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HEPATOPROTECTIVE ACTIVITY OF FUNGAL ENDOPHYTIC FRACTIONS OF *ANDROGRAPHIS PANICULATA* (BURM. F.) WALL NEES. LEAVES IN PARACETAMOL AND ETHANOL INDUCED HEPATOTOXICITY

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ABSTRACT: The most widely used drug for analgesic and antipyretic is Paracetamol, but a change in dose at high-level yields to adverse reactions such as the toxic liver. The psychoactive compound present in alcoholic drinks is ethanol. Chronic alcohol consumption and various pathological conditions vary from basic intoxication to life-threatening pathological states. Endophytes are the microorganisms present inside the plant tissues forming a mutualistic, symbiotic and trophobiotic relationship. Endophytic organisms such as fungi, bacteria, and actinomycetes are found in all types of vascular plants and grasses. The present study reveals the protective effects of previously isolated fractions of A2EA (ethyl acetate) and A2nB (*n*-butanol), from the endophyte *Preussia sp. PPV3.6* (APLF-2) isolated from *Andrographis paniculata* leaves on Paracetamol and ethanol-induced hepatotoxicity at a dose of (50 mg/kg & 100 mg/kg). A2EA and A2nB (50 mg/kg & 100 mg/kg) reversed the increased serum biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphate (SALP), total bilirubin, direct bilirubin, total cholesterol (TC) and triglycerides(TG) as compared to Paracetamol and ethanol induced treated group (*** $p<0.001$). A2EA and A2nB (50 mg/kg & 100 mg/kg) significantly increased the total protein levels. A2EA and A2nB (100 mg/kg p.o) also restored the lipid peroxidation (LPO), superoxide (SOD) and catalase (CAT) levels.

INTRODUCTION: Different types of mechanisms have been proposed for hepatotoxicity with relevance in clinical trials reported by researchers. Paracetamol (Acetaminophen) is used widely as an analgesic and antipyretic drug since years ago. In therapeutic doses, Paracetamol is metabolized into a highly reactive metabolite that is N-acetyl-p-benzoquinone imine by glucuronidation or sulfation of cytochrome P-450 system.

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Normally at small doses, N-acetyl-p-benzoquinone imine gets converted to the nontoxic element while at large doses it may react with the liver proteins thus resulting into liver injury [1, 2]. Thus, it is a cause of severe hepatic failure demanding for transplantation of liver. Another most used hepatotoxic agent relating to liver disorders is ethanol (alcohol).

An excess amount of intake of alcohol leads to severe alcoholic liver disorders. Several studies have reported for increased activity of liver enzymes destroying liver parenchyma cells [3]. The trichloromethyl radical metabolite is responsible for the toxicity of alcohol [4]. Hence, there is an emerging focus on novel compounds to search, to

be effective and reliable drug for the prevention of Paracetamol and ethanol-induced hepatotoxicity. Plants being interacting continuously with different microorganisms may be certain pathogens, out of which some are favorable to the plant itself. Out of those groups of microorganisms are the Endophytes are the microorganisms which reside in the internal tissues of the plant without causing any adverse effects are called as endophytes [5]. These endophytes may be fungi, bacteria, and actinomycetes forming a communalistic, mutualistic and trophobiotic relationship. Endophytes also show defensive mechanism against herbivores and insects. The various physiological steps are transducted leading to resistance in plants. Fungal endophytes are the most important element of plant diversity that influences diversity and structures. Endophytic fungi are becoming popular in the various research areas because of their benefits, such as promoting growth, bio-control agents and production of secondary metabolites with good yield. Due to the presence of endophytes the host plant develops the tolerance of biotic and abiotic stress with disease resistance [6]. Plant growth promotion by endophytes through phytohormone production is another way causing morphological and structural changes in the plant. Their application in agriculture is defendable due to these applications [10].

Andrographis paniculata Nees also known as Kalmegh is an important medicinally active plant belongs to the family Acanthaceae. It is also known as King of bitters and Kalmegh traditionally and is used in Indian and Chinese herbal medicine. It was reported with diterpenoids, stigmasterols, flavonoids, andrographolides [7] as the major active constituents present in the plant. The plant is mainly used in liver diseases. Many other biological activities have been reported such as antidepressant [8], anti-hyperglycemic [9], antioxidant [9], immunostimulant [10], anti-cancer [10], anti-malarial [11], cardiovascular [12], hepatoprotective activity [13], anti-inflammatory [14].

Anti-microbial activity and antibiotic susceptibility were reported from the endophytes isolated from the leaves of *Andrographis paniculata* [15]. Bacterial endophyte was also reported from *Andrographis paniculata* which was used as plant growth promoter and regulators [16]. The other endophytic fungus from *Andrographis paniculata* was used in lipid production as biodiesel precursors [17].

No work was reported on the Paracetamol and ethanol-induced hepatotoxicity till now as isolation of fungal endophytes from *Andrographis paniculata* is negligible in the literature. We have published regarding two endophytes namely, APLF-1 and APLF-2, from the leaves of *Andrographis paniculata and* screened for *in-vitro* free radical scavenging activity in our paper [18]. The fractions of APLF-2 (A2EA & A2nB) were further selected for *in-vivo* hepatoprotective activity in Paracetamol and ethanol-induced hepatotoxicity.

