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SIMULTANEOUS EVALUATION, ESTIMATION OF HEPATOPROTECTIVE EFFECTS OF *KYLLINGA BREVIFOLIA* AND ANTI-EPILEPTIC ACTIVITY OF *RORIPPA SARMENTOSA* EXTRACTS IN RATS

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ABSTRACT

Methanolic extract of whole plant of *Kyllinga brevifolia* (MEKB) was prepared and investigation of its hepatoprotective activity and acute oral toxicity was carried out in male Wistar albino rats by using CCl_4 induced hepatotoxicity. 150 and 300mg/kg, p.o doses of MEKB and 100mg/kg, p.o dose of the standard drug Silymarin were administered three times at intervals of 8hr and then 0.8ml/kg of CCl_4 was administered to all the groups except normal control for 2 days. Observation of various biochemical parameters like SGOT, SGPT, ALP, γ-GT, TP and total bilirubin to assess the hepatoprotective activity along with histopathological studies was carried out after 24h of CCl_4 treatment. As assessed by the biochemical changes and histopathological studies, MEKB at the doses of 150 and 300mg/kg inhibited CCl_4 induced liver toxicity in Wistar albino rats. The methanolic extract of whole plant of *Kyllinga brevifolia* demonstrated significant protection against CCl_4 inducd hepatocellular injury. In traditional Indian medicine, the whole plant of *Rorippa sarmentosa (DC.) Macbr.* is used to treat epilepsy. Previous studies have demonstrated that extracts of these plants was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. The aim of the present study is the investigation of the effect of ethanolic (95%) extract of *Rorippa sarmentosa (DC.) Macbr.* (EERS) on biogenic amines concentrations in rat brain after induction of seizures by MES and PTZ. The relationship between seizure activities and altered concentrations of monoamines such as noradrenaline (NA), dopamine (DA), serotonin (5-HT) and Gamma amino butyric acid (GABA) in forebrain of rats in MES and PTZ seizure models was our aim of study. In MES model, EERS (150 & 300 mg/kg) significantly restored the decreased levels of brain monoamines such as NA, DA, 5-HT and GABA. Similarly, EERS significantly increased the monoamines in forebrain of rats in PTZ model. Hence, we can conclude that there was an increase in the monoamines concentration in rat brain upon administration of the ethanolic extract of *Rorippa sarmentosa (DC.) Macbr.,* which may decrease the susceptibility to MES and PTZ induced seizure in rats.

KEYWORDS : *Kyllinga brevifolia,* Hepatoprotective, CCl_4, Silymarin, Hepatotoxicity, Antiepileptic activity, Traditional Medicine, *Rorippa sarmentosa (DC.) Macbr.,* biogenic amines, NA, DA, 5-HT and GABA.

INTRODUCTION

The liver is the largest organ in the body weighing 1200-1500g. It plays a key role in regulating homeostasis within the body. Several important functions like storage and metabolism of fats and carbohydrates, metabolism of hormones and excretion of bilirubin, detoxification of drugs and other toxins including protein synthesis are regulated by the liver. Liver diseases are associated with distortion of these metabolic functions [1,2]. Beside viruses, which are the main cause of liver diseases, the liver lesions arising from excessive drug therapy, xenobiotics, alcoholic intoxication and environmental pollution are not uncommon [3]. About 20,000 deaths are found due to liver disorders every year [4]. A crucial factor for overall health and well being is to maintain a healthy liver [5]. Thus, liver diseases are among one of the serious health problems posed to human beings and its disorders are numerous with no effective remedies [6-8]. Management of liver diseases is still a challenge to the modern medicine due to unavailability of ration therapy for treatment of liver disorders [9, 10]. Therefore, herbs play an important role in the management of various liver disorders [6]. The use of natural remedies for the treatment of various hepatic diseases has a long history and medicinal plants and their derivatives are still used all over the world [4].

Kyllinga brevifolia Rottb. (Family - Cyperaceae) is perennial sedge arising from a thin creeping rhizome bearing three-angled stems up to 30 cm high. Leaves are basal, in three ranks, long-linear (grass-like), about the same length as stems. Flowers are minute, borne in a terminal white globose head (occasionally two smaller lateral heads may also be present) up to 8 mm in diameter, subtended by three spreading leaf-like bracts up to 15 cm long. Fruit is a minute achene up to 1.5 mm long. Flowers and fruits are usually available throughout the year. It is common in damp, disturbed places such as pastures, cane fields, stream sides, etc. from near sea -level up to over 1000 m elevation. It is widely distributed throughout the tropics and common throughout in the South Pacific. Traditionally used in treatment of liver disease [11,12].

