted in a hepato-protective property by reduced hepatic TNF-α level.

The obtained results exhibited a significant decrease in hepatic tissue SOD, CAT and GPx activities in sodium arsenate intoxicated rats. These results are nearly similar to those reported by Prabu and Muthumani, (2012) who demonstrated that, oral administration of sodium arsenate in rats caused significant decrease in enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase). Moreover, Kadeyala et al., (2013) reported that, the activities of SOD, CAT, GPx and reduced glutathione level were lowered in arsenic treated rats. Also, Sannadi et al., (2013) reported that, the exposure to arsenic showed a significant decrease in the activity of antioxidant enzymes such as SOD, catalase and glutathione peroxidase. Recently, Adil et al., (2015) found that, the level of hepatic SOD and GSH were significantly decreased in sodium arsenate treated rats as compared to normal rats. Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. GPx is an essential antioxidant enzyme, which counteracts free radical generation. In addition, GPx plays principle roles in the reduction of organic hydro peroxides within membranes and lipoproteins in the presence of GSH. The decrease in the activity of GPx during sodium arsenate treatment may indicate a reduction in GSH contents and an increase in the peroxides levels. Thus, the inhibition of GPx involved in free radical removal has led to the accumulation of H_2O_2, which promoted lipid peroxidation and modulation of DNA, altered gene expression and cell death(Shila et al., 2005).Furthermore, GSH is a tripeptide non-enzymatic antioxidant that has a beneficiary role in the cell protection against arsenic-induced reactive oxygen species (Masella et al., 2005). GSH level declined in the kidney and liver of arsenic treated group compared to normal rats indicating oxidative stress resemble (Adil et al., 2015).On the other hand, catalase is a major antioxidant enzyme having heme as the prosthetic group which reduces hydrogen peroxide to molecular oxygen and water (Gutteridge, 1995) and NADH acts as a substrate or cofactor for

activation of this enzyme from its inactive form. The decrease observed arsenic intoxication also significantly reduced the CAT activity by decreasing the NADH, which is utilized in arsenic metabolism (Kirkman and Gaetani, 1984). In addition, as is known to produce superoxide radicals which also inhibit the activity of catalase (Ercal et al., 2001). Oxidative stress is an important feature of cell apoptosis and necrosis (Kandhare et al., 2015). It is well known that reactive oxygen species is generated during the process of cell death and an enhanced level of ROS occur due to the production of antioxidant and detoxifying enzymes catalase, or superoxide dismutase has a tutelary role (Kandhare et al., 2013). SOD enhances the dismutation of superoxide to H_2O_2, which is cleaved by catalase (Usoh et al., 2005). It has been revealed that the reaction of molecular oxygen with dimethylarsine (a metabolite of dimethyl arsenic acid) produce free radical species (Yamanaka and Okada, 1994), significantly decrease in the activity of SOD content may be due to the overproduction of superoxide during arsenic metabolism (Gupta and Flora, 2006). In this investigation, arsenic treated group clearly reflects significant reduction of SOD content in kidney and liver due to oxidative stress which is in accordance with the previous study (Gupta and Flora, 2006 and Prabu and Muthumani, 2012).

Treatment with n-acetyl-cysteine and silymarin individually or in combination in sodium arsenate intoxicated rats significantly increased hepatic tissue CAT, GPx and SOD activities. Ortolani et al. (2000) explained that, NAC is a well-known artificial precursor of GSH. Its antioxidant and antitoxic effects in animal models and humans have been demonstrated (Sarnstrand et al., 1995). NAC both increases GSH levels (Bernard, 1991) and acts as a powerful oxygen free radical scavenger, yielding NAC-disulfide end products (Zhang et al., 1995). In addition to that, Duru et al., (2008) investigated the protective role of NAC in a rat model. Who establish that, NAC increased SOD and GPx activities in serum and renal tissue. NAC is a very effective precursor and stimulator of glutathione synthesis and in many disorders it has been proved that NAC augments glutathione production. Furthermore, Ramos et al ., (2009) approved that, in the liver of rats treated with arsenic, pretreatment with NAC increased the levels of GSH and decreased lipid peroxidation. Pudari et al., (2013) reported that, NAC is a thiol-containing antioxidant that used to reduce different conditions of oxidative stress. Its antioxidant action is related to GSH synthesis. Hence, maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS). It is also known as potent metal chelator. NAC has a strong ability to restore the impaired pro-oxidant /antioxidant balance in metal poisoning. Also, Yerradoddi et al., (2013) observed that, N-Acetyl-cysteine (NAC) is a thiol-containing antioxidant that has been used to reduce different conditions of oxidative stress. Its antioxidant action is attributed to GSH synthesis; therefore maintaining intracellular GSH levels. Moreover, Raquel et al, (2016) establish that, NAC can alleviate different effects of arsenic toxicity on different antioxidants enzymes as catalase and glutathione peroxidase which decreased by arsenate toxicity. Who added that, NAC can decrease the effects caused by oxidative stress. So evaluation of whether the antioxidant ability of NAC was able to restore the anti- and pro-oxidant balance of the cells and thus mitigate the harmful effects of As_2O_3. This is due to the fact that NAC is a precursor of glutathione (GSH), an important enzyme of the cellular antioxidant system that is able to stimulate and maintain its intracellular levels, which detoxify ROS. Moreover, NAC has been shown to be effective in metal chelation.On the other hand, Mansour et al., (2006) suggested that, silymarin significantly elevated the decreased in activities of SOD, GPx and GSH due to oxidative stress and hepatotoxicity in rats. Also, Kiruthiga et al., (2007) suggested that, silymarin possess substantial protective effect and free radical scavenging mechanism against environmental contaminants induced oxidative stress damages. Who found that, the protective effect of silymarin is elucidated by the significant increase of the antioxidant enzymes activities. Silymarin affects intracellular glutathione which prevents lipid peroxidation of membranes (El-Shitany et al., 2008). Lastly, Rajnarayana et al., (2004) demonstrated that, silibinin could renew the impairment of antioxidant defense system in the liver of arsenic-treated rats and this returned to its antioxidant and chelating properties which could be due to the orientation of hydroxyl groups in the 3rd, 5th and 7th positions in silibinin.