MATERIALS AND METHODS:

Plant Material: The collection of leaves of *Andrographis paniculata* (Burm.f.) Wall. Nees were done from Dharwad district, Karnataka, India. The plant was authenticated by Dr. G. R. Hegde, Karnataka University, Dharwad (India). A specimen is stored in the herbarium, Postgraduate Department of Pharmacognosy (SETCPD/Ph.cog/ herb/32/12/2015).

Isolation, Fermentation, and Extraction: As previously reported [18].

Hepatoprotective Activity: Based on the previously reported [18] results of free radical scavenging activity, A2EA and A2nB were further selected for *in-vivo* hepatoprotective activity in Paracetamol and ethanol-induced hepatotoxicity.

Animals: Albino wister rats weighing 150 - 200 g were used. The inbred rats were collected from Venkateshwara Enterprises Bangalore, Karnataka. They were maintained in the animal house of SET's College of Pharmacy, Dharwad for the experimental purpose under controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 5%) and 12 h light-dark cycles. They were acclimatized for 7 d before the study. Animals were then randomized into groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*.

Acute Toxicity Studies: Acute oral toxicity of A2EA and A2nB was determined using Swiss albino mice. The animals were fasted for 12 h before the experiment and were administered with

single dose of fractions dissolved in 5% gum acacia and observed for mortality up to 48 h (short-term toxicity). Based on the short-term toxicity, the dose of the next animal was determined as per OECD guideline 420. (Reg no.: 112/PO/Re/S/1999/ CPCSEA)

Experimental Design for Paracetamol Induced Hepatotoxicity [19]:

Group, I: Normal control, rats received distilled water 5 mL/kg b.w. p.o. / 7 days.

Group II: Paracetamol control, rats received distilled water 5 mL/kg b.w. p.o./7 days (except 5th day).

Group III: Rats received the standard drug silymarin 200 mg/kg b.w. p.o. / 7 days.

Group IV: Rats received with A2EA (50 mg/kg) b.w. p.o. / 7 days.

Group V: Rats received with A2EA (100 mg/kg) b.w. p.o. / 7 days.

Group VI: Rats received with A2nB (50 mg/kg) b.w. p.o. / 7 days.

Group VII: Rats received with A2nB (100 mg/kg) b.w. p.o. / 7 days.

All the animals in the groups III-VII were pre-treated with their respective drugs for 5 consecutive days. On the 5th day of the experimental period, after the drug administration of respective treatments, all animals except those in group I was administered with Paracetamol 2 g/kg b.w. p.o. And on the 7th day, after 2 h of respective drug treatments, animals were anesthetized using diethyl ether inhalation jar. Blood was collected by puncturing retro-orbital bleeding under mild ether anesthesia, centrifuged (2500 rpm at 30 °C for 15 min) and serum was subjected to biochemical estimations. Livers were excised immediately and washed in ice-cold normal saline and placed in 10% formalin solution for histopathological study. The liver homogenate was prepared to determine the levels of endogenous enzymes.

Experimental Design for Ethanol-Induced Hepatotoxicity [20]: Animals were divided into following groups of six animals in each group (n = 6). All animals except the group I-normal control were intoxicated with 20% ethanol (3.76 g/kg/day, p.o for 18 days).

Group I: Normal Control, rats received only distilled water.

Group II: Ethanol control, rats received 20% ethanol (3.76 g/kg/ day, p.o. for 18 days).

Group III: Rats received with Silymarin (200mg/kg).

Group IV: Rats received with A2EA (50 mg/kg p.o).

Group V: Rats received with A2EA (100 mg/kg p.o).

Group VI: Rats received with A2nB (50 mg/kg p.o).

Group VII: Rats received with A2nB (100 mg/kg p.o).

All the animals were treated with respective drugs orally for a period of 18 d as reported. On the 19th day, animals were anesthetized using diethyl ether inhalation jar. Blood was collected by puncturing retro-orbital bleeding under mild ether anesthesia, centrifuged (2500 rpm at 30 °C for 15 min) and serum was subjected to biochemical estimations. Livers were excised immediately and washed in ice-cold normal saline and placed in 10% formalin solution for histopathological study. The liver homogenate was prepared to determine the levels of endogenous enzymes.

Biochemical Parameters: Serum was separated and analyzed spectrophotometrically for AST, ALT, ALP, total and direct bilirubin, total triglyceride (TG), and total protein for both the models using diagnostic kits of ERBA Diagnostics, Mannheim GMBH, Germany.

Measurement of Enzymatic and Non-Enzymatic Antioxidant Levels:

Tissue Preparation: Animals were sacrificed and were perfused transcardially with ice-cold saline. The whole liver was perfused *in-situ* with ice-cold saline, dissected out, blotted dry and immediately weighed. A 10% liver homogenate was prepared separately with ice-cold saline-EDTA using

Teflon-glass homogenizer (Yamato LSG LH-21, Japan). The homogenate was used for the estimation of proteins and lipid peroxidation. The liver homogenate was centrifuged at 10,000 rpm for 10 min and the pellet discarded. The supernatant was again centrifuged at 20,000 rpm for 1 h at 4 °C. Both the liver supernatants obtained were used for the estimation of non-enzymatic antioxidants (Lipid peroxidation) and enzymatic antioxidants (Catalase and superoxide).

Lipid Peroxidation: Thiobarbituric acid reactive substances (TBARS) in the liver homogenate were estimated by using a standard protocol. Briefly, the homogenate was incubated with 15% TCA, 0.375% TBA and 5N HCl at 95 °C for 15 min; the mixture was cooled, centrifuged and the absorbance of the supernatant measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using the formula $€= 1.56 \times 105M^{-1}\ cm^{-1}$ and expressed as TBARS (µ moles) per g of tissue [21].

