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***Andrographis paniculata* Nees Aqueous Extract Prevents the Oxidative Stress in Rats During Acute Liver Failure by Activating the Antioxidant Enzymes**

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Abstract: Acute liver failure (ALF) is the rapid onset of hepatic dysfunction in a patient without known pre-existing liver illness. ALF could result into multi-organ failure (MOF) by causing adverse effects on other organs including spleen, testis, kidney, and brain. Oxidative stress is believed to play central role in the development of MOF; however, effective treatment is not available. Present study was aimed to evaluate whether *Andrographis paniculata* Nees aqueous extract (APAE) has therapeutic potential against oxidative stress in liver, spleen and testis in a thioacetamide (TAA) induced experimental rat model of ALF.

In comparison to control groups, liver, spleen, and testis of rats with ALF showed significant decrease in the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione reductase (GR), which is followed by rise in lipid peroxidation (LPO) level and fall in total reduced glutathione (GSH) level. On the other hand, treatment of APAE normalised the LPO and GSH levels in liver, spleen and testis by activating the most antioxidant enzymes.

Findings of the present study suggested that oxidative stress causes adverse effect on TAA induced ALF as well as ALF mediated degeneration of spleen and testis. APAE has therapeutic role as an effective antioxidant in the treatment of ALF and oxidative damage occurs in spleen and testis due to ALF.

Keywords: Acute Liver Failure, *Andrographis paniculata* extract, Thioacetamide, Antioxidant enzymes, Oxidative stress, Reactive oxygen species, Free radicals

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Introduction

Liver failure or hepatic impairment is the incapability of liver to carry out its typical metabolic and synthetic function during normal

physiological condition (Thibaut *et al.*, 2022). Liver failure is a life-threatening illness that necessitates immediate medical attention. Most

frequently, liver failure develops gradually over many years. However, a rarer illness known as acute liver failure (ALF) manifests quickly (in as little as 48 h) and it can be challenging to recognise at first. The main precipitants include ammonia and free fatty acids, which are finally distributed in different tissues and organ where they may cause series of adverse effect on those tissues. ALF is a rare syndrome marked by rapid decline in normal liver function after an acute insult in patient (Dong *et al.*, 2019) leading to altered coagulation and mentation without a prior known chronic liver disease (Stravitz and Lee, 2019). The progression of the disease is accompanied by the emergence of a liver-related coagulopathy and clinically apparent altered level of consciousness brought on by hepatic encephalopathy (HE). The etiology of ALF include drug induced, metabolic, genetic, viral infections (hepatitis B, C, D, and E viruses), hemodynamic, oncologic injuries and autoimmune insults of liver (Squires *et al.*, 2018) and identifying the correct cause can be difficult. Patients with ALF develops a series of serious complications of multi-organ failure, haemorrhage, intracranial hypertension, infection, and leads to death of the patients (Rajajee *et al.*, 2017). Oxidative stress and its accompanying events play a critical role in the hepato-toxicity with different etiologies (Heidari *et al.*, 2018). Furthermore, it has been noted that TAA metabolites produce significant oxidative stress and the generation of mitochondrial dysfunction in the Thioacetamide (TAA) driven model of ALF (Jamshidzadeh *et al.*, 2017)). Generation of excessive production of reactive oxygen species (ROS) due to ALF induced by TAA can overwhelm the antioxidant defence system and harm cellular components such as proteins, lipids, and DNA which leads to disruption of cellular structure and function (Zargar *et al.*, 2017). ROS play vital role in the pathophysiology of ALF as the damaged or dead hepatocytes significantly increased oxidative stress in ALF, which again contributed to further damage and loss of hepatocytes (Tian *et al.*, 2018). As liver is one of the main metabolic regulating organs, and

due to which ALF leads to oxidative stress induced multi-organ failure and those are including spleen, testis, kidney, and brain.

Spleen is a major organ affected during liver failure as both the organ plays anatomical importance in the portal circulation and both plays crucial involvement in immune homeostasis and pathogen clearance (Nugroho 2020). Liver failure is frequently escorted by multiple complications which include hypersplenism and splenomegaly (Lee *et al.*, 2017). Hypersplenism in liver failure showed that NF- κ B p65/c-Rel signalling elevated in hyper splenic macrophages, contributing increased phagocytosis and secretion of both pro-inflammatory such as IL-1 β , IFN- γ , TNF- α , which is mediated by production of ROS and NOS due to hepatocellular damage (Lingappan, 2018).

While in the mammalian reproductive system, male infertility is major problem which is caused by excessive ROS (Ma *et al.*, 2018). The aetiology of poor fertility is heavily influenced by excessive oxidative stress and the creation of peroxynitrite (Jankovic and Stefanovic, 2014). However, the etiology of ROS increment in male infertility on the basis of peroxynitrite generation is not fully understood.

Patients suffering from ALF are often treated in intensive care unit at the hospital where liver transplant can perform, if necessary. But in many cases, controlling complications with treatment to heal liver is advisable. In recent decades many potent synthetic agents and/or treatments have been developed; one of them is acetylcysteine, which reverse the poisoning of ALF caused by acetaminophen. Other includes controlling of the disease progression but not fully cure them. Scientist all over the world continue to research new treatments to cure ALF, especially those that can minimises the need of a liver transplant. Some potent future treatments are explored, but it is important to remember that they are experimental and may not yet available. Therefore, the detection and evaluation of therapeutic drug from active components of

medicinal plant for their hepatoprotective nature is ongoing all over the globe, which may contain potential properties to cure ALF progression One of them is the *Andrographis paniculata*, which is used in the present study.

Andrographis paniculata Nees (AP) is an herbaceous plant commonly known as the “King of Bitters” found in Southern Asia (Dai *et al.*, 2019). Its leaves and roots are traditionally used for different medicinal purposes as remedy for removing toxins from body in Asia and Europe. In modern pharmacology AP has been investigated for its pharmacological properties such as antiviral, antibacterial, anti-inflammatory, hepatoprotective, emollient, and anti-fertility (Wang *et al.*, 2018). The aqueous extract of andrographolide also having significant effects on mental illness, neurological disorders and antimicrobial activity (Mittal *et al.*, 2016). Moreover, it has also been reported that the aqueous extract of AP significantly increased the activity of antioxidant defence enzymes and exhibited free radicals-scavenging ability (Nsir *et al.*, 2013). Previous report from our lab also stated that AP has significant effects on the Oxidative stress in various areas of the brain associated with HA. The aim of the present study was to look at how extract of *Andrographis paniculata* Nees protected the rat liver, spleen, and testes from oxidative damage brought on by ALF.

Materials and Methods

Animals:

Adult Sprague Dawley male albino rats (150g-170g) were selected, maintained at standard conditions in animal house and fed with recommended diet as directed by the Institutional Animal Ethical Committee (IAEC). This study has been approved by IAEC, Guru Ghasidas Vishwavidyalaya for the use of animal with Ref. No. 994/GO/Re/S/06/CPCSEA.

