

## Synergistic Activity of Green Tea and Seagrass Extract in the Regression of Alcohol Induced Liver Toxicity in Rats

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**Liver, an important organ of the body, plays a key role in the anabolism of biomolecules along with detoxification of several xenobiotics. Thus, the protection of liver from alcohol toxicity and its causative effects such as steatosis, necrosis, fibrosis, cirrhosis and steato-hepatitis is of global concern. The consumption of alcohol results in the production of ROS and RNS in the liver thereby causing oxidative stress and free radical injuries. Therefore, reliable hepato-protective agents are at demand to combat against alcohol induced toxicity. At this point, we investigated the synergistic efficacy of aqueous extracts of Green tea and seagrassin the regression of liver damage induced by alcohol intoxication. Alcohol –induced rat models were adopted to perform biochemical and histo-pathological studies. The results demonstrated tremendous decline in alcohol induced hepatotoxicity with mixed extract which indicates the synergistic and supra additive activity of phytochemicals of both green tea and seagrass.**

**Keywords:** Alcohol; Antioxidants; Camellia Sinensis; Hepato-protection; Oxidative Stress; Seagrass Halophila Beccarii.

Liver is a vital organ involved in the metabolic functions of the body (Price and Wilson, 2005). Besides, its chief role in the metabolism of biomolecules like proteins, carbohydrates and lipids and it plays a crucial role in the storage of vitamins, iron and copper along with the detoxification of several exogenous and endogenous substances. Alcohol use disorder is the foundation for considerable diseases, and the liver is the most adversely affected organ due to the effect of alcohol on diverse cellular and molecular process of liver (Wang et al., 2015). The excessive consumption of alcohol is the root cause and imperative lead factor for the progression of liver damage (Gao and Bataller, 2015). Globally,

alcohol liver diseases are one of the major health problems due to its high morbidity and mortality (Saalu et al., 2012). Liver is the only organ able to metabolize the alcohol into acetaldehyde through the catalytic function of alcohol dehydrogenase (ADH) and catalase (CAT) in microsomal ethanol oxidizing system (MEOS). Eighty percent of consumed ethanol is oxidized by the catalytic action of ADH and MOES and the remaining 20% of ethanol by catalase. During the oxidation process, the molecular oxygen accepts the released electrons and converts  $H_2O_2$  into water through the action of catalase. The acetaldehyde formed by mitochondrial enzymes then enters into citric acid cycle. However, high consumption of alcohol

leads to over production of acetaldehyde, a major toxic metabolite, which stimulates the oxidative stress. Further, the diminution in the antioxidant mechanism facilitates oxidative burst due to imbalance between pro oxidants and antioxidants (Linawati *et al.*, 2007). Hence, there is a need for natural antioxidants as dietary supplement to inhibit the free radical generation.

Literature is replete with beneficial health results of poly phenols in several pathological symptoms and diseases associated with oxidative stress such as alcohol liver diseases, cancer, neurodegenerative and cardiovascular disorders and chronic diabetes (Kim *et al.*, 2014). In addition to terrestrial plants, the marine plants like seagrasses are gaining popularity due to their adaption to the marine environment as well as possession of high levels of antioxidants, Vitamin C&E and other phytochemical compounds (Vani *et al.*, 2019). As per the literature, Green tea assumed a prominent status as herbal drink based on the abundance of poly phenols, flavonoids and phenolic compound (Paper *et al.*, 2012, Ramah *et al.*, 2014). Thus, in the present work, we aimed to evaluate the synergistic activity of seagrass *Halophila beccarii* and green tea *Camellia sinensis* extracts against alcohol induced hepatotoxicity.

## METHODS

### **Seagrass and *Camellia sinensis* aqueous extracts**

The seagrass *Halophila beccarii* plants collected from the Pulicat Lake and green tea *Camellia sinensis* leaves brought from local market were shade dried and extracted by boiling with water for one hour. The seagrass aqueous extract (SGE), green tea aqueous extract (GTE) and mixed extract (ME) of seagrass & green tea aqueous extract (50:50) were filtered, evaporated and stored at 4 °C for further use.

### **Qualitative Phytochemical analysis of *Halophila beccarii* and *Camellia sinensis***

The SGE, GTE and ME were used for qualitative detection of phenols, saponins, tannins, flavonoids, terpenoids, steroids, carboxylic acids and coumarins. Standard methods were adopted to analyze the phytochemical constituents of all extracts (Singh *et al.*, 2013).