SOD Assay: Liver homogenate (0.5 mL) was taken, and 1 mL of 50 mM sodium carbonate, 0.4 mL of 24 µm NBT, and 0.2 mL of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25 ºC. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed regarding units per mg of proteins [22].

Catalase: As per the previously published method catalase activity was determined spectrophotometrically. To 1.95 mL of 10 mM H_2O_2 in 60 mM phosphate buffer (pH=7.0), 0.05 mL of the liver homogenate was added, and the rate of degradation of H_2O_2 was followed at 240 nm/min. Catalase content regarding µ/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula

$$k = 2.303/\ \Delta t \times \log (A1/A2)\ s^{-1}$$

A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH=7.0 at 25 °C, while H_2O_2 concentration falls from 10.3 to 9.2 mM [23].

Estimation of Protein: Total tissue protein content was measured by Folin phenol reagent method as reported earlier [24].

Histopathological Studies: Liver of individual animal were excised quickly, fixed in 10% buffered neutral formalin and fixed in bovine solution. They were further processed for paraffin embedding following standard microtechnique [25]. Sections of liver stained with alum-hematoxylin and eosin were observed photomicroscopically for histopatho-logical changes.

Statistical Evaluation: The data were expressed as Mean ± S.E.M. Statistical comparisons were performed in one way ANOVA followed by Tukey's T-test using Graph Pad Prism version 5.0, USA.

RESULTS:

Hepatoprotective Activity: APLF-1 and APLF-2 endophyte were identified as *Diporthe* and *Preussia sp*. PPV3.6 respectively as reported previously [18].

Acute Toxicity (LD_{50}) Studies: Acute toxicity studies were carried out according to OECD guidelines (Up and Down method). No mortality was observed up to 2000 mg/kg body weights were for A2EA and A2nB. Hence, doses of 50 mg/kg and 100 mg/kg body weight were selected to assess the hepatoprotective activity of A2EA and A2nB fractions respectively.

Effect of APLF-2 on Serum Biochemical Parameters in Paracetamol and Ethanol-Induced Hepatotoxicity in Rats: Administration of Paracetamol with overdose (2 g/kg) and ethanol 20% ethanol (3.76 g/kg/day, p.o) significantly caused liver damage and necrosis of cells by elevating the levels of SGOT, SGPT, SALP, total and direct bilirubin, triglyceride and total proteins as compared to normal control.

A2EA and A2nB, (50 mg/kg & 100 mg/kg) reversed the elevated biochemical parameters as compared to Paracetamol treated group **Table 1** and **2**, **Fig. 1** and **2**.

TABLE 1: EFFECT OF A2EA AND A2nB ON SERUM BIOCHEMICAL PARAMETERS IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

Groups	SGPT (mg/dl)	SGOT (mg/dl)	SALP (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Triglyceride (mg/dl)	Total protein
Control (2 mL/kg)	55.26 ±1.52	137.5 ±1.40	230.2 ±2.04	0.34 ±0.09	0.16 ±0.018	53.69 ±0.83	4.63 ±0.08
Paracetamol treated (2 g/kg)	197.2 ±1.87	268.0 ±1.09	332.0 ±0.933	0.86 ±0.14	0.52 ±0.02	99.32 ±0.57	8.65 ±0.10
Silymarin (25 mg/kg)	79.60 ±1.50***	192.6 ±2.74***	281.0 ±1.97***	0.63 ±0.04***	0.36 ±0.03***	63.58 ±0.94***	5.48 ±0.09***
A2EA (50 mg/kg)	178.0 ±2.18***	219.9 ±5.28***	286.0 ±1.72***	0.54 ±007***	0.25 ±0.01***	85.89 ±4.7*	7.16 ±0.10***
A2EA 100 mg/kg)	125.7 ±2.04***	199.9 ±3.56***	265.7 ±0.92***	0.44 ±0.01***	0.18 ±0.01***	62.85 ±2.11***	6.53 ±0.08***
A2nB (50 mg/kg)	188.1 ±1.39*	238.7 ±7.73**	318.9 ±3.15***	0.64 ±0.15***	0.44 ±0.01*	77.90 ±1.53***	6.53 ±0.07***
A2nB (100 mg/kg)	105.5 ±1.54***	184.6 ±4.89***	261.1 ±1.81***	0.48 ±0.22***	0.35 ±0.01***	65.63 ±5.07***	5.65 ±0.08***

Each value represents Mean ± S.E.M (n=6) *p<0.05, ** p<0.01, ***p<0.001 compared to Paracetamol treated group. One way ANOVA followed by Tukey's multiple comparison tests

