Asian Journal of Chemistry; Vol. 35, No. 1 (2023), 203-211

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.26927

Phenolic, Flavonoids Content, Antioxidant Potential of *Murraya koenigii* L. (Curry Leave): *in vitro* and *in vivo* Protective Effects

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Received: 8 November 2022; *Accepted*: 20 December 2022; *Published online*: 27 December 2022; AJC-21099

In the current investigation, the proximate value, total phenolic content, toal flavonoid content, antioxidant potential and *in vitro* and *in vivo* protective effects of *Murraya koenigii* L. (curry leave) extracts were carried out. Flavonoids, phenols and alkaloids were recorded in ethanol, methanol, ethyl acetate, petroleum ether, benzene and acetone extracts. A higher level of total phenolic content was recorded in ethanolic fraction followed by benzene, ethyl acetate, methanol, petroleum ether and acetone fractions. The total flavonoids content was recorded in the range of 5.12 mg RE g^{-1} to 9.24 mg RE g^{-1}. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was estimated in all the solvent fractions at the concentration ranging from 312.5 µg mL^{-1} to 5000 µg mL^{-1}. Percentage inhibition was recorded in the range of 39.40 to 72.32. To study the antimutagenic property, the plate incorporation method was used to see the effects of the MKLE fraction on *S. typhimurium* trains TA 1537, TA 1535, TA 98, TA 100 and TA 102 both in the absence and presence of S9 mix (5% v/v). Higher antimutagenic property at a concentration of 5000 µg/plate with 2-nitrofluorene 7.5 µg/plate was observed against TA 98. *In vivo* study indicates the protective effects of the extract of the MKLE against chlorpyrifos-induced toxicity in brain and lungs of the experimental rats. Four animal groups 5 male and 5 females were selected, group I serve as a control, group II was given a single dose of MKLE as such for 28 consecutive days while group III was treated with chlorpyrifos at the dose level of 60 mg/kg B.wt and group IV was dosed with chlorpyrifos at the dose level of 60 mg/kg B.wt simultaneously treated with the MKLE as such twice in a day (B.D.). Chlorpyrifos administration (group III) to the rats resulted in reduced body weight. decrease Ache level increases, SGOT, SGPT, ALP were observed while no significant alterations were noticed in any parameter in the II group animals, Histopathology of brain and lung of the chlorpyrifos treated animals shows the cellular deficit in the brain tissue and mild congestion in the lung tissue. The present study concluded that curry leaves are a rich source of phenolic, alkaloids, flavonoids and exhibit antioxidant and antimutagenic activity and appeared to be beneficial to rats to a great extent in attenuating and resulting in the damage sustained by chlorpyrifos exposure.

Keywords: Phenolic, Flavonoids Content, Phytochemicals, Antioxidant activity, Chlorpyrifos, Mutagens, *Murraya koenigii*.

INTRODUCTION

The discovery of various chemical constituents that have a significant role in preventing a variety of diseases are regarded as a significant development of the present. The World Health Organization (WHO) anticipated that traditional medicine would be used by 80% of the population. The need for molecules from plants and other natural resources that are now being discovered as prospective methods of replacing synthetic compounds grows as a result of the potential side effects and health difficulties, which are associated with the available synthetic chemicals [1,2]. Numerous plant extracts and phytochemicals have been demonstrated to have beneficial benefits as superior substitutes for conventional medical therapy [3,4]. *Murraya koenigii* belongs to the *Rutaceae* family and is an herbal antidote that is being used in the Indian traditional system of ayurvedic medication [5]. Out of the total of 14 global species of genus, two species namely *M. koenigii* and *M. paniculate* are reported

in India. Among all species reported worldwide, *M. koenigii* is particularly significant because of the broad range of traditional therapeutic characteristics it possesses [6]. The plant is known to be the richest source of carbazole alkaloids and flavonoids present in the *M. koenigii* leaves and is responsible for so many biological properties like antitumor [7] antimutagenic [8], antioxidative [9] and anti-inflammatory [10], antimicrobial [11] hypolipidemic, hypoglycemic, antihypersensitive property [12]. These medicinal properties of *M. koenigii* leaves have been reported due to the presence of various chemical constituents *e.g.* carbazole alkaloids, flavonoids, terpenoids, phenolics, carotenoids, nicotinic acid, carbohydrates and vitamins. The major chemical constituents isolated from various parts of the *M. koenigii* exhibiting antioxidant potential are: mahanimbine, isomahanine, mahanine, isolongifolene, 9-formyl-3-methyl carbazole, koenine, koenigine, mukonicine, mahanimbinine, *Murraya*cinine, mukoeic acid, Murrayanine [7,9,13].

The significance of studying mutagenesis has increased as a result of the crucial function that mutations play in the development of cancer. As a result, from the perspective of cancer prevention, interest in the existence of antimutagens in foods as well as in historically used medicinal plants, herbs and spices has intensified [14,15]. However, several mutagens and carcinogens may function by producing ROS. The mechanism of mutagenesis is complex. It is therefore of enormous practical and therapeutic value to find and study plant extracts and phyto compounds with antioxidant and antimutagenic capabilities [14]. The increasing eating of fruits and vegetables has also been demonstrated to be generally protective against several malignancies. Accordingly, it is assumed that plants with strong antioxidant activity may also exhibit antimutagenic activity and mitigate the effects of mutagens and carcinogens. The antimutagenic properties of plant extracts linked to chemical constituents of plants that function as antimutagens have been reported by several authors [16-18]. However, focused efforts are necessary to investigate and utilize Indian medicinal plants in mutation-related carcinogenesis.

As per the world health organization and data available, millions of cases of pesticide poisoning occur every year and it results in more than lakhs death [19]. Human health risks vary with the type of pesticide and also with the extant vulnerability. The farmers are exposed to the chlorpyrifos during work with the chlorpyrifos due to their application in the field. Most pesticides produces harm the environment and animals by entering the food chain. It is reported that vegetables, dairy products and meat products have pesticide residues [20]. The chlorine group present in chlorpyrifos increases the compound lipid solubility and half-life in the body, resulting in a lowering of Ache level compared to other organophosphates pesticide [21]. When it comes to both target and non-target organisms, chlorpyrifos poisoning is comparable. Direct ingestion, oral administration during suicide attempts or unintentional inhalation, dermal route during spraying and the toxic effects of chlorpyrifos on both humans and animals include effects on the central nervous system, cardiovascular system and respiratory system. Exposure to chlorpyrifos can result in poisoning. After consumption, it has a variety of effects on mammals, including the suppression of the acetylcholinesterase (AChE) enzyme and other biochemical indicators [22]. Acetylcholinesterase is inhibited by chlorpyrifos poisoning by attaching to the enzyme's active site, which renders the enzyme inactive.