### **Quantitative analysis for secondary metabolites**

Quantitative analysis was carried out to

measure the prevalence of secondary metabolites in the SGE, GTE and ME. The content of flavonoids was quantitatively detected at 510 nm by using spectrophotometer with quercetin as standard as per the method described by Sahu and Saxena (Sahu and Saxena, 2013). The total phenol content of extracts was estimated by the considering gallic acid as a standard at 750 nm (McDonald *et al.*, 2001). The quantitative estimation of saponins was carried out at 544 nm with Diosgenin as a standard (Makkar *et al.*, 2007).

### **Measurement of phytonutrients (Vitamin C & Vitamin E)**

SGE, GTE and ME were used to quantify the levels vitamin C by adding the extract (100-500µg) with 3.0 ml of acidified reaction mixture containing sodium phosphate and ammonium molybdate and the levels were recorded at 695 nm after boiling the mixture 2h. The amount of vitamin E present in SGE, GTE and ME was measured at 540 nm by the addition of a thiourea (10 %) and 0.25 ml 2 % Dinitro phenyl hydrazine to the extracts (Prieto *et al.*, 1999).

### **In vitro antioxidant activity of seagrass and green tea**

The standard method was adopted for measuring the total antioxidant activity of SGE, GTE and ME [100-500 µg/ml] (Prieto *et al.*, 1999, Oyaizu 1986). The reducing power of SGE, GTE and ME was measured at 700nm by comparing with standard ascorbic acid (Oyaizu, 1986). The Free Radical Scavenging activity of the SGE, GTE and ME was measured as per the method optimized in our laboratory (Vani *et al.*, 2019). The hydrogen peroxide radical scavenging activity of the extracts was calculated by measuring the absorbance at 510 nm (Ruch *et al.*, 1989).

### **Effect of seagrass, green tea and mixed extracts on alcohol induced hepato-toxic animals**

#### **Experimental Animals**

All animals (150-200 grams) were maintained and experiments were performed as per the ethical standards (Institutional Animal Ethical Committee of Sri Padmavati Mahila visvavidyalayam, Tirupati, A.P. India (1677/Po/Re/S/2012/CPCSEA/30).

The animals were categorized as different groups with six albino rats in each. Group 1 was the normal control group (NC group). Group-II: Normal Control supplemented with mixed extract

250 mg/kg (NCME) and Group 3 was the negative control group (ALC) treated with 20% w/v alcohol (10ml/kg b.w.) 16 days. Groups 4, 5, and 6 were the intervention groups treated with alcohol (10 mL/kg. b.w) and Green tea extract, seagrass extract and mixed extract (GTE+ALC, SGE+ALC, and ME+ALC group) at a concentration of 250mg/kg.b.w. respectively for 16 days. After treatment, blood was collected from retro- orbital plexus and serum was separated for the analysis of alanine amino transaminase , Aspartate amino transaminase and Alkaline phosphatase (Rashmi et al., 2016). Then the animals were dissected to collect liver tissue to estimate the lipid peroxides, MDA content (Fraga et al., 1988), levels of antioxidants such as superoxide dismutase (Kakkar

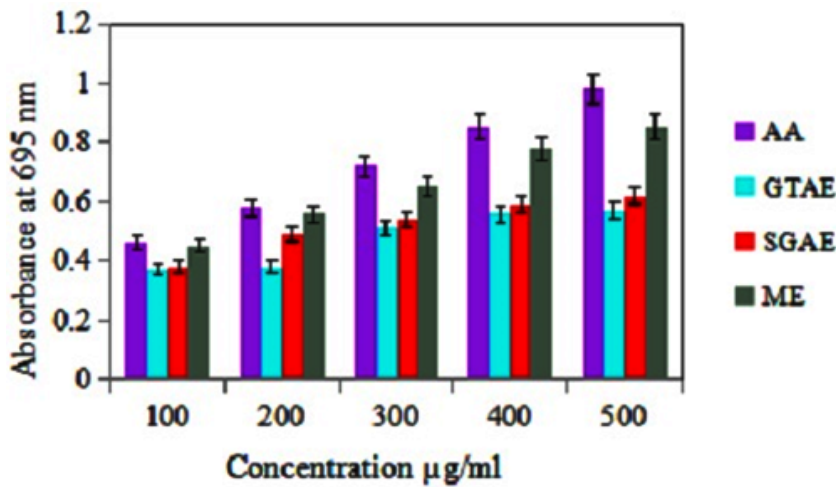
et al., 1984; Crouch et al., 1981), catalase (Sinha, 1972) and histopathological studies.