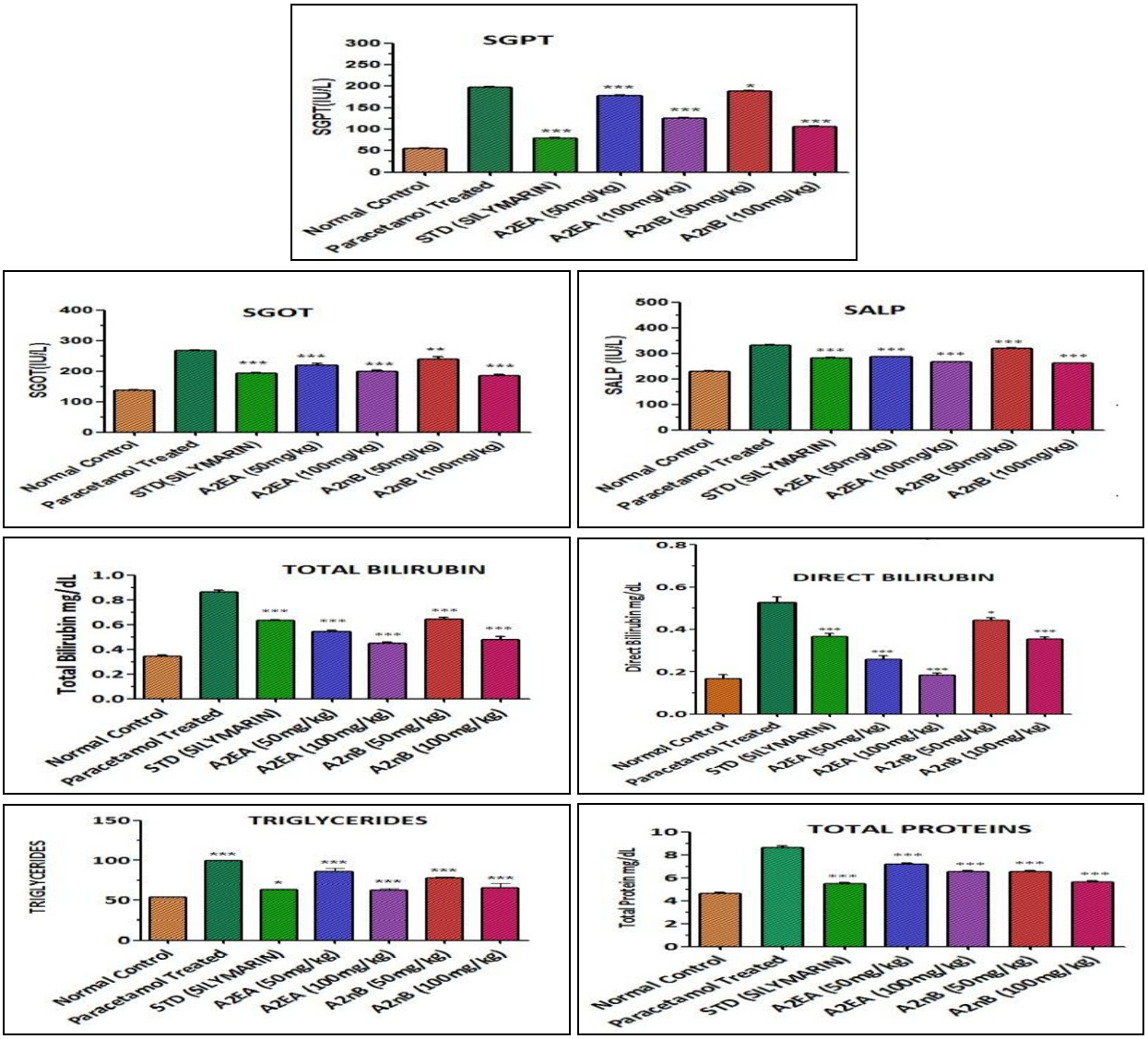

FIG. 1: EFFECT OF A2EA AND A2nB ON SERUM BIOCHEMICAL PARAMETERS IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

TABLE 2: EFFECT OF A2EA AND A2nB ON SERUM BIOCHEMICAL PARAMETERS IN ETHANOL INDUCED HEPATOTOXICITY IN RATS

Groups	SGPT (mg/dl)	SGOT (mg/dl)	SALP (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Triglyceride (mg/dl)	Total protein
Control	41.58	84.62	124.5	0.34	0.13	51.14	4.68
(2 mL/kg)	±0.33	±0.19	±0.25	±0.01	±0.09	±0.28	±0.04
Ethanol treated	75.83	253.5	222.4	0.84	0.54	73.71	7.26
(3.76 g/kg/day)	±0.23	±0.40	±0.69	±0.08	±0.07	±0.75	±0.03
Silymarin	52.22	176.0	183.9	0.63	0.24	53.45	6.18
(25 mg/kg)	±0.35***	±0.77***	±0.54***	±0.08***	±0.14***	±0.45***	±0.07***
A2EA	74.57	246.8	215.7	0.77	0.45	69.49	7.23
(50 mg/kg)	±0.45	±0.47**	±1.87**	±0.02**	±0.09***	±0.39***	±0.61
A2EA	73.16	223.0	203.1	0.45	0.34	65.10	6.85
100 mg/kg)	±0.48**	±1.02***	±1.25***	±0.09***	±0.09***	±0.28***	±0.13*
A2nB	73.82	250.9	217.6	0.52	0.49	71.50	6.68
(50 mg/kg)	±0.53	±2.05	±0.31*	±0.04***	±0.06*	±0.46*	±0.07***
A2nB	68.47	226.32	193.0	0.72	0.44	61.40	6.71
(100 mg/kg)	±0.32***	±0.66***	±0.82***	±0.07***	±0.07***	±0.30***	±0.07***

Each value represents Mean ± S.E.M (n=6) *p<0.05, ** p<0.01, ***p<0.001 compared to ethanol-treated group. One way ANOVA followed by Tukey's multiple comparison tests