Assessment of medicinal plant extracts to protect against chlorpyrifos-induced toxicity in various organs of rats have been studied extensively. Effects of plants extracts alone or in combination with chlorpyrifos at various dose level was studied. Several workers have reported ameliorating effects such as *Ocimum sanctum* [23], green tea extract [24], *Emblica officinalis* garten fruits 16 extract [25], *Ziziphora tenuior* extract [26], date fruit extract [27], pomegranate extracts [28], *Beta vulgaris* L. root extract [29] and olive leaves extract [30]. However, less work has been done on extracts of *M. koenigii* leaves against chlorpyrifos induced toxicity in rats. Keeping in view the potential application of *M. koenigii*, current study has been designed to investigate the proximate value, flavonoids content, total phenolic content, antioxidant potential of various solvent extract fractions and their *in vitro* and *in vivo* protective effects of *M. koenigii* leaves.

EXPERIMENTAL

Chlorpyriphos, sodium azide, 2-nitrofluorene, mitomycin C, 2-aminoanthracene and 9-aminoacridine were purchased from Sigma-Aldrich, India. All other chemicals such as nicotinamide adenine dinucleotide (NADP), D-glucose-6-phosphate, sodium phosphate, potassium chloride, dibasic and sodium phosphate, HCl, H_2SO_4, $FeCl_3$, NaOH and common organic solvents were of analytical grade. Bacteriological media such as Nutrient agar, minimum glucose agar, soft agar and Nutrient broth used in the study were procured from Hi-Media, India. Lyophilized S9 homogenate (liver microsomal enzyme) prepared from male SD rat (Aroclor 1254 induced) was purchased from Krishgen Biosystem, Delhi, India.

Bacterial strains: Bacterial strains used in the study were procured from MTCC, Institute of Microbial Technology, Chandigarh, India. *S. typhimurium* subculturing of TA 1535, TA 1537, TA 98, TA 100 and TA 102 was done in nutrient broth and grown overnight at 37 ºC in an incubator. Density of each culture was set to obtained 2×10^9 organisms per mL. The exact number of CFU/mL was calculated using pour plate serial dilution method. The growth requirements and the genetic identity of strains like histidine requirement, histidine and biotin requirement, sensitivity to UV radiation, ampicillin and tetracycline resistance and *rfa* mutation of *S. typhimurium* strains were checked along with the range of spontaneous revertants.

Animal ethics: Prior to initiation of the study permission for conducting animal experiment was taken from Institutional Animal Ethics Committee (IAEC) of Shri Ram Institute for Industrial Research, Delhi, India vide CPCSEA registration no. 148/po/RCBI/S/99/CPCSEA, dt. 21.07.2017.

Preparation of extracts: *M. koenigii* fresh leaves were collected from the garden of Shriram Institute for Industrial Research, Delhi, India (28º40′52.478″N, 77º12′41.900″E). The plant was authenticated and its leaves were washed with distilled water and dried under the shade at room temperature. Leaves were dried and powder was prepared using a mortal and

pistol. Dried powder (500 g) was dissolved in 3.0 L of ethanol, manually mixed and then transferred to a shaker for 3 days. Ethanol extracts were passed through a column containing silica gel to separate impurities and collected in clean flask. Similarly, methanol, ethyl acetate, petroleum ether, benzene and acetone extracts fractions were prepared. The different solvent fractions were concentrated using a rota evaporator at 50 ºC. The extraction yield was calculated and each fraction were reconstituted in a minimum amount of DMSO to prepare a stock solution for further experiments [31].

Nutritional composition: Using AOAC official techniques, the total moisture, ash, lipid, crude protein and carbohydrate contents of each *M. koenigii* leaf were calculated. Mineral content was determined using an absorption spectrophotometer [32].

Qualitative assessment of phytochemicals: Based on the appearance of white or green precipitate, alkaloids were confirmed to be present. Conc. HCl (2 mL) was added to 2 mL of *M. koenigii* leaf extract. Mayer's reagent was used in small amounts to check for the presence and absence of alkaloids. Based on the appearance dark blue or greenish black colour, using 5% $FeCl_3$ (1 mL) tannins were detected. For the detection of flavonoids, 1 mL of 2 N NaOH was added to 2 mL of *M. koenigii* leaves extract. The presence of flavonoids was confirmed based on the development of yellow colour. Based on the appearance of blue or green colour, phenols were confirmed to be present. For the assessment of phenols, 1 mL of *M. koenigii* leaf extract was mixed with 2 mL of distilled water before a few drops of 10% $FeCl_3$ were added. The presence of phenols was confirmed based on the appearance of either blue or green colour [33].

Total phenolic content (TPC) and total flavonoid content (TFC): The Folin-Ciocalteu reagent was used to calculate the TPC of *M. koenigii* leaf extracts with a few minor modifications [34]. The standard reference was gallic acid and the absorbance was measured at 600 nm. TPC was quantified as mg gallic acid equivalents per gram of dried extract (mg GAE g^{-1}). Total flavonoid content of different solvents fractions of *M. koenigii* leaves extracts was estimated by the colorimetric method by taking the absorbance at 415 nm. Rutin was used as the standard reference. TFC was expressed as mg rutin equivalents per gram of dried extract (mg RE g^{-1}).

Antioxidant activity: The antioxidant activity of different solvent fractions of *M. koenigii* leaves extracts were determined and percentage inhibition free radical scavenging activity was calculated. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was determined according to the method as previously described [34]. DPPH free radical was measured by taking the absorbance at 492 nm on a UV/visible spectrophotometer. Ascorbic acid was used as the standard reference The DPPH free radical scavenging percentage for each test sample was calculated using the following equation:

$$\% \text{ Inhibition (free radical scavenging)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c = absorbance of control, A_s = absorbance of sample.

Mutagenicity test: Using strain TA100, the MKLE fraction was examined for any potential preliminary toxicity or cytotoxicity in both the presence and absence of S9 mix (5% v/v). Based on the results, the solubility and precipitation experiments, the test concentration for cytotoxicity was chosen. Cytotoxicity against the tester strain was assessed at concentrations of 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate both in the presence (5% v/v S9 mix) and in the absence of metabolic activation system. Cytotoxicity is characterized by suppression of the bacterial lawn in the background and by observing reduction in colony counts.