Chemicals:

Chemicals that used in this present study were of molecular and/or analytical grade of the best quality purchased from SRL (India), Sigma

Chemical Co (USA) and Himedia (India). Chemicals used for biochemical parameters are Coomassie Brilliant Blue R-250 (CBB), TEMED (N NN N-tetramethylethylene diamine) and phenyl methyl sulphonyl fluoride (PMSF) etc., and some are carried out using kit supplied by Sigma Chemical Co.

Preparation of APAE and dosage:

Andrographis paniculata Nees aqueous extract used in the present study was supplied by Nature and Nurture Healthcare Pvt Ltd., New Delhi, India in powder form. The plant specimen was identified to be the same one recorded in the herbarium of Royal Botanic Garden, Kew, Specimen no. K000545980. The extraction of the plant herb part was done following the method of Hawthorne *et al.* (1993), using hydro distillation process and filtration of the extract was done through NLT 100% 40 mesh screen. The rate of evaporation loss during drying of the extract was 1g/105°C/2h. The main compound isolated from this extract was 10% W/W Andrographolide.

The extract was introduced in rats orally by a metal gavage needle at a fixed dosage concentration of 250 mg/ kg b.w. daily up to seven days prior to induction of ALF. *Induction of ALF:*

ALF was induced by intraperitoneal injection of 300 mg TAA/kg b.w. prepared in 0.9% NaCl, once up to two days at an interval of 24 h as described earlier (Singh *et al.*, 2014); At the same time rats in the control group were received equivalent volume of physiological saline.

Experimental Design:

In this ALF experimental model, rats were categorized into four groups: Control group, ALF group, APAE control group, APAE pre-treatment with ALF group. Each group contains 5-7 rats and maintained in separate cages. All rats were acclimatized for one week prior to administration of therapeutic drug and induction of ALF. Rats of APAE control and APAE with ALF group were pre-treated for 7 days with APAE orally as mention above. Rats of ALF group and APAE pre-treatment with ALF groups are administrated with

aforementioned dosage of TAA to induce ALF after the pre-treatment of APAE. After 30 min of final injection all rats were sacrificed by decapitation method, then liver, spleen, and testis were dissected out, then tissue were washed with ice cold saline (0.9% NaCl) and stored at -80°C for further studies.

Preparation of tissue extracts:

Tissue extracts were prepared by homogenizing with 0.02M Tris-Cl (pH 7.4) buffer with protease inhibitors. The homogenate of the organs was centrifuged for 45 min at 35,000 g at 4°C , then supernatants obtained were collected and stored at -80°C for use in different parameters.

Biochemical Estimations:

Lipid peroxidation assay:

Malondialdehyde (MDA), a stable product of lipid peroxidation was estimated using the method of Heath and Packer (1968) with some alteration. Where, 1 mole of MDA reacts with 2 moles of thiobarbutaric acid (TBA) in an acid solution and forms trimethionine (pink color) substance having maximum absorption at 620 nm. Briefly, 1.5 ml of 0.5% TBA was diluted in 20% TCA with 0.5 ml of tissue extract and it was incubated at 95°C for 25 min. Reaction was stopped by incubating the reaction mixture on ice. Thereafter, sample were centrifuge for 5 min (15000 g, 4°C) to clear the solution. Absorbance was recorded at a wavelength of 450 and 620 nm. OD620 values were subtracted from the MDA-TBA complex values at 532 nm. Then the level of MDA concentration was calculated by using the Lambert-Beer law. The lipid peroxidation levels were expressed as μM MDA/g wet wt.

GSH Level:

Total thiol was estimated by the method of Sedlak and Lindsay (1968) with some alteration. 1.5 ml of 0.2 M Tris buffer, pH 8.2, and 0.1 ml of 0.01 M 5,5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) were combined with 0.1 ml of tissue extracts. The final volume of mixture was diluted to 10 ml by adding methanol and incubated for 30 min. Then it was

centrifuged at 3,000 rpm for 15 min and absorbance was recorded at 412 nm. To calculate GSH (reduced glutathione) molar extinction coefficient of 13,100 was used and units as nmol/mg protein.

SOD:

The activity of superoxide dismutase (SOD; EC: 1.15.1.1) was measured by using the standardized protocol of our lab. The reaction mixture containing 0.1 ml tissue extracts, 0.02 M sodium pyrophosphate buffer (pH 8.3), 30 μM nitroblue tetrazolium (NBT), and 6.2 μM phenazine methosulphate (PMS). Then the reaction was initiated by adding 50 μM NADH at 30°C and stopped at 90 s by adding 2.0 ml glacial acetic acid to the reaction mixture. The reaction mixture was stirred and shaken with 4 ml of n-butanol, then it is allowed to stand for 10 min. Then the butanol layer was separated by centrifugation. Absorbance at 560 nm was measured using butanol as blank. The activity of SOD was expressed as one unit/mg of protein, and one unit represents the quantity of the enzyme that provided 50% inhibition of NBT degradation per minute.

Catalase:

The activity of Catalase (EC: 1.11.1.6) was measured by using a reaction mixture of 0.01 M Potassium phosphate buffer (pH 7.0) and 0.1 ml tissue extract (Singh *et al.*, 2008). Then the reaction was started by adding 0.8 M H_2O_2 and to stopped it after 60s, 2 ml of dichromate acetic acid reagent was added to the reaction mixture. Then the tubes containing reaction mixture were incubated in a boiling water bath for 10 min. Following that, the tubes were cooled to room temperature, and absorbance at 240 nm was measured. Catalase activity was quantified as μM of H_2O_2 consumed/min/mg protein after comparing the results to a reference plot (with a range of 10 - 160 μM of H_2O_2).

GR assay:

Using Carlberg and Mannervik's method, glutathione reductase (GR; EC: 1.6.4.2) activity was assessed (Carlberg and Mannervik 1985).

Where the component of the reaction mixture of 1 ml are, 0.2 M sodium phosphate buffer (pH 7.0), 0.2 mM EDTA, 1 mM oxidised glutathione (GSSG), and 0.2 mM NADPH. The process started when the tissue extract was added, and the oxidation of NADPH was observed as a 5 min drop in absorbance at 340 nm. Nonspecific oxidation of NADPH was corrected by the absorbance obtained in the absence of GSSG. The enzyme activity was expressed as units/mg protein, and the unit of the enzyme in this process was defined as a mole NADP/min/ at 30°C.

NOx Level:

To estimate the nitric oxide level in sample, the level of NO₂ was measured by using Gries reagent following the method described earlier (Singh and Trigun, 2010). Separately, 100 µl of tissue fractions and 400 µl of 50 mM carbonate buffer were combined (pH 9.0). After centrifuging tubes, supernatant (400 µl) was collected and incubated with Griess reagent (200 µl 1% sulfanilamide prepared in 2.5% H₃PO₄ and 200 µl 0.1% N-naphthylethylenediamine). Following the incubation, absorbance was measured at 545 nm in comparison to a blank that had all the components but no sample.