**Histopathological observations of lung tissue**

For histopathological examination, small sections (0.5 cm) of hepatic tissues sliced from lobe edges were processed for paraffin embedding, fixed with 10% formaldehyde and staining was carried out using hematoxylin and eosin (Bancroft and Cook HC, 1984).

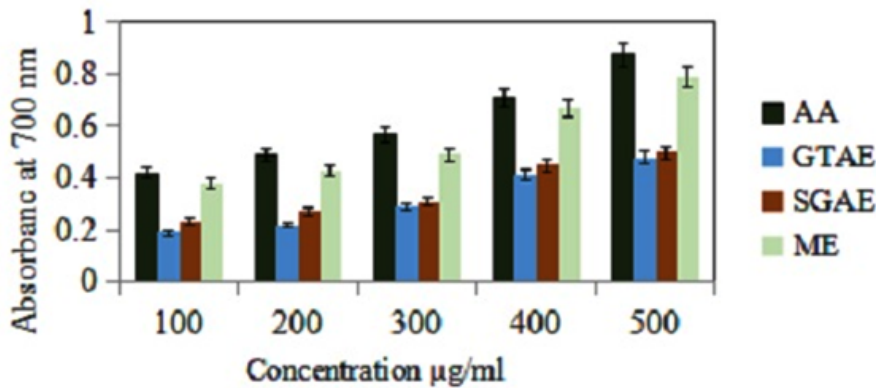
**Statistical analysis**

Three independent data sets were generated for all the experiments and n=6 unless otherwise noted. The mean ± SEM of the experimental data was calculated using Graph pad prism (7.0). One-Way ANOVA followed by Dunnet’s test was followed to compare with control



Values are expressed as mean ± SEM of statistical significance 1% level (p<0.05).

**Fig. 1.** Antioxidant activity of Seagrass, Green tea and mixed extracts.



Values are expressed as mean ± SEM of independent experiments, statistical significance 1% level (p<0.05).

**Fig. 2.** Reducing power of Seagrass, Green tea and mixed extract

and Statistical significance is denoted by an asterisk (\*) when  $p$  values are  $*p < 0.03$  to  $0.05$ ,  $*p < 0.001$ ,  $***p < 0.0001$  and  $****p < 0.00001$ .

## RESULTS

### Phytochemical profile of Green tea and Seagrass

The extracts of Green tea and Seagrass exhibited the abundance of alkaloids, phenols, saponins, flavonoids, tannins, coumarins, glycosides, steroids, terpenoids (Table 1.).

**Table 1.** Phytochemical profile of SGE, GTE and ME

S. No	Phytochemical Constituents	GTE	SGE	ME
1	Alkaloids	-	+	++
2	Cardiac glycosides	+	-	+
3	Coumarins	-	+	+
4	Flavonoids	+	+	++
5	Glycosides	+	+	++
6	Phenols	+	+	++
7	Saponins	-	+	++
8	Steroids	-	+	++
9	Tannins	-	+	++
10	Terpenoids	+	+	++

Compared with green tea, seagrass extract showed larger amount of coumarins, saponins and tannins. Both the extracts are enriched with vitamin C and vitamin E. All the secondary metabolites and vitamins seem to be abundant in the mixed extract compared to seagrass and green tea extract (Table 2).

### In vitro anti-oxidant potential of SGE, GTE and ME

The mixed extract of seagrass and green tea demonstrated significant antioxidant activity compared parent extract and levels were highly considerable on comparison with standard ascorbic acid (Fig.1).

The DPPH and  $H_2O_2$  scavenging assay are simple, fast and widely used methods for measuring the free radical scavenging ability. All the three extracts exhibited dose-dependent free radical scavenging activity and 50% scavenging of DPPH and  $H_2O_2$  was noticed with 300  $\mu\text{g/ml}$  of Mixed extract (Table 3).