The mutagenicity test was carried out as two separate investigations, initial mutation assay and confirmatory mutation assay, based on the results of the cytotoxicity test, the treatment was performed both in the absence and in the presence of metabolic activation system. Plates were maintained in triplicate for each concentration of MKLE Fraction, positive controls (Table-1) and MKLE + positive control. The following materials were mixed in a test tube and poured in the order given below onto a minimal glucose agar plate. The test was performed as per the plate incorporation assay [35]. For the test with metabolic activation, 0.1 mL of standard bacterial suspension, 0.1 mL of test solution and 0.5 mL of S-9 mix were added to 2.0 mL of histidine-deficient overlay top agar previously melted and cooled to 45 ºC. For the assay, without metabolic activation, 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.4) was added to overlay agar in place of S-9 mix. In both instances, the mixture was quickly shaken and rapidly transferred to Petri plates containing 15 mL of minimal glucose agar. The overlay agar was allowed to solidify before the plates were inverted and placed in an incubator at 37 ºC for 72 h. After incubation, the plates were examined to assess and count the number of histidine-independent colonies (revertants) [35,36]. Inhibition of mutagenicity was calculated as follows:

$$\text{Inhibition (\%)} = 100 - \left\{ \left(\frac{T}{M} \right) \times 100 \right\}$$

where T denotes revertant observed in the plate having both plant fraction and mutagen, and M denotes revertant recorded in the plate having mutagen.

TABLE-1
POSITIVE CONTROLS

S. typhimurium strain	-S9	+S9
TA98	2-Nitrofluorene (7.5 µg/plate)	2-Aminoanthracene (5 µg/plate)
TA100	Sodium azide (5 µg/plate)	2-Aminoanthracene (5 µg/plate)
TA102	Mitomycin C (0.5 µg/plate)	2-Aminoanthracene (10 µg/plate)
TA1535	Sodium azide (5 µg/plate)	2-Aminoanthracene (10 µg/plate)
TA1537	9-Aminoacridine (75 µg/plate)	2-Aminoanthracene (10 µg/plate)

***In vivo* protective effects of MKLE extracts against chlorpyrifos induced toxicity:** Ten animals (5 male and 5 female) per group were taken for conducting the study. Group I serve as a control, group II was given a single dose of MKLE as such for 28 consecutive days while group III was treated with chlorpyrifos at the dose level of 60 mg/kg B.wt and group

IV was dosed with chlorpyrifos at the dose level of 60 mg/kg B.wt simultaneously treated with the MKLE as such twice in a day (B.D.) [37]. Animals selected for this study were within the protocol-specified body weight (range 190-240 g) and age 8-12 weeks. Before accepting animals for the experiment, the Animal house In-charge declared Wistar rats to be healthy and then released the animals for experiment. The Wistar rats were received and kept for a period of 5 days for acclimatization in the experimental room. Veterinary examination of all animals was recorded on the day of receipt and during the acclimatization period. All the animals were housed in a group of three in polypropylene rat cages (Table-2). The toxicity was confirmed by the brain enzyme (Ache) and by the liver function test. G3 animals were dosed with the antidote/MKLE Fraction and G4 animals were given toxicant (chlorpyrifos) and simultaneously treated with MKLE Fraction for 28 days to treat the toxic effect [38,39]. During this study, all the animals were monitored daily till the end of the experiment, at the end of 28 days all the animals were sacrificed by CO_2 asphyxiation and blood was collected for the biochemical analysis, brain and lung tissue were collected and preserved in 10 % neutral buffered formalin for the histopathology examination. The parameters SGOT (U/L), SGPT (U/L), SAP (U/L), ACHE (U/L), Glu (mg/dl, TP (g/dl), urea (mg/dl), CRET (mg/dl), Na^+ (mEq/L), K^+ (mEq/L), Cl^- (mEq/L) were estimated. To study the histopathology findings the tissue was processed and then embedded in paraffin wax and sectioned at 3-5 µ and stained with the hematoxyline and eosin methods.

TABLE-2
EXPERIMENTAL GROUPS

Group-I: Vehicle control	0.0 mg/kg Body weight
Group-II: *Murraya koiningi* leave ethanol extract (MKLE)	As such (1 mL/100 g animal)
Group-III: (Toxicant/Chlorpyrifos)	60 mg/kg Body weight
Group-IV: (Treatment)	60 mg/kg Body weight (CPS)
(Chlorpyrifos and MKLE)	As such (1 mL/100 g animal)

Statistical analysis: The entire statistical analysis was carried out using Minitab 16.0. Standard errors and one-way ANOVA were calculated.

RESULTS AND DISCUSSION

Proximate analysis and mineral contents: The moisture, ash, crude protein, fat and carbohydrate content of fresh leaves of *M. koenigii* were analyzed using proximate analysis; ash content (2.23 ± 0.03 g/100 g), Moisture content 64.2 ± 0.32 g/100 g), protein (14.2 ± 0.34 g/100 g), carbohydrate (2.7 ± 0.29 g/100 g) however, fat content was recorded 10.2 ± 2.5 g/100 g (Fig. 1) mineral contents of *M. koenigii* was also assessed. Minerals K (21.50 mg/g dry weight), Na (0.052 mg/g dry weight), Fe (0.05 mg/g dry weight), P (3.64 mg/g dry weight), Ca (20.89 mg/g dry weight), Mg (3.14 mg/g dry weight) S (3.42 mg/g dry weight), Mn (0.01 mg/g dry weight), Zn (0.02 mg/g dry weight), Cu (0.01 mg/g dry weight) and Al (0.07 mg/g dry weight).

Qualitative estimation of phytochemicals: Solvent fractions of *M. koenigii* leaves were evaluated for the presence and absence of flavonoids, phenols, alkaloids, tannins, quinones (Table-3). Flavonoids, phenols and alkaloids were recorded in ethanol, methanol, ethyl acetate, petroleum ether, benzene and acetone fractions. However, tannins was observed only in ethanol and methanol fractions.