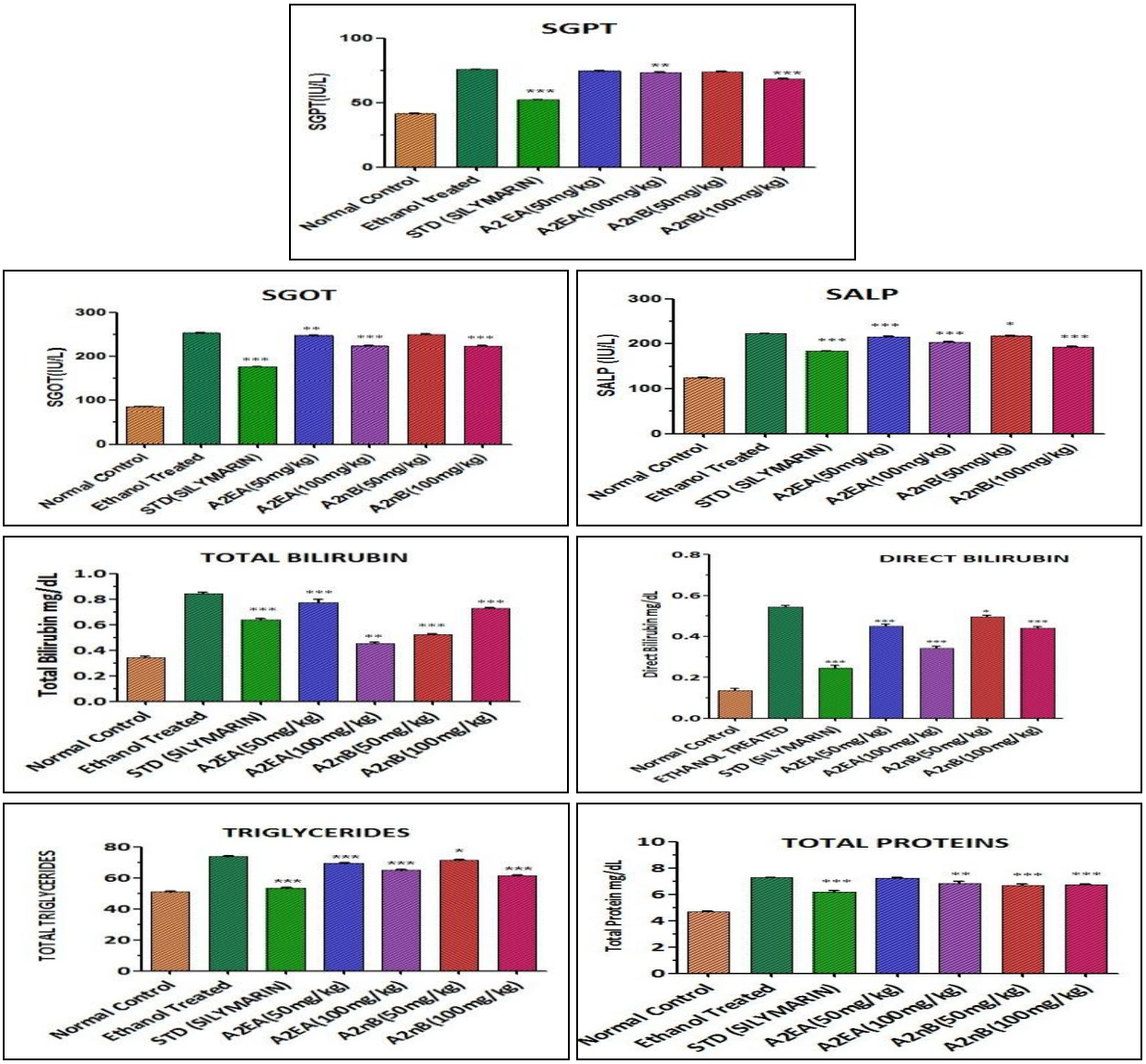

FIG. 2: EFFECT OF A2EA AND A2NB ON SERUM BIOCHEMICAL PARAMETERS IN ETHANOL INDUCED HEPATOTOXICITY IN RATS

Effect of A2EA And A2nB on Endogenous Antioxidant Enzymes in Paracetamol and Ethanol-Induced Hepatotoxicity: There was a marked increase in LPO level (46.79 ± 1.35) in Paracetamol treated group and (22.67 ± 0.80) in the ethanol-treated group. A2EA (100 mg/kg) and A2nB (50 & 100 mg/kg) significantly inhibited *in-vivo* lipid peroxidation, (***p<0.001). A2EA (50 mg/kg) showed significance at (**p<0.01) value; these values were compared to Silymarin. The SOD levels were decreased in Paracetamol treated (26.09 ± 0.73) and in ethanol-treated (30.67 ± 0.21) groups. A2EA and A2nB (50 & 100 mg/kg) showed significance value, as **p<0.01, when compared to Silymarin.

TABLE 3: EFFECT OF A2EA AND A2nB ON LPO, GSH, AND CAT LEVELS IN PARACETAMOL INDUCED RATS

Groups	LPO level (µmol/mg/ protein)	SOD level (µmol/mg/ protein)	CAT level (µmol/mg/ protein)
Control (2 g/kg)	15.53 ±1.02	42.30 ±0.63	74.80 ±0.28
Paracetamol treated (2 mg/kg)	46.79 ±1.35	26.09 ±0.73	44.85 ±0.41
Silymarin (25 mg/kg)	16.45 ±0.24***	52.84 ±0.32***	71.58 ±0.63***
A2EA (50mg/kg)	32.70 ±0.75**	27.89 ±0.57**	48.93 ±1.38*
A2EA (100 mg/kg)	26.84 ±0.49***	30.43 ±0.79	64.00 ±0.51***
A2nB (50 mg/kg)	35.71 ±1.43***	27.62 ±0.55**	48.94 ±0.48**
A2nB (100 mg/kg)	28.99 ±0.99***	30.30 ±0.84	64.35 ±0.75***