Statistical Analysis:

All the experiments were conducted thrice using four replicas of each sample from each experimental group for all parameters used in this study. The data shown here as mean ± SD where n=3. Variance between groups were calculated through one-way analysis of variance (ANOVA) using Sigma plot 14.5 software. The minimum probability of p<0.01 and p<0.05 were taken as significant level between groups.

Results

LPO activity:

The level of lipid peroxidation was significantly increased (p<0.05) in the liver, spleen and testis of acute ALF group (Figs. 1a, 2a, 3a, respectively). On administration of APAE on ALF treatment group there was significant decrease (p<0.05) in the

activity of lipid peroxidation in the liver, spleen and testis as well of ALF groups (Figs. 1a, 2a, 3a, respectively).

GSH Level:

The GSH level of acute ALF group decreased significantly in liver (p<0.01), spleen (p<0.001) as well as in testis (p<0.01) as compared to control group (Figs.1b, 2b, 3b, respectively). On administration of APAE on ALF treatment group it restored the GSH level significantly (p<0.01) towards that of control (Figs.1b, 2b, 3b, respectively).

SOD activity:

The level of SOD in ALF treated rats decreased significantly in the liver (<0.01), spleen (p<0.001) and testis (p<0.01) (Figs. 1c, 2c, 3c, respectively) as compared to the control group. In ALF treatment groups, dosing of APAE significantly restored the SOD level in liver (p<0.05), spleen (p<0.01) and testis (p<0.01) towards control group (Figs. 1c, 2c, 3c, respectively).

Catalase activity:

In ALF groups, the level of catalase decreased significantly in the liver (p<0.05), spleen (p<0.01), and testis (p<0.05) (Figs. 1d, 2d, 3d, respectively). The treatment of APAE significantly (p<0.05) restored the catalase activity in liver, spleen, and testis of ALF treated groups (Figs. 1d, 2d, 3d, respectively).

GR activity:

The activity of Glutathione reductase significantly decreased in the liver (p<0.05) and testis (p<0.01) in ALF group (Figs. 1e, 3e, respectively) however, in spleen it was significantly increased (Fig. 2e). On administration of APAE to ALF groups, there was significant (p<0.05) increase in the activity of Glutathione reductase in testis of rats (Fig. 3e) and seems to be static in liver and spleen (Fig. 1e, 2e, respectively).

NO Level:

The NO level increased significantly in liver (p<0.05), spleen (p<0.01) and in testis (p<0.01) as

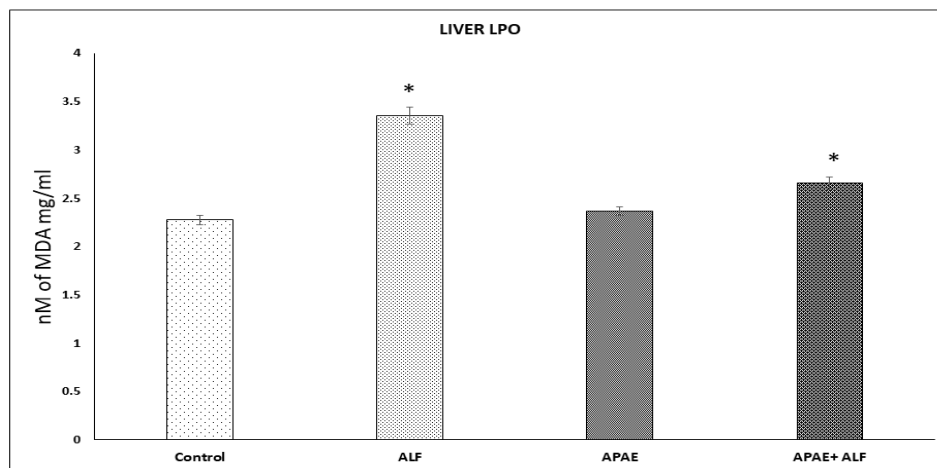


Fig. 1(a): Level of MDA; stable product of LPO significantly increased in liver of ALF group rats however, on treatment of APAE to the ALF treatment group significantly lower the level of MDA. Values represent mean $\pm$ SD where n = 3. Level of significance was determined by one-way ANOVA; *p<0.05.

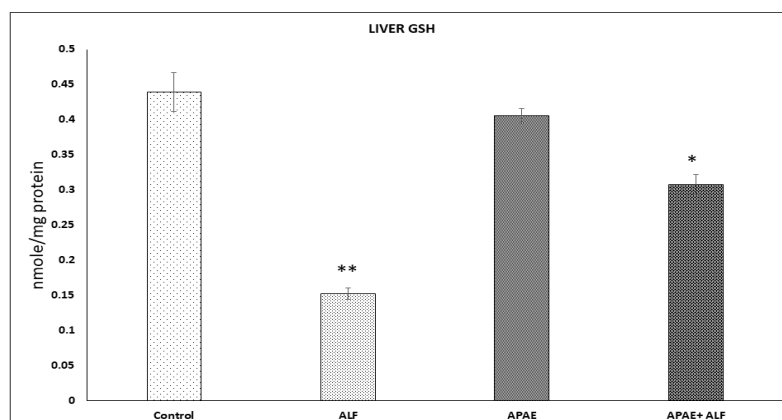


Fig. 1(b): Level of Glutathione; which is significantly decreased in liver of ALF group rats however, on treatment of APAE to the ALF treatment group significantly restore the level of Glutathione towards that of control group rats. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05 and **p < 0.01.

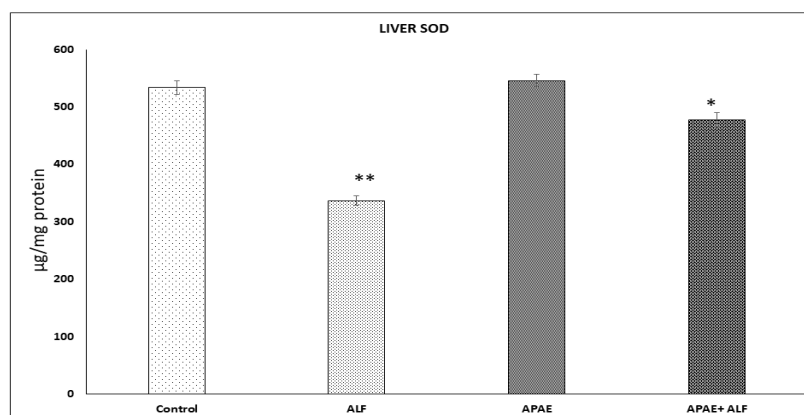


Fig. 1(c): Activity of Superoxide dismutase; which is significantly decreased in liver of ALF group rats however, on treatment of APAE to the ALF treatment group significantly restore the activity of SOD in liver of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05 and **p < 0.01.