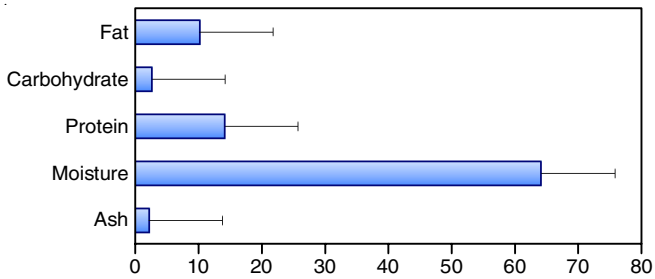

Fig. 1. Proximate composition (g/100 g) of fresh leaves of *M. koenigii*

TABLE-3
QUALITATIVE ASSESSMENT OF MAJOR PHYTOCHEMICALS OF *M. koenigii* LEAVE FRACTIONS

Extracts	Flavonoids	Phenols	Alkaloids	Tannins
Ethanol	Present	Present	Present	Present
Methanol	Present	Present	Present	Present
Ethyl acetate	Present	Present	Present	Absent
Petroleum ether	Present	Present	Present	Absent
Benzene	Present	Present	Present	Absent
Acetone	Present	Present	Present	Absent

Total phenolic and flavonoid content of *M. koenigii* leave fractions: TPC and TFC were calculated in all solvent fractions of *M. koenigii* leaves dried powder. The results of TPC and TFC are summarized in Table-4. All experiments were performed in triplicate, Values are the means of ± SD and values given in each column differ significantly ($p < 0.05$). A higher level of TPC content (mg GAE g^{-1}) was recorded in ethanol fraction followed by benzene, ethyl acetate, methanol, petroleum ether and acetone fractions. The TFC level was recorded in the range of 5.12 mg RE g^{-1} to 9.24 mg RE g^{-1}. Order of TFC level in different solvent fraction are ethanol < methanol < benzene < ethyl acetate < petroleum ether < acetone.

TABLE-4
QUANTITATIVE ESTIMATION OF TPC AND TFC OF *M. koenigii* LEAVE EXTRACTS

Solvent fractions	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg RE g^{-1})
Ethanol	85.13 ± 0.12	9.24 ± 0.23
Methanol	65.23 ± 0.23	8.12 ± 0.21
Ethyl acetate	66.23 ± 0.43	7.54 ± 0.22
Petroleum ether	62.45 ± 0.54	6.88 ± 0.45
Benzene	66.43 ± 0.87	7.67 ± 0.12
Acetone	48.32 ± 0.43	5.12 ± 0.34

Antioxidant activity: The DPPH free radical scavenging activity was estimated in all the solvent fractions in the concentration ranging from 312.5 to 5000 µg mL^{-1}. The percentage inhibition was recorded in the range of 39.40 to 72.32. Data of all solvent fractions are summarized in Table-5. All the experiments were performed in triplicate, values are means of ± SD and values given in each column differ significantly ($p < 0.05$).

TABLE-5
ANTIOXIDANT POTENTIAL DATA OF *M. koenigii* LEAVE EXTRACTS

Solvent fractions	Concentrations of extracts (µg mL^{-1})				
	5000	2500	1250	625	312.5
Ethanol	72.32 ± 0.23	68.34 ± 045	62.23 ± 0.78	59.34 ± 0.43	50.34 ± 0.65
Methanol	68.21 ± 0.12	58.12 ± 082	52.32 ± 0.56	54.34 ± 0.42	48.34 ± 0.34
Ethyl acetate	67.23 ± 0.12	59.34 ± 0.12	48.43 ± 0.12	53.23 ± 0.12	49.23 ± 0.12
Petroleum ether	59.23 ± 0.12	51.43 ± 0.12	48.21 ± 0.12	53.45 ± 0.12	48.43 ± 0.12
Benzene	62.32 ± 0.12	54.32 ± 0.12	48.65 ± 0.12	45.45 ± 0.12	40.34 ± 0.12
Acetone	57.43 ± 0.12	48.45 ± 0.12	43.12 ± 0.12	41.34 ± 0.12	39.40 ± 0.12

In present study, the moisture, ash, crude protein, fat, carbohydrate content and minerals composition (K, Na, Fe, P, Ca, Mg, S, Mn, Zn, Cu, Al) of fresh leaves of *M. koenigii* are presented in Fig. 2. Among all tested parameters moisture content was found to be higher. Varying levels of proximate composition and minerals contents of *M. koenigii* have also been reported by other researchers also [32,40,41]. Moreover, flavonoids, phenols, alkaloids were almost present in all organic fractions. However, tannins was observed only in ethanolic and methanolic fractions only. Using quercetin as a positive control, 80% scavenging activity displayed by the ethanolic extract of *M. koenigii*, which is comparable to the actions of the reference antioxidant quercetin [42].

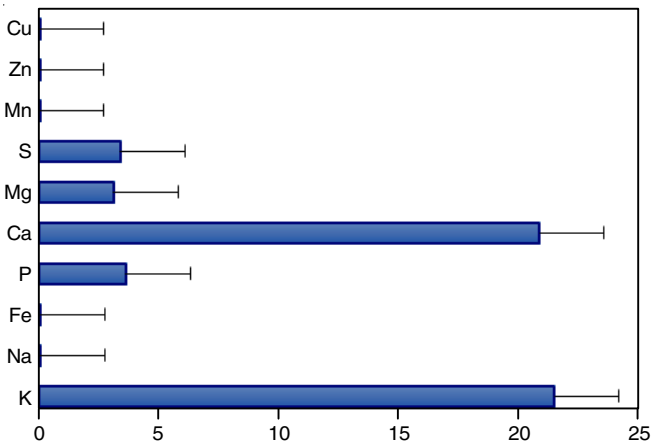

Fig. 2. Mineral contents (mg/g dry weight) of fresh leaves of *M. koenigii*

Mutagenic activity: Pretest was performed with TA 100 *S. typhimurium* test strains with eight concentrations (39, 78, 156, 312, 625, 1250, 2500 and 5000 µg/plate) and dose log interval of two in duplicate plates both in the presence (5%v/v) and absence of metabolic activation system. The results were evaluated based on the revertant count reduction and diminution of the bacterial background lawn. Clearing or diminution of bacterial background lawn and reduction in revertant colony count was not observed in the tested concentrations when compared with negative control. All the negative control plates were normal and no inhibition was observed. Based on the results (Table-6), 5000 µg/plate was selected as the highest dose for the main study both in the absence and presence of metabolic activation system.