### Reducing power of Mixed aqueous extract

The antioxidant potential of secondary metabolites is calculated based on the reducing power which reduces ferric ions to ferrous ions by their electron donating activity. The mixed extract demonstrated equivalent reducing power

**Table 2.** Abundance of phytochemical constituents in SGE, GTE&ME

Compounds	GTE mg/g	SGE mg/g	ME mg/g
Phenols	6.44 $\pm$ 0.035	8.15 $\pm$ 0.025	19.44 $\pm$ 0.032
Flavonoids	4.25 $\pm$ 0.048	6.05 $\pm$ 0.025	11.59 $\pm$ 0.035
Saponins	2.02 $\pm$ 0.025	5.04 $\pm$ 0.082	13.02 $\pm$ 0.062
Tannins	0.36 $\pm$ 0.025	3.15 $\pm$ 0.025	5.75 $\pm$ 0.045
Vitamin C	5.84 $\pm$ 0.047	7.15 $\pm$ 0.055	10.96 $\pm$ 0.110
Vitamin E	4.95 $\pm$ 0.108	18.50 $\pm$ 0.080	23.86 $\pm$ 1.202

Values are expressed as mean  $\pm$  SEM of independent experiments, statistical significance 1% level ( $p \leq 0.05$ ).

**Table 3.** Free radical scavenging by SGE, GTE and ME

Conc $\mu\text{g/ml}$	Scavenging activity (%) on DPPH				Scavenging activity (%) on $H_2O_2$			
	GTE	SGE	ME	Ascorbic acid	GTE	SGE	ME	Ascorbic acid
100	23	25	37	40	21	26	49	40
200	25	35	42	47	25	28	56	47
300	37	42	51	55	38	40	57	55
400	45	53	57	67	42	45	63	67
500	52	60	65	75	50	55	70	75

Values are expressed as mean  $\pm$  SEM of independent experiments, statistical significance 1% level ( $p \leq 0.05$ ).

on comparison with ascorbic acid standard (Fig. 2).

#### Protective effect of SGE,GTE and ME on liver bio markers in Alcohol induced Rats

The markedly increased activities of hepatic biomarker enzymes confirm the liver damage caused by alcohol toxicity. Alcohol induced rats were supplemented with SGE, GTE and mixed extract independently at a dose level of 250 mg/kg bwt for about 16 days. When compared with SGE and GTE, mixed extract represented significant decrease in AST, ALT, ALP levels of alcohol induced rats (Table 4).

#### Protective effect of SGE,GTE &ME on liver Antioxidant enzymes and TBARS

The range of liver antioxidant enzymes such as SOD, catalase and TBARS levels was measured to detect the protective effect of SGE, GTE&ME on hepatotoxicity and oxidative damage induced by alcohol. The alcohol control group (ALC) showed 50 % reduction in the expression level of antioxidant enzymes on comparison with normal control group. Administration of SGE, GTE and ME significantly improved the level of antioxidant enzymes as compared to

alcohol group. Lipid peroxidation levels were increased in ALC group due to alcohol toxicity. The augmented level of TBARS is an indicative of oxidative damage caused by peroxidation of lipids on oxidative stress. The TBARS levels were brought to the normal by the administration of mixed extract when compared with seagrass and green tea extracts which confirms the synergistic effect of seagrass and green tea in the alleviation of hepatotoxicity induced by alcohol (Table 5).

#### Histopathological examination

The hepatocyte cells are found to be normal with clearly visible nuclei, central vein, hepatic stroma, parenchyma and portal triad in control group (Fig.3A). Normal control rats treated with mixed extract showed normal appearance of hepatocytes (Fig.3B). The animal groups treated with alcohol showed disarranged hepatocytes with cytomegaly along with vacuolated degenerating cells with fatty changes called steatosis and necrosis of hepatic cells (Fig.3C). Appearance of steatosis with small and large droplets of fat is classical hallmark of alcoholic hepatitis in alcohol induced rats (Fig.3D). The administration of mixed extract stand for several changes like drastic

**Table 4.** Effect of SGE, GTAE and ME on the Liver Function Tests as a marker of liver damage in alcohol induced hepatotoxicity in rats

Groups	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Group-I: Control	40.2 ± 1.01	30.5 ± 0.09	94.7 ± 0.05
Group- II: Control + ME	30.7 ± 1.09*	21.8 ± 0.5*	90.5 ± 1.04**
Group- III: Alcohol Control(ALC)	70.4 ± 1.08**	80.1 ± 0.1*	210.5 ± 0.07*
Group-IV: ALC + GTE	50.7 ± 1.02*	39.2 ± 0.8*	170.4 ± 1.09*
Group-V: ALC + SGE	45.7 ± 1.04*	32.8 ± 0.9*	152.8 ± 1.3*
Group-VI: ALC +ME	42.9 ± 1.07**	31.6 ± 1.01**	145.4 ± 1.5**

Values are expressed as mean ± SEM of independent experiments, statistical significance 1% level ( $p \leq 0.05$ ).