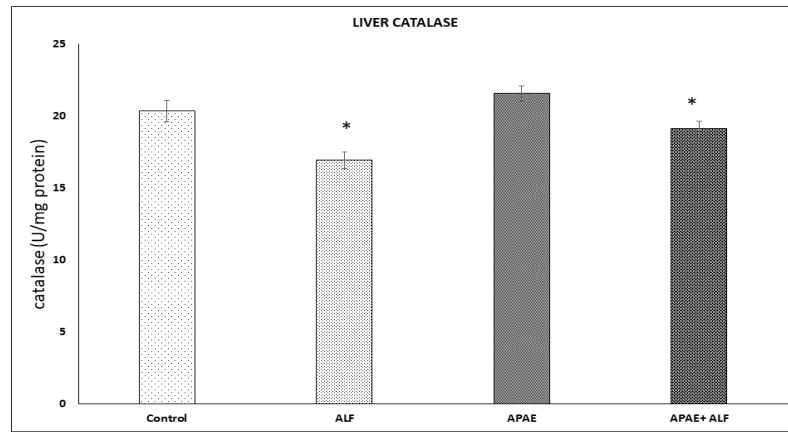


Fig. 1(d): Catalase activity in liver of ALF rat model; where catalase activity significantly decreased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group significantly restore the activity of catalase in liver of rats with ALF. Values represent mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$.

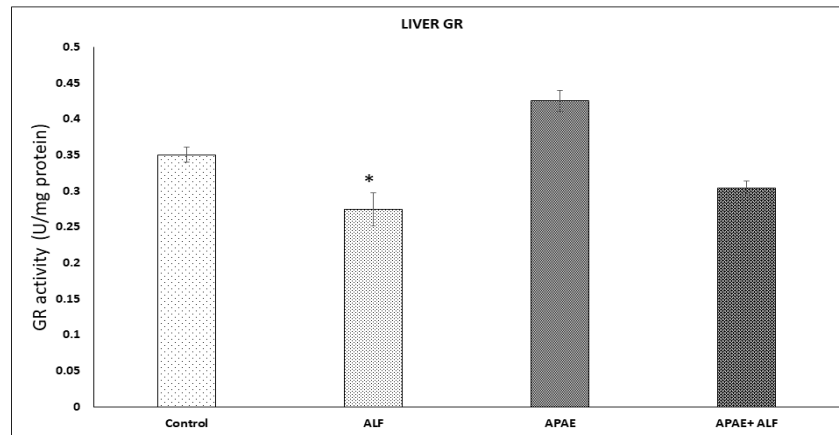


Fig. 1(e): Activity of Glutathione reductase in liver of ALF rat model; where GR activity significantly decreased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group the activity of catalase seems to be static in liver of rats with ALF. Values represent mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$.

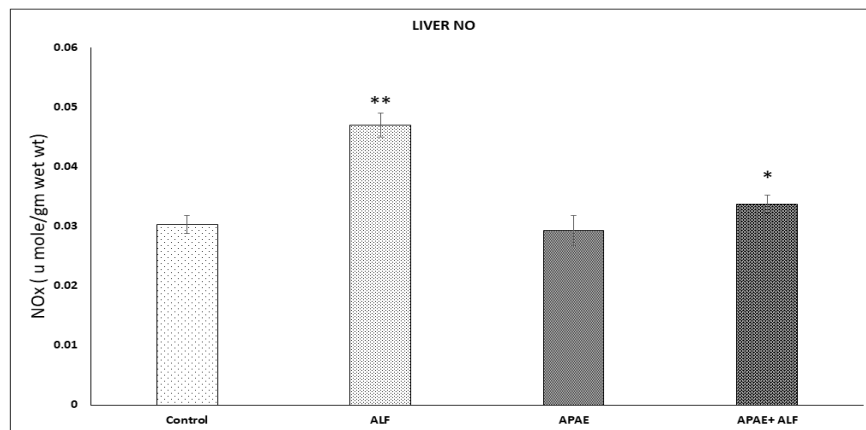


Fig. 1(f): Level of NOx in liver of ALF rat model; where NOx level significantly increased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group the NOx level significantly decreased in liver of rats with ALF. Values represent here as mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$ & ** $p < 0.01$.

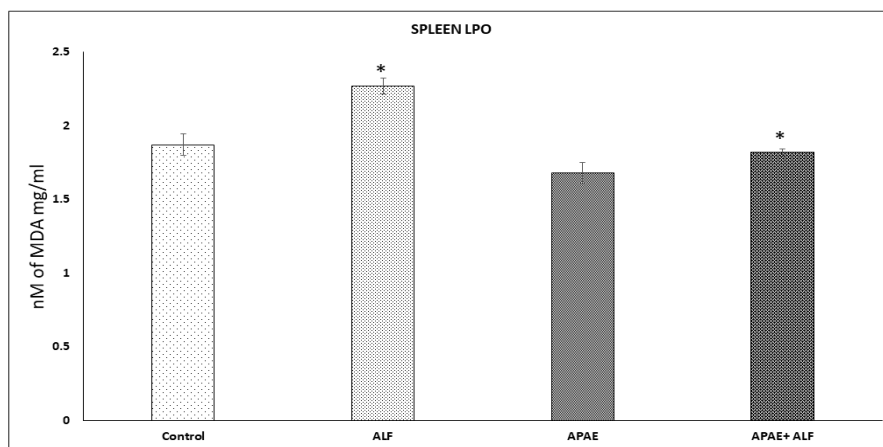


Fig. 2 (a): Level of MDA; stable product of LPO significantly increased in spleen of ALF group rats. however, on treatment of APAE to the ALF treatment group significantly lower the level of MDA towards that of control group rats. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05.

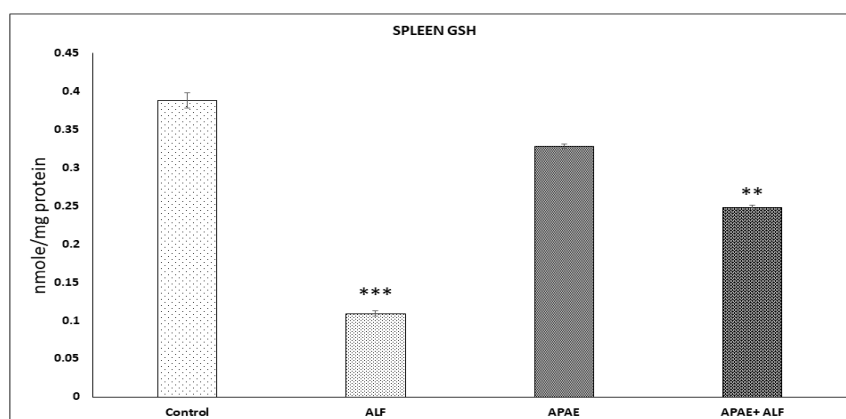


Fig. 2(b): Glutathione level in spleen of ALF rat model; where GSH level significantly decreased in spleen of ALF group rats. however, on treatment of APAE to the ALF treatment group significantly restore the level of Glutathione towards that of control group rats. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; **p<0.01 and ***p < 0.001.