Epilepsy affects about 0.5 to 1.0% of the world's population and is among the most prevalent of the serious neurological disorders [38]. In developing countries the prevalence of epilepsy is generally higher than in developed countries [39]. Epileptic seizures are paroxysmal clinic events arising from neuronal hyper excitability and hypersynchrony of the cerebral cortex, either locally (partial seizures) or diffusely in both hemispheres (generalised seizures). During a seizure the agitated neuronal activity is caused by a sudden imbalance between the inhibitory and excitatory signals in the brain with δ-aminobutyric acid (GABA), noradrenaline, serotonin, and dopamine respectively; being the most important neurotransmitters involved [40].

Both dopamine and serotonin have convincingly been implicated in the pathophysiology of seizures, though their role in the treatment of epilepsy remains controversial. [41-45]. All the currently available antiepileptic drugs are synthetic molecules. In traditional medicine, medicinal plants are used for the therapy of epilepsy and have shown to possess promising anticonvulsant activities. Screening of anticonvulsant activity using animal models can be an invaluable source for search of new antiepileptic compounds. Previous study on the ethanolic (95%) extract of whole plant of *Rorippa sarmentosa (DC.) Macbr.* (EERS) was based on reporting of acute toxicity and screening for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats [6]. The present study was carried out to analyze the effect of *Rorippa sarmentosa (DC.) Macbr.* on biogenic amines concentrations in rat brain after induction of seizure by MES & PTZ model.

Rorippa sarmentosa (DC.) Macbr. Linn. (Family: Brassicaceae) is common in damp waste areas from near sea-level to more than 1000 m elevation. Probably native to Melanesia and now widely distributed throughout most of the tropical Pacific. In Fiji, the plant is used to induce miscarriages and to cure convulsions in children [46-48].

Therefore, the present study was aimed to establish the effect of *Rorippa sarmentosa (DC.) Macbr.* on biogenic amines levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS [48-62]

Plant collection

In the month of March 2016, the whole plant of *Kyllinga brevifolia* was collected from Hyderabad, Telangana. The voucher specimen of the plant was deposited at the college, for further reference. The plant material of *Rorippa sarmentosa (DC.) Macbr.* was collected from Deshmukhi in the month of April 2017. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

A coarse powder of the whole plant of *Kyllinga brevifolia* was obtained by pulverization in grinder-mixer after it was dried in shade. A 40 mesh sieve was taken and the powder was passed through it. 230gm of powder was weighed accurately and transferred into a Soxhlet apparatus. It was subjected to continuous hot extraction with methanol as solvent for 48h. Using rotary evaporator the extract was evaporated under reduced pressure until all the solvent has been removed. The percentage yield of methanolic extract of *Kyllinga brevifolia* was found to be 15.5%w/w. Similarly, the whole plants of *Rorippa sarmentosa (DC.) Macbr.* were made into dry powder after adequate separation and shade drying. The dry powder was passed through 40 mesh sieve. 80gm of the powder was accurately weighed and subjected to continuous hot extraction in Soxhlet Apparatus using ethanol as solvent. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed. Percentage yield of ethanolic (95%) extract of *Rorippa sarmentosa (DC.) Macbr.* was found to be 20.5 % w/w.

Animals used

The animals were kept in polypropylene cages and maintained in a well-ventilated room with 12:12 hour light/dark cycle. Standard pellet feed and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA. Albino wistar rats (150-200g) of either sex were employed in the study.

Acute Toxicity Study

As per the OECD guideline no. 423 (Acute Toxic Class Method), the acute toxicity of methanolic extracts of *Kyllinga brevifolia* was determined. Even at 1500mg/kg dose the test extract was not mortal. Hence, 1/10th (150mg/kg) and 1/5th (300mg/kg) of this dose were selected for further study [13].

Carbon tetrachloride induced hepatotoxicity in rats

Evaluation of liver protective effect was carried out using the carbon tetrachloride (CCl_4) model described by *Rao and Mishra* [14]. Wistar albino rats (150-200g) were divided into five groups and received the following treatments; group-I served as normal control; received vehicle only. Group-II served as untreated group; received only CCl_4, to analyze the severity of toxicity produced by carbon tetrachloride administration. Groups III-V served as treated groups; received MEKB orally at the dose of 150 and 300mg/kg, p.o. and standard drug Silymarin orally at a dose of 100mg/kg, p.o. three times at 8h intervals. 0.8 ml/kg of Carbon tetrachloride diluted with liquid paraffin (1:1) was administered p.o. for 2 days to all animal groups except for normal control. After 24h of carbon tetrachloride treatment, the retro-orbital sinus was punctured and blood was collected from all groups of rats. Separation of serum was executed by centrifugation at 2500rpm at 37^{0}C for 15min and analyzed for various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by *Reitman and Frankel* method [15], alkaline phosphatase (ALP) by *Kind and King* method [16], and bilirubin by *Malloy and Evelyn* method [17].