Each value represents Mean ± S.E.M (n=6) *p<0.05, ** p<0.01, ***p<0.001 compared to Paracetamol treated group. One way ANOVA followed by Tukey's multiple comparison tests

TABLE 4: EFFECT OF A2EA AND A2nB ON LPO, GSH AND CAT LEVELS IN ETHANOL INDUCED RATS

Groups	LPO level (µmol/mg/ protein)	SOD level (µmol/mg/ protein)	CAT level (µmol/mg/ protein)
Control (2 mL/kg)	22.67 ±0.80	30.67 ±0.21	65.31 ±0.33
Ethanol treated (3.76 g/kg/day)	52.54 ±0.32	25.29 ±0.39	27.95 ±0.35
Silymarin (25 mg/kg)	32.64 ±0.40***	35.98 ±0.30***	55.78 ±0.20***
A2EA (50 mg/kg)	50.90 ±0.61	26.62 ±0.33	29.66 ±0.29*
A2EA 100 mg/kg)	43.11 ±0.84***	29.67 ±0.32***	42.93 ±0.20
A2nB (50 mg/kg)	48.64 ±0.65*	27.37 ±0.50**	29.52 ±0.69***
A2nB (100 mg/kg)	37.24 ±1.35***	32.47 ±0.10***	45.18 ±0.41***

Each value represents Mean ± S.E.M (n=6) *p<0.05, ** p<0.01, ***p<0.001 compared to ethanol-treated group. One way ANOVA followed by Tukey's multiple comparison tests

There was a marked depletion of CAT levels in Paracetamol treated (44.85 ± 0.41) and ethanol treated (65.31 ± 0.33) groups. A2EA (100 mg/kg), A2nB (100 mg/kg), increased the CAT activity significantly (***p<0.001) **Table 3** and **4**.

Histopathology of Liver for Paracetamol Induced Hepatotoxicity in Rats: Normal arrangement of cell morphology, with no inflammation, was observed. Hepatic vein, central vein, kupffer cells, and portal triads are in a normal position with fine chromatin **Fig. 3**. In Paracetamol treated group, degeneration of fatty cells with aggregates. Dilation of the central vein, sinusoid, ballooning, and inflammation of hepatocytes is noticed. No cellular boundaries are seen **Fig. 3**. In standard Silymarin (100 mg/kg) treated group there is a regeneration of hepatic cells. No inflammation, portal triad, central vein, and sinusoidal congestion mildly were observed. The hepatic globular structure was found to be normal. Regeneration of hepatocytes was also found in **Fig. 3**. A2EA treated livers (50 mg/kg) showed the appearance of normal hepatocytes around the necrosis and fatty vacuoles **Fig. 3**. A2EA treated livers (100 mg/kg) showed the disappearance of fibrous septae and normal hepatocytes were seen. Mild congestion of central vein and sinusoid are seen **Fig. 3**. There was a decrease in the inflammation of hepatic cells in A2nB (50 mg/kg) treated livers. The injured liver was restored **Fig. 3**. A2nB (100 mg/kg) treated livers regenerated the hepatocytes to normal with mild inflammation and ballooning **Fig. 3**.

Histopathology of Liver for Ethanol-Induced Hepatotoxicity in Rats: The normal liver was found to have normal hepatocytes, mild central vein, and sinusoid congestion, no inflammation was found. The normal hepatic structure was found in **Fig. 4**. In ethanol (2mL / 100g) treated group, dilation of the sinusoid, ballooning, cell degeneration was observed. Inflammation of the central vein with fatty cells was also seen in **Fig. 4**. In standard Silymarin (100 mg/kg) treated group no inflammation of fatty was cells seen. Mild congestion of central vein sinusoidal and portal triad was observed. The hepatic architecture was normal **Fig. 4**. A2EA treated livers (50 mg/kg) showed the appearance of regeneration of hepatocytes around the necrosis and fatty vacuoles. Normal renal tubules were found **Fig. 4**.

A2EA treated livers (100 mg/kg) showed less inflammatory cells with regeneration **Fig. 4**. A2nB (50 mg/kg) treated livers injured liver cells were restored **Fig. 4**. A2nB (100 mg/kg) treated livers showed regeneration of cells, no inflammation has seen **Fig. 4**.