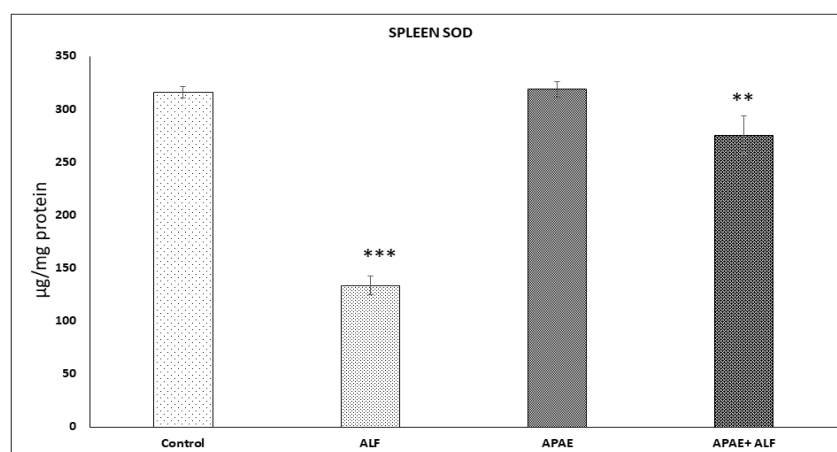


Fig. 2(c): Activity of SOD; which is significantly decreased in spleen of ALF group rats however, on treatment of APAE to the ALF treatment group significantly restore the activity of SOD in liver of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; **p<0.01 and ***p < 0.001.

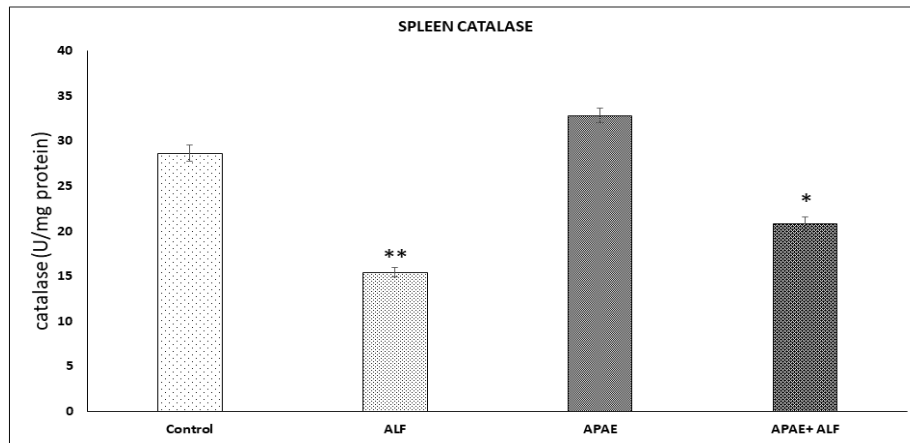


Fig. 2(d): Activity of catalase in spleen of ALF rat model; where catalase activity significantly decreased in spleen of ALF group rats. However, on treatment of APAE to the ALF treatment group significantly restore the catalase activity in spleen of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05 and **p<0.01.

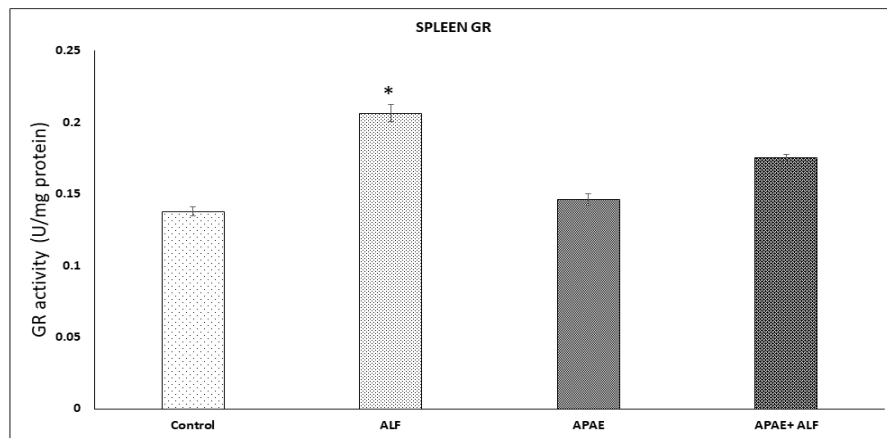


Fig. 2(e): Glutathione reductase activity in spleen of ALF rat model; where GR activity significantly increased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group the activity of catalase seems to be static in spleen of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05.

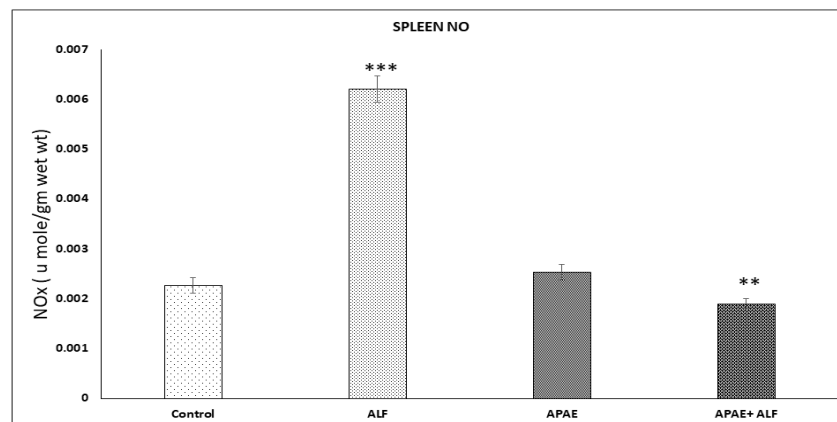


Fig. 2(f): Level of NOx in spleen of ALF rat model; where NOx level significantly increased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group the NOx level significantly decreased in spleen of rats with ALF. Values represented mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; **p<0.01 and ***p<0.001.

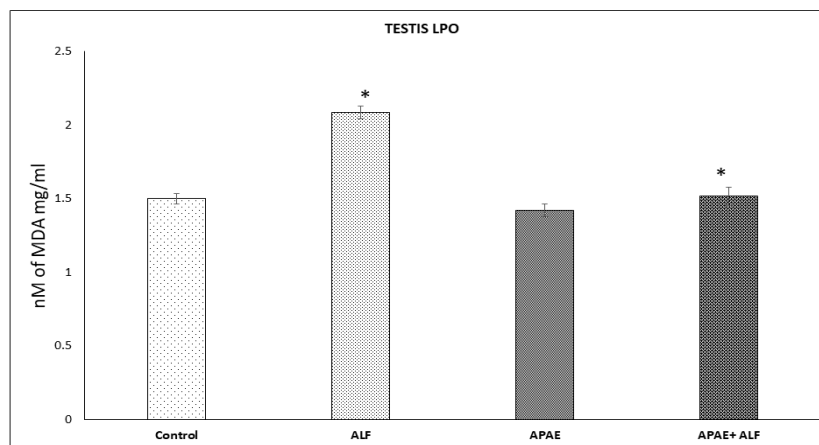


Fig. 3(a): Level of MDA; stable product of LPO significantly increased in the testis of ALF group rats. However, on treatment of APAE to the ALF treatment group significantly lower the level of MDA in the testis of ALF rat model. Values represent mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$.