Statistical analysis

Mean ± standard error mean (S.E.M) was used to express the data. One way and multiple way analysis of variance (ANOVA) was used to assess the Significance of differences among the group. The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance. The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

Experimental design

Albino wistar rats were divided into four groups of three animals each. Vehicle control (1% w/v SCMC, 1ml/100 g) was administered to Group-I, whereas standard drug (Phenytoin, 25mg/kg) *i.p* was given to Group-II, Group-III and IV, received 95% ethanolic extract of the whole plant of *Rorippa sarmentosa (DC.) Macbr.* (L.) (150 and 300 mg/kg b.w) *p.o* respectively for 14 days. On the 14th day, Seizures are induced to all the groups by using an Electro convulsiometer. The duration of various phases of epilepsy were observed.

Pentylenetetrazole (90mg/kg b.w, *s.c*) was administered to other groups to induce clonic convulsions after above respective treatment. Animals were observed for a period of 30mins post– PTZ administration.

A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine

After observing the convulsion on the 14th day, the rats of all groups were sacrificed; whole brain was dissected out and the forebrain was separated. A weighed quantity of tissue was taken and homogenized in 0.1 mL hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. The tube was centrifuged after 10 mins of vigorous shaking under same conditions to obtain two separate phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay [10].

Nor-Adrenaline and Dopamine Assay

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was continued after two minutes by addition of 0.01ml Na_2SO_3 in 5m NaOH. After 1.5 minutes, acetic acid was added. The solution was then heated to 100°C for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette as with 5-HT: in some cases, the readings were limited to the excitation maxima. 395-485nm for NA and 330-375nm for DA uncorrected instrument values [10].

Serotonin Assay

O-pthaldialdehyde (OPT) method was employed and few modifications were done in the reagent concentration along with the changes in the proportions of the solvent. From the OPT reagent 0.025ml were added to 0.02ml of the HCl extract. The fluorophore was developed by heating to 100°C for 10 min. Upon reaching equilibrium with the ambient temperature, excitation / estimation spectra or intensity reading of samples were carried out at 360-470 nm[10].

Estimation of brain GABA content

The brain amino butyric acid (GABA content was estimated according to the method of Lowe et al., (1958) [11]. Decapitation method was used to sacrifice the animals and brains were rapidly removed, and the forebrain region was separated. It was blotted,

weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000rpm for 10min at 0°C. 0.1ml sample of tissue extract was placed in 0.2ml of 0.14 M Ninhydrin solution in 0.5M corbonate-bicorbonate 1 buffer (pH9.95), maintained in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartarate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). Fluorescence at 377/455nm in a spectofluorimeter was recorded after 10min.

RESULTS

Acute toxicity study

In the study involving acute toxicity, the animals that treated with the higher dose of 1500mg/kg of MEKB did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. No mortality was reported in the above-mentioned dose at the end of the 14 days of observation.

Effect of MEKB on CCl_4 – induced hepatotoxicity

The results of MEKB on Carbon tetrachloride-induced hepatotoxicity were represented in Table 1. The levels of SGOT, SGPT, ALP, γ-GT and total bilirubin exhibited a significant increase (P<0.001) where as there was a decrease in the levels of TP in the animals that were treated with only CCl_4 when compared to the normal control group after 24h of CCl_4 treatment, indicating hepatocellular damage. A significant reduction (P<0.001) in the CCl_4 induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increase in the TP was shown by the MEKB at tested doses (group-III & IV) when compared to the animals treated only with CCl_4 (group-II) after 24h of CCl_4 treatment. Overall, MEKB at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin

Table-1: Effects of MEKB on alternation of hepatic enzyme and serum bilirubin in rat after 24h of CCl_4 treatment