Results of Trial I: Trial I was studied with different concentrations *i.e.* 312, 625, 1250, 2500 and 5000 µg/plate of test item (MKLE) along with the negative and positive controls with the tester strains TA 1537, TA 1535, TA 98, TA 100 and TA 102 by the plate incorporation method both in the presence and

TABLE-6
GENOTOXIC PROPERTIES OF MKLE (His$^+$ REVERTANT IN THE ABSENCE AND PRESENCE OF METABOLIC ACTIVATION -TRIAL-1)

Concentration (µg/plate)	TA1535	TA1537	TA98	TA100	TA102
	Mean count of His$^+$ Revertant in the absence of metabolic activation				
VC	12.34 ± 2.25	12.00 ± 3.00	25.00 ± 2.8	123.00 ± 3.25	227.23 ± 8.12
MKLE 312.5	13.00 ± 3.00	11.00 ± 2.50	27.24 ± 1.22	129.12 ± 4.87	222.00 ± 5.00
MKLE 625	12.87 ± 2.43	13.00 ± 3.00	29.00 ± 4.00	121.12 ± 4.56	227.45 ± 3.43
MKLE 1250	13.00 ± 2.00	11.23 ± 3.12	25.23 ± 4.34	113.00 ± 4.00	213.00 ± 4.00
MKLE 2500	11.20 ± 2.22	12.00 ± 2.00	23.00 ± 2.00	109.78 ± 2.20	217.23 ± 1.22
MKLE 5000	13.00 ± 2.00	10.00 ± 3.00	22.12 ± 4.12	107.22 ± 7.00	201.98 ± 7.12
PC	272.00 ± 4.87	278.32 ± 8.12	472.00 ± 9.00	545.00 ± 18.12	633.00 ± 11.00
	In the Presence of Metabolic Activation (+ S9)				
VC	11.20 ± 2.54	11.23 ± 2.12	27.34 ± 4.82	123.21 ± 3.45	230.00 ± 5.00
MKLE 312.5	10.56 ± 2.12	11.23 ± 2.00	30.23 ± 2.97	136.00 ± 5.00	227.00 ± 4.12
MKLE 625	13.20 ± 2.17	10.20 ± 2.34	27.32 ± 2.67	115.00 ± 3.00	210.54 ± 4.00
MKLE 1250	12.23 ± 2.10	11.32 ± 2.08	26.00 ± 3.00	116.43 ± 4.34	212.00 ± 3.00
MKLE 2500	11.20 ± 2.00	12.00 ± 2.00	22.00 ± 2.00	115.20 ± 3.00	205.12 ± 3.00
MKLE 5000	10.43 ± 2.00	10.20 ± 1.00	22.00 ± 3.00	109.00 ± 6.00	205.76 ± 6.00
PC	255.00 ± 10.00	297.00 ± 12.00	512.12 ± 10.00	621.00 ± 12.12	667.34 ± 7.23

VC = Vehicle control (acetone); PC = Positive control for -S9 TA98 (2-Nitrofluorene 7.5 µg/plate) -S9 TA100 (Sodium azide 5 µg/plate) -S9 TA102 (Mitomycin C 0.5 µg/plate) -S9 TA1535 (Sodium azide 5 µg/plate) -S9 TA1537 (9-Aminoacridine 75 µg/plate) for +S9 TA98, TA 100 (2-Aminoanthracene 5 µg/plate) +S9 TA 102, TA 1535, TA 1537 (2-Aminoanthracene 10 µg/plate).

absence of metabolic activation system. The plates were treated and incubated at 37 ± 2 ºC for 48 h. After incubation, the colonies were counted manually. A significant increase in revertant colony number was not observed in all the tester strains (Table-6). An evaluation of the genotoxic properties of MKLE extract found non-mutagenic using *S. typhimurium*.

Results of Trial II: Trial II was performed by following the pre-incubation method both in the presence (5% v/v S9 mix) and absence of a metabolic activation system. The test doses were the same as the main study trial I after treatment plates were incubated at 37 ± 2 ºC for 48 h. After incubation, the colonies were counted manually. All of the tester strains TA1537, TA1535, TA98, TA100 and TA102 did not show a significant increase in the number of revertant colonies at any of the tested doses both in the presence (5 % v/v S9 mix) and in the absence of metabolic activation system (Table-7). There was no dose-dependent increase in the revertant colony numbers. A substantial increase in colony count was observed in all the positive controls when compared with the negative control. An evaluation of the genotoxic properties of MKLE an extract found non-mutagenic using *S. typhimurium.*

Antimutagenic properties: Varying levels of antimutagenic properties of the ethanolic fraction of *M. koenigii* leaves on the mutagens were recorded against five different strains of *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102 (Table-8). Experiments were conducted in the absence and presence of metabolic activation higher antimutagenic property at concentration 5000 µg/plate with 2-nitrofluorene 7.5 µg/plate was recorded against TA 98. Followed by TA 100, TA 1537, TA 1535 and TA 102 with varying concentrations of mutagens. Studies conducted earlier were focusing only on the one or two species of *S. typhimurium* with limited numbers of known mutagens. In this study, all five bacterial strains of *S. typhimurium* with six mutagens have been studied to generate complete data sets of antimutagenic property of MKLE. Present results are comparable with the findings of recent study [8]

TABLE-7
GENOTOXIC PROPERTIES OF *Murraya koenigii* LEAVES EXTRACT (His^+ REVERTANT IN THE ABSENCE AND PRESENCE OF METABOLIC ACTIVATION -TRIAL-2)

Concentration (µg/plate)	TA1535	TA1537	TA98	TA100	TA102
Mean count of His^+ Revertant in the absence of metabolic activation (- S9)					
VC	12.76 ± 1.00	10.23 ± 1.00	30.00 ± 3.00	130.23 ± 5.00	232.12 ± 2.65
MKLE 312.5	13.43 ± 1.12	12.32 ± 3.00	29.00 ± 2.09	130.00 ± 2.87	212.45 ± 11.00
MKLE 625	10.32 ± 1.23	10.43 ± 3.54	27.43 ± 2.09	120.00 ± 6.00	223.00 ± 5.00
MKLE 1250	11.34 ± 2.00	12.43 ± 2.00	29.00 ± 5.00	122.43 ± 2.00	214.87 ± 3.00
MKLE 2500	10.43 ± 2.00	12.98 ± 2.00	24.00 ± 3.00	116.00 ± 4.00	201.00 ± 2.00
MKLE 5000	11.76 ± 2.00	11.00 ± 2.00	21.98 ± 3.43	119.00 ± 2.00	209.00 ± 2.87
PC	247.00 ± 10.00	246.00 ± 11.87	472.45 ± 11.32	538.32 ± 15.65	663.32 ± 13.65
Mean count of His^+ Revertant in the presence of metabolic activation (+ S9)					
VC	10.87 ± 1.00	11.32 ± 1.23	26.00 ± 2.00	132.09 ± 5.00	24.00 ± 2.00
MKLE 312.5	11.00 ± 1.00	11.032 ± 2.00	32.00 ± 4.00	133.00 ± 3.00	240.32 ± 10.11
MKLE 625	9.89 ± 1.00	12.00 ± 2.00	23.32 ± 3.00	119.20 ± 7.00	227.00 ± 9.00
MKLE 1250	12.89 ± 4.00	11.23 ± 1.20	24.00 ± 2.00	109.87 ± 7.00	221.43 ± 5.00
MKLE 2500	12.32 ± 2.00	10.23 ± 2.00	25.12 ± 2.98	103.00 ± 5.00	202.12 ± 6.98
MKLE 5000	10.87 ± 2.00	10.80 ± 1.00	23.00 ± 2.00	112.00 ± 10.54	205.87 ± 2.00
PC	250.08 ± 8.00	280.23 ± 10.10	530.20 ± 10.12	640.00 ± 10.20	673.12 ± 10.00