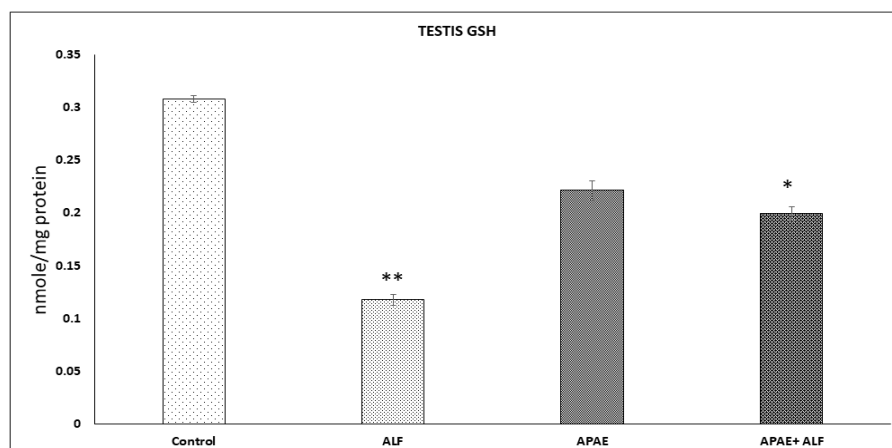


Fig. 3(b): Level of Glutathione; which is significantly decreased in testis of ALF group rats. However, on treatment of APAE to the ALF treatment group significantly restore the level of Glutathione towards that of control group rats. Values represent mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$ and ** $p < 0.01$.

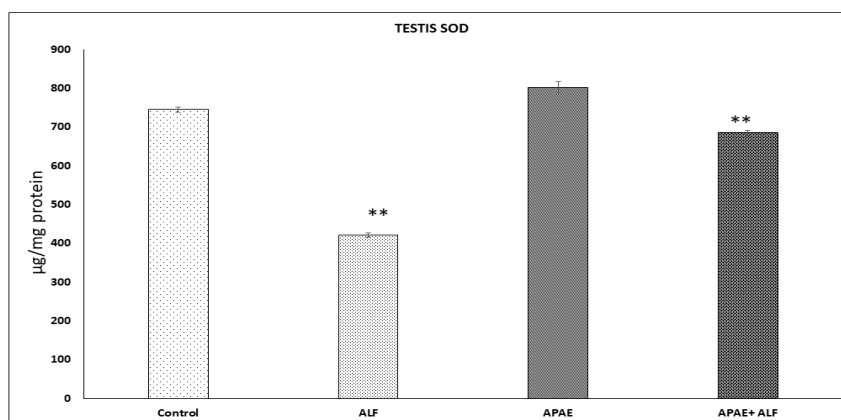


Fig. 3(c): Activity of SOD; which is significantly decreased in testis of ALF group rats however, on treatment of APAE to the ALF treatment group significantly restore the activity of SOD in testis of rats with ALF. Values represent mean $\pm$ SD where $n = 3$. Level of significance was measured by one-way ANOVA; * $p < 0.05$ and ** $p < 0.01$.

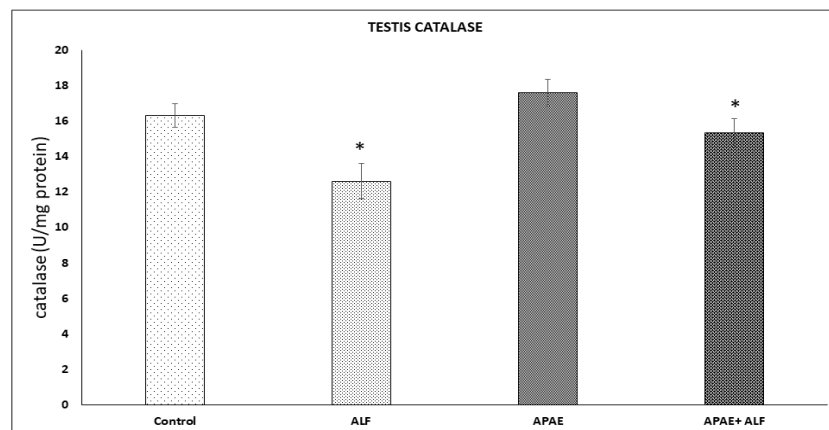


Fig. 3(d): Catalase activity in testis of ALF rat model; where catalase activity significantly decreased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group significantly restore the catalase activity in testis of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05.

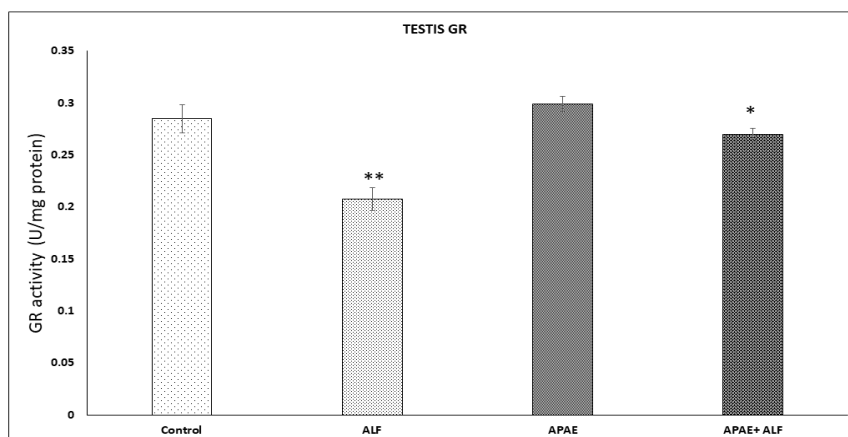


Fig. 3(e): GR activity in testis of ALF rat model; where GR activity significantly decreased in case of ALF group rats. However, on treatment of APAE to the ALF treatment group the catalase activity significantly increased in testis of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05 and **p<0.01.

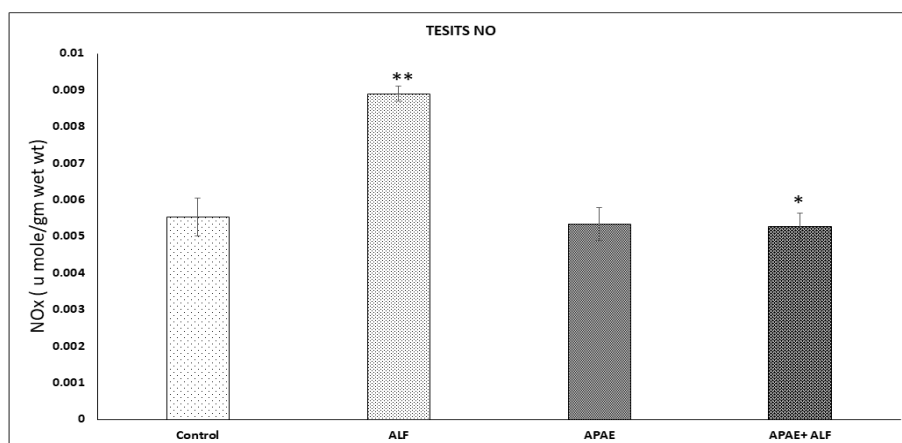


Fig. 3(f): Represent the NOx level in testis of ALF rat model; where level of NOx significantly increased in testis of ALF group rats. However, on treatment of APAE to the ALF treatment group the NOx level significantly decreased in testis of rats with ALF. Values represent mean $\pm$ SD where n = 3. Level of significance was measured by one-way ANOVA; *p<0.05 and **p<0.01.

compared to control group (Figs. 1f, 2f, 3f, respectively). On administration of APAE on ALF treatment group it lowered the NO level significantly towards that of control in liver ($p<0.05$), spleen ($p<0.01$) and testis ($p<0.05$) (Figs. 1f, 2f, 3f, respectively).