Treatment of rats with sodium arsenate caused a significant increase in liver L-malondialdehyde (L-MDA) concentration when compared with control normal group. MDA, an end-product of lipid peroxidation mediated by oxygen free radicals, is believed to be an important cause of destruction and damage to cell membranes and has been suggested to be a contributing factor in the development of sepsis mediated tissue injury (Sener et al., 2005). Lipid peroxidation is a vital marker for toxicity induced by various xenobiotics and is thought to be a consequence of oxidative

stress initiated when the dynamic balance between peroxidant and antioxidant mechanism is impaired. It has been demonstrated previously that, arsenic treatment to animals increased the levels of LPO, even at low doses leading to apoptosis and necrosis of brain cells, suggesting cellular injury by action of free radicals. Therefore, increase in LPO observed in the present study following arsenic treatment to rats, could be a consequence of increased free radical production and/or consequent suppression in the activity of antioxidant defense enzymes and glutathione levels (Shveta et al., 2016). As confirmed by Xinjuan et al., (2014) who reported that, oxidative stress may have caused further lipid peroxidation, directly damaging the membranes of cells and organelles and leading to the release of reactive aldehydes with potent pro inflammatory and pro-fibrotic properties. Iwan et al., (2015) indicated that, the increasing of heavy metal concentration exposure lead to an increase in MDA level. Likewise, Morakinyo et al., (2010) indicated that, arsenate treatment significantly elevated lipid peroxidation index, MDA, in comparison with the control. An increased MDA concentration might be a result of decreased production of antioxidants in the sodium arsenate treated rats. Furthermore, Sana et al., (2014) demonstrated that, arsenic administration to rats resulted in an increase in the production of malondialdehyde (MDA).The major effect of arsenic on the cellular membrane is believed to be peroxidation of membrane lipids.

However, NAC or and silymarin treatment to arsenic intoxicated rats significantly decreased hepatic tissue MDA concentration when compared with sodium arsenate exposed group. These findings are in agreement with the results reported by Fatemeh et al, (2012) who demonstrated that, NAC dramatically suppressed the increases in (ROS), lipid peroxidation, caspase cascade activity which happened due to arsenate toxicity. Also, Cibele et al., (2016) observed that, a process that leads to lipid peroxidation and DNA damage in exposed cells, remediation with NAC can possibly alleviate the adverse sides caused by As_2O_3. Moreover, Flora, (1999) demonstrated that, treatment with NAC partially reversed arsenic-induced alterations in hepatic MDA. Furthermore, NAC and silymarin, due to their antioxidant effects preventing lipid peroxidation, protected membrane integrity and normalized thromboplastic activity (Toklu et al., 2008). Younis et al., (2016) reported that, the administration of silymarin resulted in a hepato-protective property by decreased hepatic MDA levels. Additionally, Nencini et al., (2007) showed that,silymarin restored increased MDA and decreased GSH levels to the normal tissue. This could be due to the excellent antioxidant properties of silymarin. This suggestion was confirmed by Alessandro et al., (2017) who reported that, silybin acts through the turning-off of pro-inflammatory signals, derived from nuclear factor-κB (NF- κB) activation, involved in the induction of the synthesis of cytokines such as tumor necrosis factor- α (TNF-α), interleukin (IL)-1, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) .Furthermore, silybin induces apoptosis through the modulation of cytoplasmatic levels of bcl-2-like protein 4 (Bax) and B-cell lymphoma 2 (Bcl-2) proteins, cytochrome c release and caspase- 3 and 9 activation .The antioxidant activity is due to its capacity to act as both free radical scavenging and lipid peroxidation inhibitors, as demonstrated in vitro and in vivo.

5. CONCLUSION:

The potential ameliorating effect of NAC and or silymarine as powerful antioxidant agents in combating free radical-induced oxidative stress and tissue injury is a result of arsenic toxicity. The histopathological studies in the kidneys and liver of rats also supported that NAC and or silymarin markedly reduced the Arsenic induced pathological changes and preserved the normal histological architecture of the kidney and liver tissues. Thus, N-acetylcysteine and silymarin administration in intoxicated rats suppress the inflammatory process, inhibiting the apoptosis,ROS and many other inflammatory markers which are contributing factors in organ failure. Therefore, we recommended that, N-acetylcysteine and silymarin are very essential and should be used with safe therapeutic dose which may elevate the undesirable and dangerous toxic effects during heavy metals exposure

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