VC = Vehicle control (acetone); PC = Positive control for -S9 TA98 (2-Nitrofluorene 7.5 µg/plate) -S9 TA100 (Sodium azide 5 µg/plate) -S9 TA102 (Mitomycin C 0.5 µg/plate) -S9 TA1535 (Sodium azide 5 µg/plate) -S9 TA1537 (9-Aminoacridine 75 µg/plate) for +S9 TA98, TA 100 (2-Aminoanthracene 5 µg/plate) +S9 TA 102, TA 1535, TA 1537 (2-Aminoanthracene 10 µg/plate).

TABLE-8
EFFECTS OF ETHANOLIC FRACTION OF *Murraya koenigii* LEAVES ON THE MUTAGENICITY INDUCED BY KNOWN MUTAGENS

Concentration (µg/plate)	TA1535	TA1537	TA98	TA100	TA102
Mean count of His^+ Revertant in the absence of metabolic activation					
PC	272.00 ± 4.87	278.32 ± 8.12	472.00 ± 9.00	545.00 ± 18.12	633.00 ± 11.00
MKLE 1250 + PC	180.12 ± 3.34 (33%)*	190.12 ± 3.12 (31%)*	290.23 ± 5.12 (38 %)*	380.34 ± 6.45 (30 %)*	460.54 ± 6.23 (27 %)*
MKLE 2500 + PC	160.32 ± 3.10 (41 %)*	150.32 ± 3.33 (46 %)*	245.34 ± 4.56 (48 %)*	290.00 ± 5.12 (46 %)*	410.00 ± 5.23 (35 %)*
MKLE 5000 + PC	137.23 ± 3.12 (50 %)*	125.24 ± 2.32 (55 %)*	198.00 ± 4.34 (58 %)*	277.12 ± 4.12 (49 %)*	380.34 ± 4.23 (40 %)*
In the presence of metabolic activation (+ S9)					
PC	255.00 ± 10.00	297.00 ± 12.00	512.12 ± 10.00	621.00 ± 12.12	667.34 ± 7.23
MKLE 1250 + PC	190.43 ± 4.12 (25%)*	188.32 ± 4.12 (37%)*	286.65 ± 3.23 (44 %)*	390.23 ± 4.23 (37 %)*	477.23 ± 5.12 (28 %)*
MKLE 2500 + PC	158.45 ± 4.20 (38 %)*	156.43 ± 4.22 (47%)*	255.76 ± 4.12 (50%)*	299.12 ± 5.22 (52 %)*	422.12 ± 3.33 (37 %)*
MKLE 5000 + PC	129.45 ± 5.45 (49 %)*	129.34 ± 4.45 (56 %)*	192.32 ± 5.23 (62 %)*	270.34 ± 4.98 (57 %)*	390.23 ± 3.45 (42 %)*

VC = Vehicle control (acetone); PC = Positive control for -S9 TA98 (2-Nitrofluorene 7.5 µg/plate) -S9 TA100 (Sodium azide 5 µg/plate) -S9 TA102 (Mitomycin C 0.5 µg/plate) -S9 TA1535 (Sodium azide 5 µg/plate) -S9 TA1537 (9-Aminoacridine 75 µg/plate) for +S9 TA98, TA 100 (2-Aminoanthracene 5 µg/plate) +S9 TA 102, TA 1535, TA 1537 (2-Aminoanthracene 10 µg/plate) *% Inhibition

conducted in which oxycarotenoid-rich extracts *M. koenigii* were tested using two strains of *S. typhimurium* strains *viz*. TA 1535 and TA 98 with 20 µg/plate of nitro-*o*-phenylenediamine and 1 µg/plate of N-methyl- N-nitro-N-nitrosoguanidine with significant inhibition *i.e.* 90.3% against TA 1535 and 67.1% against TA 98.

***In vivo* protective effect of *M. koenigii* leaves extract:** No toxic sign was observed in the animal of the treatment group when compared with their control counterpart. Results of mean body weight data of male and female rats are presented in Table-9. Biochemical parameters such as SGOT, SGPT, SAP, ACHE, Glu, TP, urea, CRET, Na^+, K^+, Cl^- were estimated in the serum sample and shown in Table-10. During the investigation, the level of SGPT, SGOT, SAP was increased and the level of ACHE gets slightly lower after 28 days of exposure to Cps. and however, in the herbal extract-treated group (at the dose level of 5000 mg/kg b.wt) serum SGPT, SGOT, SAP and ache level was significantly normal as compared to that in cps induced animal group. During the investigation, the level of SGPT, SGOT, SAP were increased and the level of ACHE gets slightly lower after 28 days of exposure to Cps. and however, in the herbal extract-treated group (at the dose level of 2000 mg/kg b.wt) serum SGPT, SGOT, SAP and ache level was significantly normal as compared to that in cps induced animal group. Fig. 3 shows the normal architecture of lung alveoli. Histopathology findings the tissue was processed and then embedded in paraffin wax and sectioned at 3-5µ and stained with hematoxyline and eosin method. Fig. 4 demonstrated the normal architecture of brain cells.

In this study, the neurotoxic effect of organophosphate compound *i.e.* chlorpyriphos was investigated by studying the biochemical investigations of liver and brain enzymes and histopathological examination of brain and lung tissue. This study concluded that ethanolic extract from *M. koenigii* leaves (curry leaves) lowered the serum SGOT, SGPT and APL and also inhibit the Ache enzyme, thus a combination of ache and liver enzyme normalize effect exhibited by *M. koenigii* leaves extract proves that the MKLE is the beneficial herbal antidote to cure the ACHE activity.