Group (n=3)	SGOT (U/L	SGPT (U/L)	ALP (U/L)	γ–ГТ (IY/Λ)	TP (gm/dl)	Total Bilurubin (mg/dl)
Group-I (Normal Control)	25.21 ± 0.12**	15.22 ± 0.02**	184.4 ± 0.05**	45.17 ± 1.04**	6.23 ± 0.12**	0.72 ± 0.02**
Group-II (CCl_4: 0.8ml/kg)	60.45 ± 0.14	37.15 ± 0.12	412.46 ± 0.14	96.24 ± 0.07	2.05 ± 0.12	3.70 ± 0.01
Group-III (MEKB 150mg/kg)	41.17 ± 0.14**	28.44 ± 0.04**	259.5 ± 0.10**	62.94 ± 0.15**	3.78 ± 0.41**	1.22 ± 0.02**
Group-IV (MEKB 300mg/kg)	35.28 ±0.05**	23.74 ± 0.14**	231.24 ±0.12**	56.17 ± 0.1**	5.45 ± 0.05**	0.86 ± 0.03**
Group-V(Silymarin 100mg/kg)	31.45 ± 0.04**	21.13 ± 0.42**	192.48 ±0.3**	50.64 ±0.14**	7.145 ± 0.12**	0.76 ± 0.01**

*Values are expressed as mean ± SEM of 6 rats in each group. ** p<0.001, as compared to CCl_4-treated group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate tranaminase, ALP = Alkaline phosphatase, γ-GT = Gamma glutamyl transpeptidase, TP = Total proteins.*

Table: 1. Effect of EERS on neurotransmitters levels in rat brain after MES induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control(SCMC 1ml/100gm)	768.66±4.29	645.74±2.18	192.32±2.34	289.24±1.48
II	MES (SCMC 1ml/100gm)	435.29±2.84 ^{a**}	485.12±2.28 ^{a**}	74.25±2.16 ^{a**}	235.31±2.18 ^{a**}
III	Phenytoin 25mg/kg,*i.p*	590.45±3.15 ^{b**}	692.14±2.14 ^{b**}	102.38±2.54 ^{b**}	290.24±2.24 ^{b**}
IV	EERS 300 mg/kg,*p.o*	572.16±3.47 ^{b**}	652.24±2.21 ^{b**}	90.27±0.19 ^{b**}	280.27±1.28 ^{b**}
V	EERS 150 mg/kg,*p.o*	730.19±3.27 ^{b*}	576.32±3.69 ^{b*}	84.64±0.48 ^{b*}	275.22±2.45 ^{b**}

*Values are expressed as mean ± SEM of six observations. Comparison between: **a**- Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.*

Table: 2. Effect of EERS on neurotransmitters levels in rat brain after PTZ induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control (SCMC 1ml/100gm)	769.24±2.39	848.42±3.56	187.05±2.21	273.83±1.12
II	MES(SCMC 1ml/100gm)	532.26±3.57 ^{a**}	576.23±4.15 ^{a**}	96.15±3.21 ^{a**}	201.42±1.24 ^{a**}
III	Diazepam (4mg/kg), *p.o*	612.46±3.29 ^{b**}	845.14±3.28 ^{b**}	128.21±2.68 ^{b**}	274.13±1.12 ^{b**}
IV	EERS 300 mg/kg,*p.o*	748.39±1.34 ^{b*}	895.26±2.37 ^{b**}	115.20±2.18 ^{b**}	288±1.27 ^{b**}
V	EERS 150 mg/kg,*p.o*	752.16±2.12 ^{bns}	769.15±4.15 ^{b**}	114.12±1.44 ^{b*}	255.56±1.12 ^{b**}

*Values are expressed as mean ± SEM of six observations. Comparison between: **a** - Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.*

DISCUSSIONS AND CONCLUSIONS

The role of biogenic amines in epileptogenesis and in recurrent seizure activity is well-documented. Deficiencies in gamma amino butyric acid (GABA), noradrenaline (NA), dopamine (DA) and/or serotonin (5-hydroxy- tryptamine or 5-HT) can be induced spontaneously and experimentally. Many experimental procedures have been designed to increase monoaminergic activity and they have proven antiepileptic properties [12-17]. In the present study, monoamine levels in the brain were restored by established antiepileptic drugs such as Phenytoin and diazepam [18]. Similarly, significant increased monoamine levels (p<0.05 & p<0.01) were observed in the forebrain of rats due to EERS. Drugs that increase the brain contents of GABA exhibit anticonvulsant activity against seizures induced by MES and PTZ [19]. MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures [20].