Discussion

ALF is a significant global health issue that burdens patients and those who provide care for them with significant illness burden and medical expenses. Therefore, a major area of concern in medical science is the study of its pathophysiology and prospective treatment agents. The establishment of ALF animal model for better understanding of pathogenesis and check the potential of its therapeutic agents is necessary. Onset of ALF causes oxidative stress in the liver and other organs, moreover the TAA metabolite produces oxidative stress and causes liver injury by destruction of hepatocytes and histological damages including infiltration of neutrophils and lymphocytes, necrosis and hepatocyte vacuolation in rats (Sepehrinezhad *et al.*, 2021). Hepatocytes injury causes production of ROS, which activates downstream signalling pathway causing mitochondrial dysfunctions and induction of apoptosis in hepatocytes (Lv *et al.*, 2019). Mitochondrial dysfunction results in the decline of antioxidants enzymes due to hike in the ROS production. In the present study we intended to see how ALF induced oxidative stress affects different organs in rat model and the therapeutic role of APAE against the oxidative stress caused by liver failure in rats. For induction of ALF rat model, we used a fixed dosage of TAA standardized in our lab. For therapeutic treatment group, 250 mg/kg bw of APAE was given to normal and ALF rats by orally with oral gavage.

In normal physiological condition, liver metabolizes different metabolites and removes toxic substances from body. But in case of liver failure, it is unable to process its normal physiological process due to hepatocellular damage. Hepatic injury causes production of ROS and oxidative stress in case of liver injury (Onuoha

et al., 2022), which causes decline in the antioxidant level of the liver. This oxidative stress due to hepatocellular damage in liver further have adverse effects on different organs like spleen and testis of rats and leads to multi-organ failure (MOF). The rate of production of ROS may directly relate to the lipid peroxidation (LPO) level. MDA, the stable product of LPO, through which the level of LPO can be observed. In this study there was significant increase in the MDA level in liver, spleen, and testis of ALF induced rats as compared to the control groups. However, in case of ALF group pre-treated with APAE significantly lower the MDA level in liver, spleen, and testis. Glutathione (GSH) is a natural antioxidant present inside the organ and has a central role in ROS regulation. GPx is an enzyme which utilised the GSH and convert it into oxidised GSSG during free radical formation, and so, it is one of the other oxidative stress markers for analysing the oxidative stress in this present study. The hydrogen peroxide (H_2O_2) converts into H_2O and O_2 molecule with the help of GPx by utilizing the GSH. Then another enzyme GR play the role in converting back the GSSG into GSH so the total GSH/GSSG ration depends on the synchronous activity of GPx and GR. In the present study, the GSH level significantly decreased in ALF groups of liver, spleen and testis. This may corelate with the oxidative stress caused by the liver failure and generation of free radicals in the spleen and testis of rats, which further leads to damage of these tissues. However, when the APAE is given to the rats of ALF group, the level of GSH was significantly increased in these organs. Likewise, the GR activity in the liver and testis of rats with ALF showed significant decline but interestingly there was increase in the activity in case of spleen. Treatment of APAE restored the GR activity in these three organs, liver, spleen and testis toward the control. The treatment of APAE to the ALF induced rats, restored the natural antioxidant GSH levels and GR activity to normal, which suggest the antioxidant nature of APAE. This finding clearly shows that APAE restore the GSH level in ALF groups on the liver, spleen, and testis of rats and

also increases the activity of GR by lowering production of ROS and free radicals due to oxidative damage in the liver, and liver failure mediated oxidative stress in spleen, and testis of rats with ALF. It has been stated that the leaf extracts of AP upregulate the antioxidant status in the liver, spleen, testis, heart, kidney, and pancreas (Lv *et al.*, 2019). These findings of the present study may conclude that the bioactive component of the AP helps in the protection of these organs against the oxidative damage caused by ALF.

The SOD, catalase and GPx, the antioxidant enzyme complex is considered to be the initial defence mechanism against the oxidative damage in the body due to ROS and free radicals generated during ALF in the liver, spleen, and testis. Both the enzyme SOD and catalase, scavenge free radicals and defend the cells against oxidative damage. First the action of antioxidant enzyme SOD reduces the oxidative damage in cells by converting H_2O_2 into water (Ighodaro and Akinloye, 2018). Further, the activity of catalase lowers the ROS by converting the H_2O_2 into water molecule. In the present study activity of both enzymes, SOD and catalase significantly reduced in the liver, spleen and testis of ALF rats, which is also reported by Onuoha *et al.* (2022) as they have stated that the activity of SOD and catalase decreased in the ALF rat model. However, on treatment of APAE to the ALF rats, significantly restored the antioxidant status by lowering the liver failure induced oxidative stress in these organs. Nitric oxide (NO) is a crucial molecule involved in signalling mechanism in immune system, and effect on intra- and intercellular physiological mechanism (Gantner *et al.*, 2020). However, NO overproduction causes cytotoxicity and plays a number of roles in inflammatory immunological reactions (Yao *et al.*, 2022). In the present study level of NO in liver, spleen, and testis in ALF rats was significantly high as compared to those of control rats. However, treatment of APAE to the ALF groups significantly reduced the NO level in these organs of rats. It has also been stated by Hanh *et al.* (2020) that flavonoids from AP prevent the NO

overproduction and helps in lowering the cytotoxicity and prevent cell damage. The observed result suggested that, the bioactive component of AP is lowering the oxidative damage caused by TAA induced liver failure by activating antioxidant enzymes and also lowers the NO level and prevents the cytotoxicity and inflammation caused by the hyperproduction of NO.

Conclusion

The present study indicated that TAA induced liver failure caused the liver injury and causes oxidative stress in liver. Oxidative stress induced through liver failure has adverse effect on different organs like spleen and testis. The treatment of APAE in the ALF model actively ameliorates the oxidative damage in these organs of rats caused due to ALF by activating antioxidant enzymes complex. Hence, it can be suggested that the APAE could be a treatment for the patients with ALF.