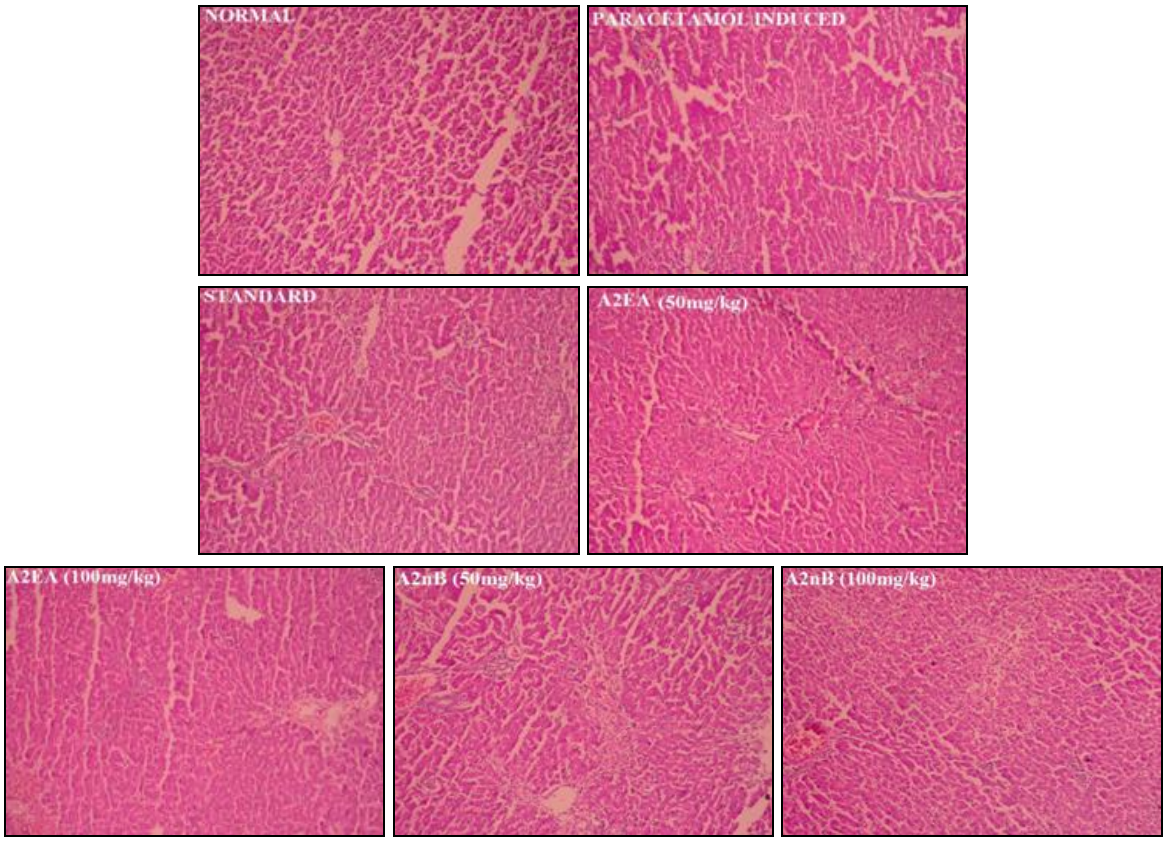

FIG. 3: HISTOPATHOLOGICAL PHOTOGRAPHS OF LIVERS IN PARACETAMOL INDUCED HEPATOTOXICITY

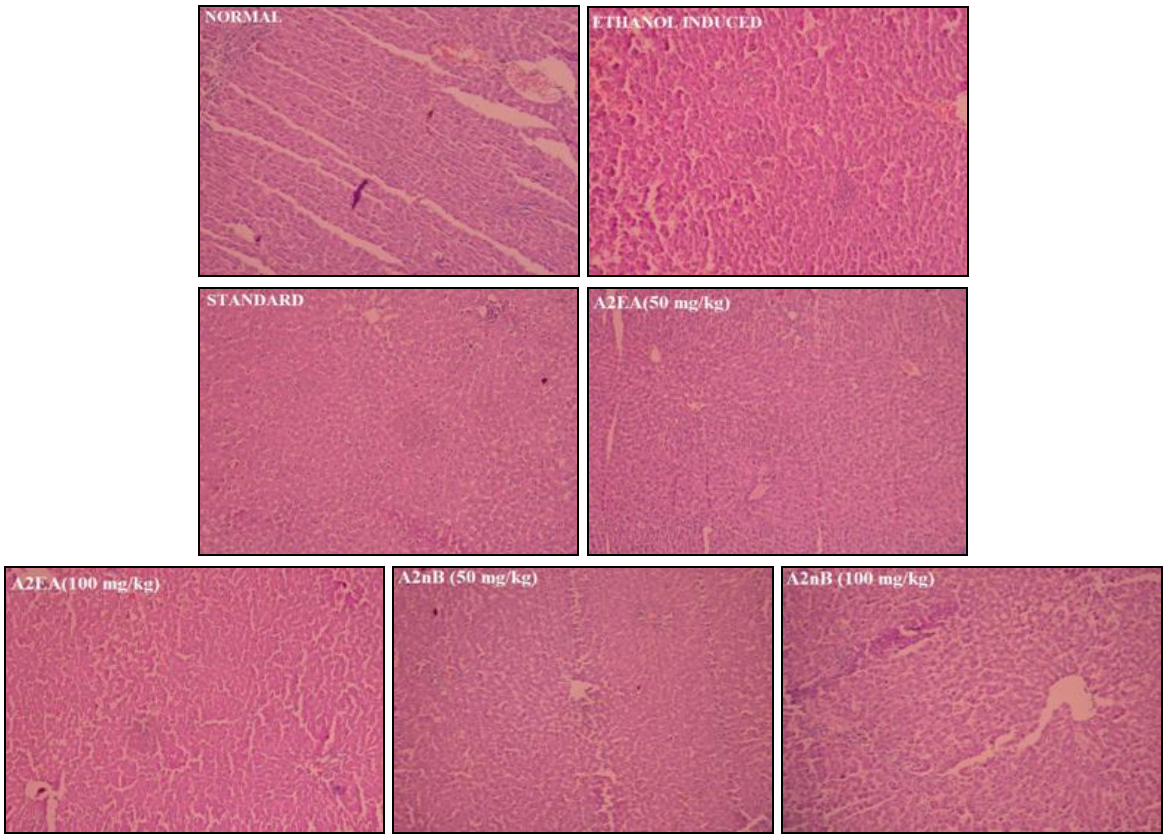

FIG. 4: HISTOPATHOLOGICAL PHOTOGRAPHS OF LIVERS IN ETHANOL INDUCED HEPATOTOXICITY

DISCUSSION: Endophytes are those micro-organisms that reside in the interior of plants especially leaves, stems and roots showing no harmful effects. All the classes of vascular plants are found to have endophytes. It includes different organisms such as fungi; bacteria, *Actinomycetes*, and *Mycoplasma* are reported as plant endophytes [26]. The role of fungal endophytes reported from plants occurred in different environmental conditions such as xerophytic, tropic, temperate and aquatic. Endophytic fungi are to be the richest source of newer antimicrobial metabolites. Some plants metabolites induce resistance when associated with endophytes. They also quickly activate defense system after pathogen challenge. Some of the fungal endophytes can protect drought conditions. Large groups of these endophytes can produce some secondary metabolites either same or novel as present in the plant.

Paracetamol is a common antipyretic and analgesic drug, if taken at high dose leads to liver damage [27]. Paracetamol gets converted to water-soluble metabolites and secreted in the urine by drug metabolizing enzymes. In the therapeutic dose, Paracetamol is converted by drug metabolizing enzymes to water-soluble metabolites and secreted in the urine [28]. Paracetamol acts by binding covalently with its toxic metabolites, *i.e.* n acetyl-p-benzoquinone amine to the sulfhydryl group of the protein resulting in lipid peroxidation and necrosis of liver cell. The functions of hepatic cells get disturbed by an overdose of Paracetamol due to liver damage. As a result plasma membrane starts leaking causing an increase in serum enzyme levels [29].

Ethanol (Alcohol) produces some detrimental changes in the liver. Chronic administration of alcohol leads to the development of steatosis, alcoholic hepatitis, and cirrhosis resulting in weight and volume change [30]. Reactive oxygen species (ROS) such as superoxide and hydroxyl radical gets accumulated on the administration of alcohol. ROS causes lipid peroxidation, oxidation of DNA and protein destroying hepatic cells [31]. Cytochrome P-450 is one of the major sites in the production of ROS in liver responding to alcohol. The activity of cytochrome P-450 increases the long-term intake of alcohol [32]. Acute toxicity studies concluded the nontoxicity of A2EA and A2nB as per OECD guidelines. Therefore, the doses selected for the *in-vivo* studies were 50 mg/kg and 100 mg/kg.