Conclusion

The presence of flavonoids, phenols and alkaloids in different organic solvent fractions, ethanol, methanol, ethyl acetate, petroleum ether, benzene and acetone of *Murraya koenigii* L. (Curry Leave) were investigated. The TPC content was higher in ethanolic fraction followed by benzene, ethyl acetate, methanol, petroleum ether and acetone fractions, while the TFC level was recorded in the range of 5.12 mg RE g^{-1} to 9.24 mg RE g^{-1}. The DPPH free radical scavenging activity was also estimated in all solvent fractions in the concentration ranging from 312.5 to 5000 µg mL^{-1}. The percentage inhibition was recorded in the range of 39.40 to 72.32. The higher anti-

TABLE-9
MEAN BODY WEIGHT DATA OF MALE AND FEMALE RATS

Week group	0	1	2	3	4
Male rats					
C	185.2 ± 5.25	201.84 ± 5.97	217.94 ± 6.57	233.62 ± 6.46	249.88 ± 6.12
CPS 60 + MKLE 2000	189.84 ± 5.88	195.98 ± 6.27	203.76 ± 5.59	211.34 ± 6.16	218.56 ± 5.43
Female rats					
C	185.58 ± 8.91	196.06 ± 9.94	207.1 ± 9.80	217.72 ± 10.11	229.24 ± 10.28
CPS 60 + MKLE 2000	187.74 ± 6.56	195.6 ± 6.76	203.48 ± 6.91	211.18 ± 7.80	218.14 ± 7.80

TABLE-10
EFFECT OF MKLE AND CPS ON BIOCHEMICAL PARAMETERS OF MALE AND FEMALE WISTAR RATS

Groups	SGOT (U/L)	SGPT (U/L)	SAP (U/L)	ACHE (U/L)	Glu (mg/dl)	TP (g/dl)	Urea (mg/dl)	CRET (mg/dl)	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)
Male wistar rats											
Control	50.4	48.20	113.2	629.0	98.6	7.02	39.06	0.930	140.0	4.42	100.20
CPS 60	118.4	106.80	112.6	284.4	106.0	7.08	37.40	0.900	140.0	4.32	99.80
MKLE 1000	50.2	53.12	113.4	630.4	101.8	6.98	39.12	0.924	140.2	4.28	100.00
MKLE 2000	49.3	52.12	112.5	623.2	102.2	6.28	40.40	0.912	139.2	4.12	100.12
MKLE 3000	51.8	49.42	119.9	610.2	99.4	7.12	38.74	0.856	139.0	4.50	99.40
CPS 60 + MKLE 1000	52.8	54.20	121.2	316.4	104.0	7.28	39.50	0.858	141.0	4.20	101.68
CPS 60 + MKLE 2000	41.0	43.40	129.4	408.0	100.6	7.62	42.80	0.880	137.6	4.72	98.60
CPS 60 + MKLE 3000	45.6	42.60	126.6	423.0	98.8	7.58	41.60	0.940	138.2	4.44	101.60
Female wistar rats											
Control	48.2	50.2	110.1	615.0	99.8	7.00	34.40	0.890	138.8	4.44	98.6
CPS 60	110.2	110.2	109.1	280.4	97.6	6.94	39.20	0.820	139.0	4.24	101.4
MKLE 1000	47.0	50.1	110.8	625.1	99.4	7.20	38.74	0.856	139.0	4.50	99.3
MKLE 2000	47.0	53.12	104.2	620.2	99.8	7.90	37.14	0.845	138.0	4.60	99.5
MKLE 3000	51.9	53.65	113.4	627.4	99.7	7.12	38.44	0.836	140.0	4.80	99.4
CPS 60 + MKLE 1000	54.2	54.00	138.0	321.4	80.0	7.36	40.56	0.962	143.4	4.26	101.4
CPS 60 + MKLE 2000	45.6	42.60	126.6	423.0	98.8	7.58	41.60	0.940	138.2	4.44	101.6
CPS 60 + MKLE 3000	41.4	42.00	136.6	410.8	97.2	7.26	39.60	0.734	140.0	4.82	81.6

