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Unveiling the Biochemical, Physiological and Pathological Responses to Single Acute Dose of Chloroformic Extract of *Murraya koenigii*

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Abstract

Murraya koenigii is a distinctly valued plant for its medicinal properties which is mostly considered for its hypoglycaemic, hepatoprotective and antioxidant abilities. Studies have shown *M. koenigii* to be harmless for consumption up to 1000 mg/kg. "Despite the pharmacological advantages of *M. koenigii*, there was limited proof regarding the harmful impacts." Therefore, the present investigation was conducted to concentrate on the toxicity of chloroformic extract in rats. The study includes quantitative analysis of all bioactive components like Flavanoids, Saponins, Phenols, Tannins, Glycosides and Terpenoids. An acute toxicity study was conducted on female Wistar rats, administering a single oral dose of 200mg/kg and 2000 mg/kg of Chloroformic extract. The rats were monitored for 14 consecutive days for any alterations in behaviour. At the conclusion of the experiment, the body weights were measured, haematological and biochemical tests were performed. Histopathological abnormalities were observed in sections of the liver and kidney. There were no fatalities noted throughout the experiment. Rats exhibited reduced activity and loss of appetite 24 hours following the dispensation of a solitary acute dose of 2000 mg/kg body weight. Our results suggest that the extract derived from *M. koenigii* using chloroform as a solvent, given at a dose of 2000mg/kg, presents lymphocytic infiltration in the liver. However, 200 mg/kg bw of Chloroformic extract was safe and therefore, can be used in the evaluation of its medicinal properties.

Major Findings: 1. Chloroformic extract of *Murraya koenigii* exhibited high concentrations of Tannins, Terpenoids, Phenols and Saponins. 2. No significant changes in the body weight, biochemical and hematological analysis of the groups treated with 200 and 2000 mg/kg bw of CEMK. 3. Histopathological examination revealed lymphocytic infiltration in liver, and slight oedema in kidney tissues of rats treated with single dose of 2000 mg/kg bw CEMK. 4. LD50 of the *Murraya koenigii* drug can be considered to be more than 2000 mg/kg bw as it was observed to be safe given once orally.

Keywords: Behaviour, Chloroform Extract, Limit Test, Lymphocytic Infiltration, *Murraya koenigii*, Quantitative Phytochemical analysis

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1. Introduction

Murraya koenigii is a distinctly valuable plant due to its distinct aroma and medicinal properties. It has been an integral part of Indian cuisine for centuries and has played a versatile role in traditional medicine. The plant comprises diverse chemical constituents that interact intricately to generate their pharmacodynamic[1]. Multiple active components that are accountable for their therapeutic attributes have been acknowledged and described. *M. koenigii* has been found to possess antioxidant, cytotoxic, antimicrobial, antibacterial[2], anti- ulcer, positive inotropic, and cholesterol-reducing activities[3]. Alkaloids such as mahanine, koenine, koenigine, koenidine, girinimbiol, girinimibine, koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline[4,5], as well as coumarin glycosides such as scopotin and murrayanine[6], are among the basic constituents derived from *M. koenigii* leaves. Furthermore, it has been documented to exhibit antioxidant, antifungal, larvicidal, anticarcinogenic, hypoglycaemic, anti-lipid peroxidative, hypolipidemic, anti-hypertensive, hepatoprotective, and preventive impact against carbon tetrachloride-prompted hepatic discomfiture in rats[7]. A study of Pinto *et al.*[8] has revealed that the pancreatic inhibition assay of chloroformic extract of *M. koenigii* exhibited 53.42% while the aqueous and methanolic extract exhibited 33 and 51%, respectively. Thus, proving the hypolipidemic property of *M. koenigii in vitro*. In a study performed by Phatak *et al.*[9], *M. koenigii* chloroformic extract has demonstrated potential cardioprotective effects in the prevention of lead-induced anaemia, cardiac ailments, and ischemic heart disease by mitigating the harmful effects on blood toxicity. Another study showcased the impact of the methanol extract on the overall behaviour of mice which was significantly different from that of the crude powder. The crude powder of Curry Leaves consistently exhibited mild to moderate hypoactivity or reduced locomotion at all dose levels[1]. In another study, there were no fatalities reported within 24 h after administering doses of 500 and 1500 mg/kg of chloroformic extract of *M. koenigii*. However, a 50% mortality rate was recorded at a dose of 2500 mg/kg[10].

The initial toxicity test, known as acute toxicity, is conducted to determine the maximum dosage of *M. koenigii* leaves that can be safely used in therapy[11].