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References

- Carlberg I and Mannervik B. (1985) Glutathione reductase. *Methods Enzymol.* 113: 484-490.
- Dai Y, Chen SR, Chai L, Zhao J, Wang Y and Wang Y. (2019) Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide. *Crit Rev Food Sci Nutr.* 59(sup1): S17-S29.
- Dong V, Nanchal R and Karvellas CJ. (2019) Pathophysiology of Acute Liver Failure. *Nutr Clin Pract.* 35(1): 24-29.
- Gantner BN, LaFond KM and Bonini MG. (2020) Nitric oxide in cellular adaptation and disease. *Redox Biol.* 34: 101550.
- Hanh TTH, My NTT, Cham PT, Quang TH, Cuong NX, Huong TT, Nam NH, and Minh CV. (2020) Diterpenoids and flavonoids from *Andrographis paniculata*. *Chem Pharm Bull (Tokyo)* 68(1): 96-99.
- Hawthorne SB, Rickkola ML, Screnius K, Holm Y, Hiltunen R and Hartonen K. (1993) Comparison of

- hydrodistillation and supercritical fluid extraction for the determination of essential oils in aromatic plants. *J Chromatography A*. 634(2): 297-308.
- Heath RL and Packer L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 125(1): 189-198.
- Heidari R, Niknahad H, Sadeghi A, Mohammadi H, Ghanbarinejad V, Ommati MM, Hosseini A, Azarpira N, Khodaei F, Farshad O, Rashidi E, Siavashpour A, Najibi A, Ahmadi A and Jamshidzadeh A. (2018) Betaine treatment protects liver through regulating mitochondrial function and counteracting oxidative stress in acute and chronic animal models of hepatic injury. *Biomed Pharmacother*. 103: 75-86.
- Ighodaro OM and Akinloye OA. (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med*. 54(4): 287-293.
- Jamshidzadeh A, Heidari R, Abasvali M, Zarei M, Ommati MM, Abdoli N, Khodaei F, Yeganeh Y, Jafari F, Zarei A, Latifpour Z, Mardani E, Azarpira N, Asadi B and Najibi A. (2017) Taurine treatment preserves brain and liver mitochondrial function in a rat model of fulminant hepatic failure and hyperammonemia, *Biomed Pharmacother*. 86: 514-520.
- Jankovic Velickovic L and Stefanovic V. (2014) Hypoxia and spermatogenesis. *Int Urology Nephrol*. 46: 887-894.
- Li L, Duan M, Chen W, Jiang A, Li X, Yang J and Li Z. (2017) The spleen in liver cirrhosis: revisiting an old enemy with novel targets. *J Transl Med*. 15(1): 111.
- Lingappan K. (2018) NF- κ B in oxidative stress. *Curr Opin Toxicol*. 7: 81-86.
- Lv H, Yang H, Wang Z, Feng H, Deng X, Cheng G and Ci X. (2019) Nrf2 signaling and autophagy are complementary in protecting lipopolysaccharide/d-galactosamine-induced acute liver injury by licochalcone A. *Cell Death Dis*. 10(4): 313.
- Ma Q, Shao H, Feng Y, Zhang L, Li P, Hu X, Ma Z, Lou H, Zeng X and Luo G. (2018) A new bioluminescent imaging technology for studying oxidative stress in the testis and its impacts on fertility. *Free Radic Biol Med*. 124: 51-60.
- Mittal SPK, Khole S, Jagadish N, Ghosh D, Gadgil V, Sinkar V and Ghaskadbi SS. (2016) Andrographolide protects liver cells from H₂O₂ induced cell death by upregulation of Nrf-2/HO-1 mediated via adenosine A_{2a} receptor signalling. *Biochim Biophys Acta*. 1860(11 Pt A): 2377-2390.
- Nasir A, Abubakar MG, Shehu RA, Aliyu U and Toge BK. (2013) Hepatoprotective effect of the aqueous leaf extract of *Andrographis paniculata* Nees against carbon tetrachloride-induced hepatotoxicity in rats. *Nigerian J Basic Appl Sci*. 21(1): 45-54.
- Nugroho A. (2020) Splenectomy in liver cirrhosis with splenomegaly and hypersplenism. In: *Liver Pathology*, (eds.) Gayam V. and Engin O., IntechOpen. <https://doi.org/10.5772/intechopen.94337>.
- Onuoha SC, Chukwuma CC and Monanu MO. (2022) Biochemical investigation of Wistar albino rats orally exposed to bonny light crude oil and *Andrographis paniculata* leaf extract. *Biotechnol*. 21(4): 182-197.
- Rajajee V, Fontana RJ, Courey AJ and Patil PG. (2017) Protocol based invasive intracranial pressure monitoring in acute liver failure: feasibility, safety and impact on management. *Crit Care* 21(1): 178.
- Sedlak J and Lindsay RH. (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 25(1): 192-205.
- Sepehrinezhad A, Shahbazi A, Sahab Negah S, Joghataei MT and Larsen FS. (2021) Drug-induced-acute liver failure: A critical appraisal of the thioacetamide model for the study of hepatic encephalopathy. *Toxicol Rep*. 8: 962-970.
- Singh S and Trigun SK. (2010) Activation of neuronal nitric oxide synthase in cerebellum of chronic hepatic encephalopathy rats is associated with up-regulation of NADPH-producing pathway. *Cerebellum* 9(3): 384-397.
- Singh S, Koiri RK and Trigun SK. (2008) Acute and chronic hyperammonemia modulate antioxidant enzymes differently in cerebral cortex and cerebellum. *Neurochem Res*. 33(1): 103-113.
- Singh S, Mondal P and Trigun SK. (2014) Acute liver failure in rats activates glutamine-glutamate cycle but declines antioxidant enzymes to induce oxidative stress in cerebral cortex and cerebellum. *PLoS One* 9(4): e95855.
- Squires JE, McKiernan P and Squires RH. (2018) Acute liver failure. *Clin Liver Dis*. 22(4): 773-805.
- Stravitz RT and Lee WM. (2019) Acute liver failure. *Lancet* 394(10201): 869-881.
- Thibaut R, Gage MC, Pineda-Torra I, Chabrier G, Venteclef N and Alzaid F. (2022) Liver macrophages and inflammation in physiology and pathophysiology of non-alcoholic fatty liver disease. *FEBS J*. 289(11): 3024-3057.
- Tian Z, Chen Y, Yao N, Hu C, Wu Y, Guo D, Liu J, Yang Y, Chen T, Zhao Y and He Y. (2018) Role of mitophagy regulation by ROS in hepatic stellate cells during acute liver failure. *Am J Physiol Gastrointest Liver*

Physiol 315(3): G374-G384.

Wang Y, Jiao J, Yang Y, Yang M and Zheng Q. (2018) Screening and identification for immunological active components from *Andrographis herba* using macrophage biospecific extraction coupled with UPLC/Q-TOF-MS. *Molecules* 23(5): 1047.

Yao X, Jin G, Liu D, Zhang X, Yang Y, Chen Y, Duan Z, Bi Y, Yan F, Yang Y, Zhang H, Dong G, Li S, Cheng S, Tang H, Hong F and Si C. (2022) Inducible nitric oxide

synthase regulates macrophage polarization via the MAPK signals in concanavalin A-induced hepatitis. *Immun Inflamm Dis.* 10(7): e643.

Zargar S, Wani TA, Alamro AA and Ganaie MA. (2017) Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. *Int J Immunopathol Pharmacol.* 30(3): 207-214.