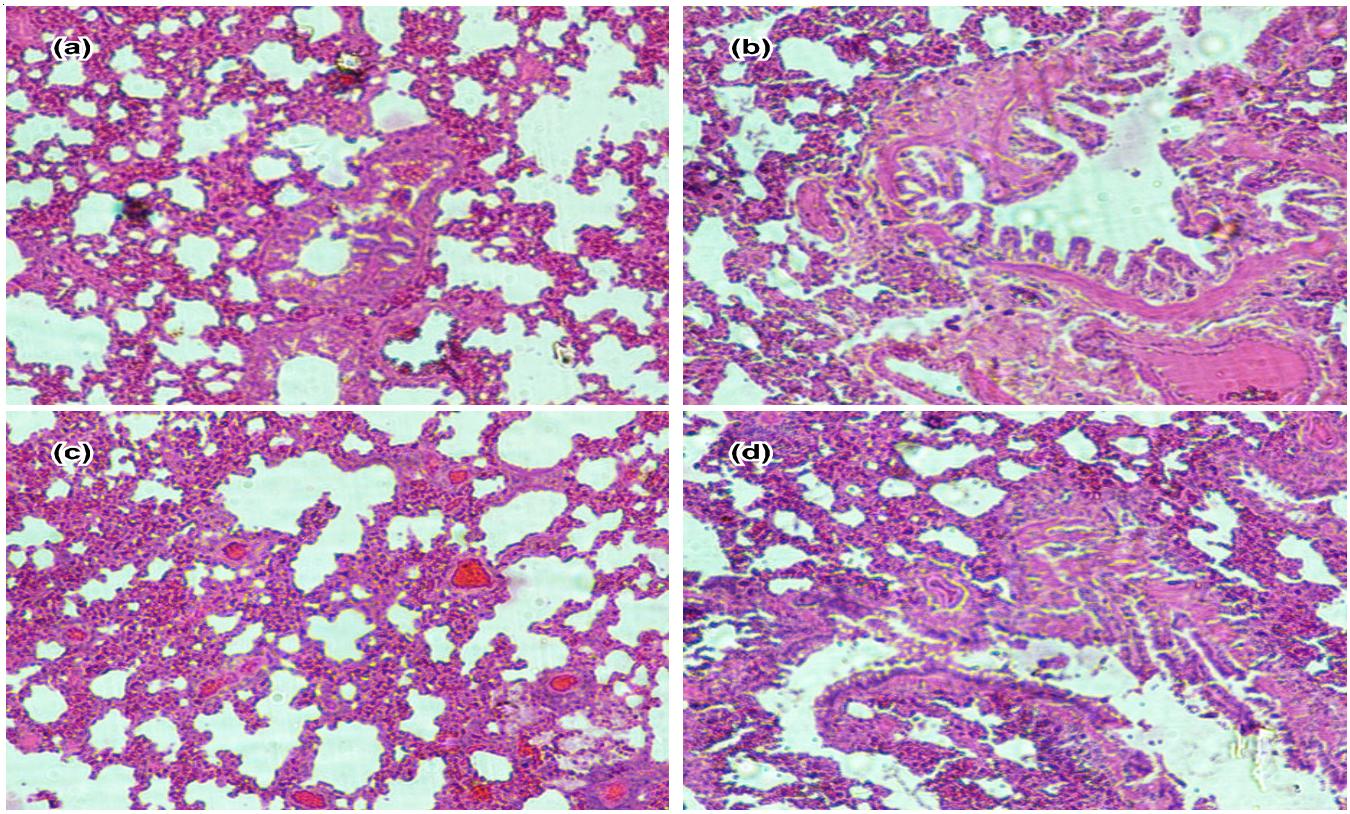

Fig. 3. High power photomicrograph of lung showing normal architecture of lung alveoli: (a) vehicle control; (b) MKLE (2000 mg/kg b.wt); (c) lung showing early peripheral involvement in the blood vessel with few lymphocytes (mild congestion) CPC-60 mg/kg b.wt; (d) lung showing competitively normal lung alveoli. *M. koenigii*- 2000 mg/kg b.wt (H & E, 40 X)

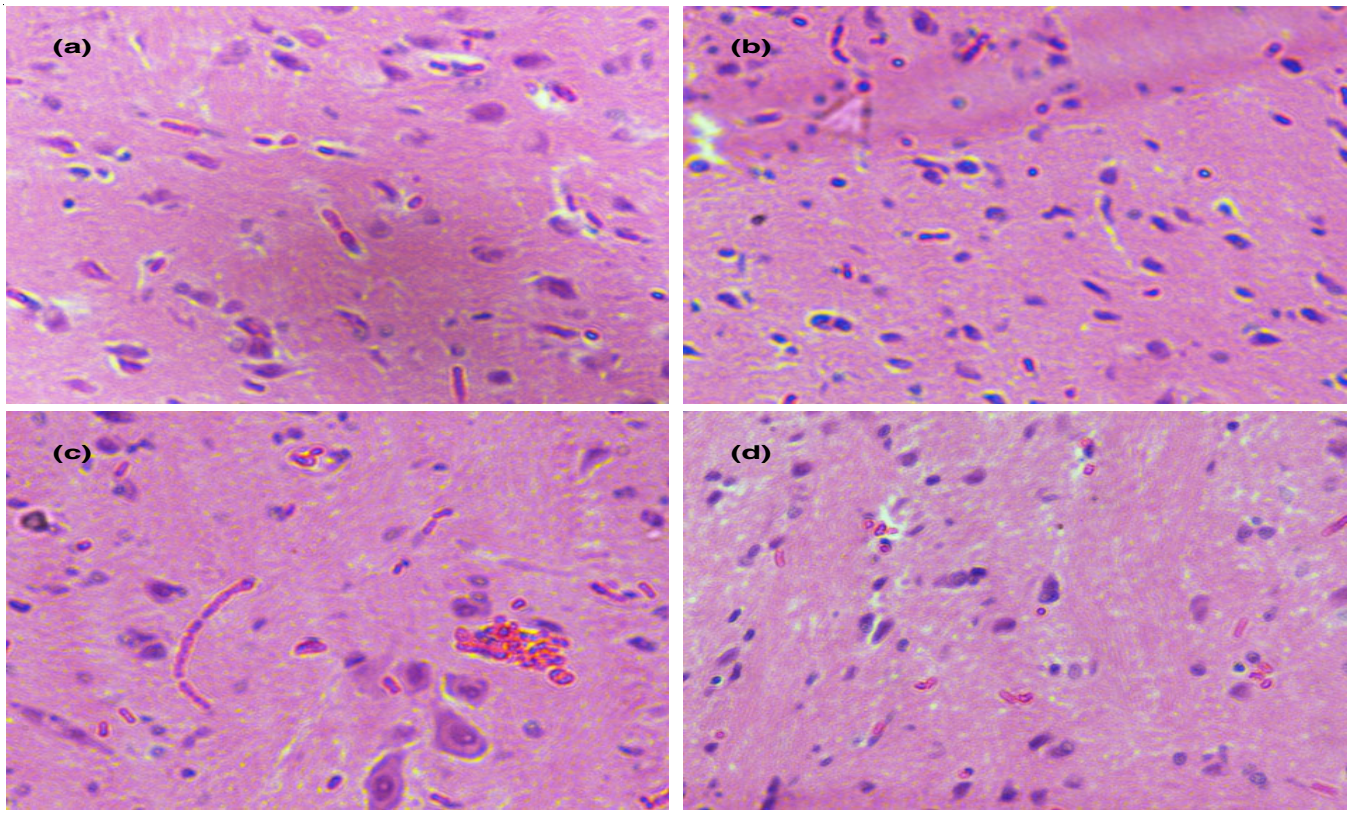

Fig. 4. High power photomicrograph of brain showing the normal architecture of brain cells; (a) vehicle control; (b) MKLE 2000 mg/kg b.wt.; (c) brain showing cellular deficits (CPS 60 mg/kg b.wt.pretreated); (d) brain showing the competitively normal architecture of brain cells. *M. koenigii*- 2000 mg/kg b.wt H & E, 40

mutagenic properties were observed against TA 98 at the concentration of 5000 g/plate with 2-nitrofluorene 7.5 g/plate. The extract of *M. koenigii* leaves has been found effective to protect against chlorpyrifos-induced toxicity in the brain and lungs of the experimental Wister rats. The study affirms that extract dose-dependently protects the biochemical parameters like SGOT, SGPT and ALP and shows currying effect of brain and lung tissue (neurotoxicity and pulmonary toxicity). Histopathology of the brain and lung of the chlorpyrifos treated animals shows the cellular deficit in the brain tissue and mild congestion in the lung tissue. The study concluded that *M. koenigii* L. leaves are an abundant source of phenolics, alkaloids and flavonoids exhibit antioxidant and antimutagenic activity and appear to be extremely helpful to rats in attenuating and causing the damage incurred by chlorpyrifos exposure.

ACKNOWLEDGEMENTS

The authors are thankful to the Shri Ram Institute of Industrial Research, Delhi, India for providing the facility for this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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