In the present study, Paracetamol and ethanol caused a significant elevation in the levels of SGOT, SGPT, SALP, total and direct bilirubin, and triglycerides level. Pretreatment with A2EA and A2nB (50 mg/kg & 100 mg/kg) fractions exhibited a significant reduction in the elevated levels caused by Paracetamol and ethanol-induced changes. These fractions showed membrane stabilizing activity as there was a decrease in serum levels. The regeneration has started taking place when there is a reduction in biochemical parameters. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of the regeneration process. Lipid peroxidation (LPO) is the oxidative degradation of lipids. Here the free radicals take the electrons from the lipids in cell membranes, resulting in cell damage. There is a raise in LPO in Paracetamol and ethanol-induced hepatotoxicity. Malondialdehyde (MDA) level is the main marker to generate free radical in LPO liver damage when treated with both Paracetamol and ethanol. The enzymes increase the oxidation of proteins during liver damage. Thus, altering the polyunsaturated fatty acids to other fatty acids and causing diminished fluid, leading to cell death.

The results revealed that treatment of rats with A2EA and A2nB to Paracetamol and ethanol administration significantly decreased these changes. The antioxidant mechanism may be responsible for the hepatoprotective activity of A2EA and A2nB. SOD and CAT enzyme levels were decreased by A2EA and A2nB (50 and 100 mg/kg) as a rise in Paracetamol and ethanol-induced groups by ROS. The protein expression levels were also determined by Western blotting analysis of liver tissue. A2EA and A2nB (50 and 100 mg/kg) administration significantly improved protein expressions of the two antioxidant enzymes in the liver. Our results suggested that A2EA and A2nB (50 and 100 mg/kg) may decline oxidative stress by elevating the activity and protein expression of the antioxidant enzymes *in-vivo.*

Normal hepatocytes cell morphology was observed in normal liver compared to Paracetamol and ethanol-induced groups where induced groups

showed fatty cell degeneration, central vein and sinusoid congestion, inflammation, and ballooning of hepatocytes. A2EA (50 mg/kg and 100 mg/kg) regenerated the hepatic cells surrounding the necrosis and fatty vacuoles, showing mild congestion of central vein and sinusoid. A2nB (50mg/kg and 100 mg/kg) also decrease in the inflammation of liver cells. Degeneration of the inflamed liver was restored with mild ballooning. Thus, A2EA and A2nB (50 and 100 mg/kg) altered the virulent effects of an excess dose of Paracetamol and ethanol on the hepatic morphology and architecture.

CONCLUSION: A2EA and A2nB showed hepatoprotective activity in Paracetamol and ethanol-induced model from the isolated fungi APLF-2 from the leaves of *Andrographis paniculata*. Membrane stabilization mechanisms and antioxidant activity may be responsible for potent hepatoprotective activity possessed by A2EA and A2nB which justifies that it can be used in the treatment of liver disorders. Further investigations will be carried out to isolate and identify secondary metabolites responsible for hepatoprotective activity concerning endophytes.

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CONFLICT OF INTEREST: None

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