**Table 5.** Effect of SGE, GTE and ME on liver antioxidant enzymes in Alcohol induced rats

Groups	SOD	Catalase	TBARS
Group-I: Control	30.36 ± 0.37	56.4 ± 0.33	0.19 ± 0.002
Group- II: Control + ME	24.34 ± 0.33*	52.13 ± 0.31*	0.20 ± 0.05*
Group- III: Alcohol Control(ALC)	15.28 ± 0.05**	23.4 ± 0.07*	43.4 ± 0.07*
Group-IV: Alcohol + GTE	18.41 ± 0.07*	46.9 ± 0.42*	0.30 ± 0.02*
Group-V: Alcohol + SGE	22.54 ± 0.27*	47.7 ± 0.065*	0.26 ± 0.04*
Group-VI: Alcohol +ME	29.02 ± 0.31*	52.8 ± 0.20*	0.25 ± 0.01*

Values are expressed as mean ± SEM of statistical significance 1% level ( $p \leq 0.05$ )

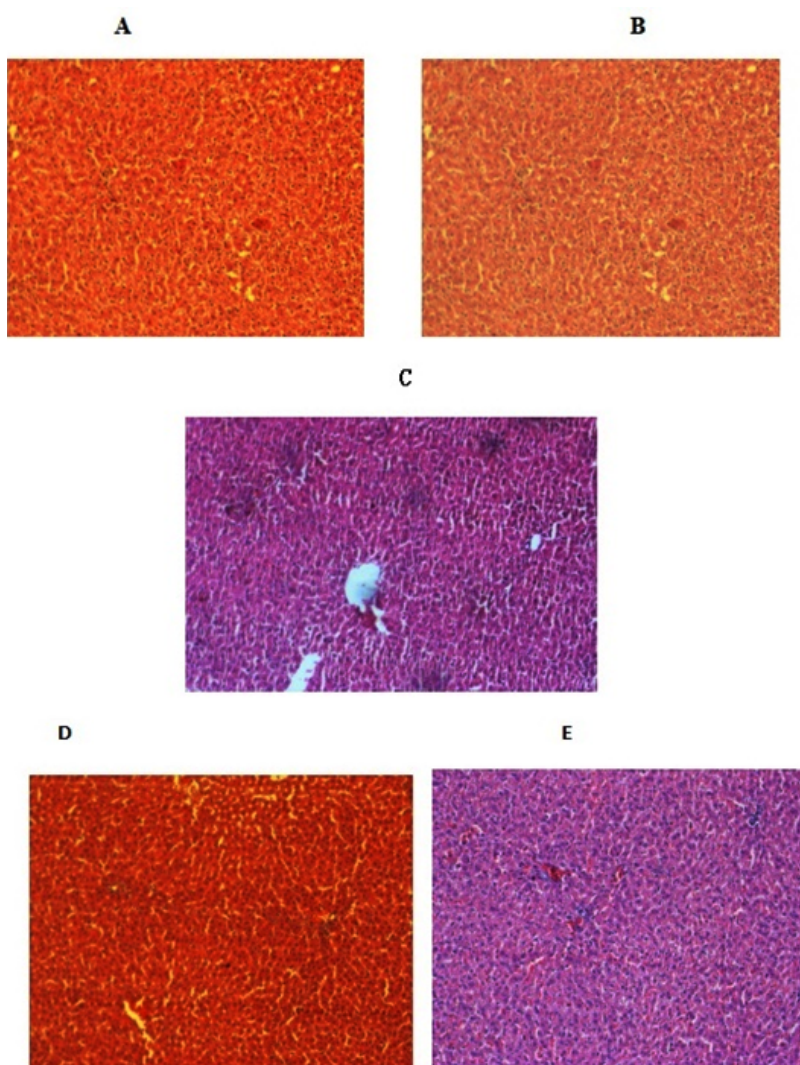
reduction in the vacuolar changes associated with cytomegaly, disappearance of cloudy swelling, fatty changes and areas of regeneration (Fig.3E)

### DISCUSSION

In this work, seagrass and green tea were selected based on the presence of phytochemical constituents such as phenols, flavonoids, tannins and saponins. The antioxidant activity of aqueous extracts of seagrass and green tea represents positive relationship between regulation of

oxidative stress and secondary metabolites such as phenols and flavonoids (Zahari *et al.*, 2016). Due to correlation between phytochemicals and the antioxidant activity of seagrass and green tea, the hepato protective potential of the mixed extract was determined using the alcohol-induced hepato toxic rats as animal models.