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect [21]. In addition to the GABA binding site, the $GABA_A$ receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates, ethanol etc., [22]. The effect of *Rorippa sarmentosa (DC.) Macbr.* extract on brain GABA content was studied. The extract showed significant (p<0.05 & p<0.01) increased GABA content in brain dose dependently. The elevation of brain GABA content signifies the anticonvulsant activity of *Rorippa sarmentosa (DC.) Macbr.*

A greater susceptibility to seizures induced by the chemoconvulsant PTZ and electroconvulsive shock was observed in Norepinephrine-lesioned rats [23]. The antiepileptic role of endogenous Norepinephrine was inferred from studies that showed harmful effects of Norepinephrine system on seizures induced by electrical stimulation or systemic administration of chemoconvulsants [24,25]. The antiepileptic activity of *Rorippa sarmentosa (DC.) Macbr.* extract was proven by the significantly (p<0.05 & p<0.01) increased levels of NA in forebrain due to EERS. Chen *et al.*,[26] demonstrated that pre-treatment with the

monoamine-depleting agent reserpine decreased the epileptic threshold to PTZ and caffeine in mice. Reserpine lacks specificity, since this drug also depletes serotonin (5-HT) and DA, in addition to NE. Therefore, a multiple deficit of monoamines could be due to increased seizure susceptibility [27]. The present studies confirmed and extended these results. It became clear that EERS significantly increased the serotonin (5-HT) and DA and NA. Susceptibility to various epileptic stimuli was significantly decreased.

In conclusion biogenic amines participate in the control of Maximal electroshock and pentylenetetrazole induced seizure in rat models. Our findings support the hypothesis that decreased the monoamines levels in rat brain after induction of seizure. Monoamines such as NA, DA, 5-HT and GABA levels were significantly restored on forebrain in *Rorippa sarmentosa (DC.) Macbr.* extract treated rats. Thus EERS increases the seizure threshold and decreased the susceptibility to MES and PTZ induced seizure in rats. It can be suggested that ethanol extract of whole plant of *Rorippa sarmentosa (DC.) Macbr. L.* possesses antiepileptic properties that may be due to restored biogenic amines levels in rat brain. These results support the ethno medical uses of the plant in the treatment of epilepsy. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

Liver is the vital organ of metabolism and excretion. It produces and secretes bile; it also produces fibrinogen, prothrombin, heparin and sulfuric acid ester. It also converts sugar into glycogen [18]. Any changes in anatomy or functions of liver are characterized by cirrhosis, jaundice, tumors, liver cell necrosis and hepatitis, metabolic and degenerative lesion etc. The management of hepatic diseases is still a challenge to the modern medicines [10, 19]. Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders [20, 21]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is *Kyllinga brevifolia*. The present studies were performed to investigate the hepatoprotective activity of methanolic extract of whole plant *Kyllinga brevifolia* in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver diseases.

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [22]. CCl_4 is a potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl_3.) by liver cytochrome P-450 dependent monooxygenases. This CCl_3 free radical combined with cellular lipids and proteins in the presence of oxygen to produce lipid peroxides [23]. Thus, antioxidant or free radical generation inhibition is important in protection against CCl_4 induced liver lesion [24]. The flavonoids constituents possess free radical scavenging properties [25]. In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also Bilurubin release in circulation [26,27]. The estimation of gamma glutamyl transpeptidase (γ-GT) is a important screening test with a high negative predictive value for hepatic disease [28].

Administration of hepatotoxins CCl_4 elevated the serum levels of SGOT, SGPT, ALP, γ-GT and bilurubin as well as decreases total serum proteins (TP) significantly [29,30]. The rise in serum enzymes level and bilurubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [31]. In our investigation, the biochemical changes were observed after 24h. of CCl_4 treatment. Thereby, it was found that the animal groups which are pretreated with MEKB at the dose of 150 and 300mg/kg (groups-III and IV) as well as silymarin at the dose of 100mg/kg (group-V) for three times at 8h. intervals, resulted in significant decreased hepatic enzymes such as SGOT, SGPT, ALP and γ-GT and also total bilurubin; as well as increased total serum proteins (TP) as compared to animals treated only with CCl_4 (group-II). These results give us the suggestion that, the animals which are pretreated with MEKB as well as silymarin, showed a protection against the injurious effects of CCl_4 that may results from the interference with cytochrome P-450. These biochemical restoration may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation [32, 33]. Silymarin is a known hepatoprotective drug. It is reported to have a protective effect on the plasma membrane of hepatocytes [34].

It is concluded from the data, that the methanolic extract of whole plant of *Kyllinga brevifolia* possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

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