The elevated levels of serum bio markers, a hallmark for hepatic damage, confirmed the seriously affected Liver functions in alcohol-treated animals (Vidhya and Indira, 2009). The oxidative stress, as a result of generation of ROS, is elevated



**Fig. 3.** A. Normal control B. Normal control treated with mixed extract C. Appearance of Steatosis, composed of small and large droplet fat are classic hallmarks of alcoholic hepatitis in alcohol induced rats. D. Greater extent of reduction in cloudy swelling, fatty changes exhibit recovery. E. Lesser sinusoidal congestion and fatty change in alcohol intoxicant rats treated with Mixed extract

due to alcohol intoxication which facilitates the diffusion of ions and leakage of ALT and AST from cytoplasm into blood after hepatic cellular damage (Vermaulen et al., 1992). However, treatment with mixed extract has decreased the levels of hepatic markers as compared to the alcohol induced control group. Our results are in agreement with the extract of *Silybum marianum* plant which possess antioxidant activity due to presence of flavonoids (Soufy, 2012, Ighodaro and Omole, 2012). Similar trend in the decreased levels of hepatic enzymes by quercetin was reported in ethanol induced toxicity (Pari and Karthikesan, 2007). The protective effect of mixed extract, in the alcohol mediated hepatotoxicity, is mainly due to the stabilization of cell membrane which prevents the leakage of hepatic enzymes and their translocation into the serum.

Similarly, total antioxidant activity is one of the primary indicator of oxidative stress that indicates the collective cause of all antioxidants found in a cellular system (Remmer et al., 1989). Thus, the quantitative estimation of total antioxidant activity presents the status of exact endogenous antioxidant status. In alcohol induced hepatotoxicity, alcohol causes the hepatic damage by cytokines induced inflammatory mediators mainly TNF- $\alpha$  from hepatic Kupffer cells. Chronic alcohol administration results in hepatotoxicity, due to auto oxidation of hepatic cells by lipid peroxidation which generate prooxidants and reduces antioxidant levels (Eswar Kumar et al., 2013). The oxidative stress of liver was augmented by alcohol intoxication, as represented by 50% reduction in the expression level of antioxidant enzymes and increased MDA content of liver tissues relative to the normal control group. The lipid peroxidation generates higher levels of TBARS levels cause significant deformations in hepatic and extra hepatic cellular metabolism and further results in whole cell deformity and death (Maruthappan and Sakthi Shree, 2009, Arun and Balasubramanian, 2011). Superoxide dismutase (SOD) expression is the representative index for damage in liver cells as it plays a key role in alleviating the toxicity generated by free radicals (Kharpate et al., 2007). Administration of rats with the mixed extract and extracts of seagrass and green tea has decreased the elevated liver MDA and increased the expression of antioxidant enzymes

such as SOD and catalase with varied degrees, and the maximum effect was found with the mixed extract of seagrass and green tea.

The biochemical investigations were also correspondingly correlated with the histopathological observations. The animal group treated with alcohol showed disarranged hepatocytes with cytomegaly along with vacuolated degenerating cells with fatty changes called steatosis and necrosis of hepatic cells. Appearance of Steatosis with small and large droplets of fat is classical hallmark of alcoholic hepatitis in alcohol induced rats. The administration of mixed extract stand for several changes like drastic reduction in the vacuolar changes associated with cytomegaly and disappearance of steatosis.

## CONCLUSION

The conspicuous decrease of Alcohol-elevated ALT, AST and ALP levels due to the administration of mixed extract represents the stabilization of plasma membranes, repair of hepatocellular damage along with the improvement in biliary dysfunction. With the support from biochemical and histopathological observations, the current paper demonstrated the synergistic activity of seagrass and green tea extracts in the regression of hepatotoxicity induced by alcohol intoxication.

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### Conflict of Interest

The authors declare that there is no conflict of interest.

### Authors' Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

### Funding source

None.

### Data Availability

All datasets analyzed during this study are included in the manuscript.

### Ethics Statement

This study was carried out in accordance with the recommendations of (Institutional Animal Ethical Committee of Sri Padmavati Mahila visvavidyalayam, Tirupati, A.P. India guidelines under the protocol number (1677/Po/Re/S/2012/CPCSEA/30).

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