Through this test, the LD50 value of the extract from *M. koenigii* leaves can be determined[12]. The liver assumes a crucial function in the metabolism and elimination of medications. The intake, metabolism, and elimination of drugs collectively establish a state of dynamic equilibrium that is essential for maintaining homeostasis[13]. "To achieve dynamic homeostasis, precise regulation of drug or xenobiotic metabolism by xenobiotic metabolizing enzymes is essential." Any disruption in the functioning of these enzymes can result in an imbalance, leading to an increased production of free radicals. These free radicals can then bind to macromolecules like DNA, causing mutations, damaging lipids and membranes, or altering the activities of proteins. The adverse reactions caused by drugs that are harmful to the liver can be categorized as acute reactions (characterized by cell necrosis), fat accumulation, infiltration of lymphocytes, oedema and miscellaneous reactions[14].

To facilitate the use of chloroformic extract of *M. koenigii* as a hypolipidemic agent, it is necessary to establish its safety which holds significant phytotherapeutic value, it is necessary to conduct an assessment. Hence, this particular study aimed to determine the toxicity of the chloroformic extract as a single acute administration of high and low doses derived from the leaves of *M. koenigii*.

2. Materials and Methods

2.1 Collection

Leaves from various areas in South Goa were gathered for the purpose of this study. The herbarium was prepared following the guidelines given on the website of a botanical survey of India. A branch containing leaves was dipped in mercuric chloride/methanol and was neatly pressed in between sheets of paper for 15 days for drying. Then, the specimen was pasted on a herbarium sheet. "A label containing the details of habitat, location of collection, and flower type was affixed." This herbarium was deposited at the Botany Department, Goa University for identification, and a certificate of identification was procured from eminent botanist, Prof. Janardhanan. Subsequently, the leaves were carefully washed and left to dry in the shade for a duration of two weeks. Once completely dried, the leaves were finely powdered.

2.2 Extraction

Approximately 25 g of dried and powdered *M. koenigii* leaves were utilized to create a crude extract through the

continuous operation of a Soxhlet apparatus for 10 reflex cycles. The plant material was permeated by chloroform, which dissolved the constituents. Subsequently, the organic solvent was concentrated to dryness at a temperature range of 40°C to 45°C under low pressure with the use of a rotary evaporator[15]. The yield derived from this was 6.0%.

2.3 Phytochemical Analysis

The following biomolecules are identified in the extracts through phytochemical analysis utilizing established quantitative methods.

2.3.1 Phenolic Estimation

The determination of total phenolic components was performed using the Folin-Ciocalteu colorimetric technique as described by Singleton and Rossi, 1965[16]. Two hundred microlitres (µL) of *M. koenigii* extracts were deposited in screw cap test tubes for this operation. Subsequently, the test tubes were filled with 1.0 mL of Folin-Ciocalteau reagent (diluted 1:1 with water) and 1.0 mL of a 7.5% washing soda (Na_2CO_3) solution. Subsequently, the tubes were comprehensively mixed using a vortex mixer and then incubated for 2 hr. Following incubation, the absorbance of the samples was measured at 726 nm using a spectrometer (Beckman, USA). The total phenolic content was then quantified in milligrams of Gallic Acid Equivalents (GAE) per gram of dried material (Figure 1)[17].

$$\text{Total phenolic content} = \frac{\text{Amount of gallic acid} \times \text{Volume of extract}}{\text{mass}}$$

2.3.2 Flavonoid Analysis

The total flavonoid content was evaluated using the aluminium chloride colorimetric technique described by Lin and Tang[18]. The Aluminium chloride technique was used to ascertain the total flavonoid concentration of the sample extracts. Aliquots of 1.5 ml of extracts were combined with equivalent quantities of a 2% $AlCl_3$ 6H2O solution (2 g in 100 ml methanol). The mixture was agitated briskly, and the absorbance at 367 nm was measured after 10 min of incubation. Quercetin was used as the standard, with a concentration range established between 0.05 and 0.45 mg/mL. Flavonoid content was derived from the standard curve (Figure 2). The total flavonoid content of the plant extracts was expressed as milligrams of Quercetin Equivalents (QE) per gram of dry weight obtained[19].

$$\text{Total flavonoid content} = \frac{\text{Concentration of quercetin} \times \text{Volume of extract}}{\text{mass}}$$

2.3.3 Determination of Glycosides

The cardiac glycosides in each plant extract were quantitatively assessed using the modified methodology of Solich *et al.* To confirm the presence of cardiac glycosides, 100 µL of each extract was combined with 1 mL of newly made Baljet's reagent (composed of 95 mL of 1% picric acid and 5 mL of 10% NaOH). After one hour, the mixture was diluted with 2 mL of distilled water, and the absorbance was measured at 495 nm using a Shimadzu UV/VIS spectrophotometer model UV 1800 (Kyoto, Japan). To produce the standard curve, 10 mL of various doses (12.5-100 mg/L) of digoxin were formulated (Figure 3). Total glycosides from triplicate samples were quantified as mg of digoxin per g of dried extracts[17,20].

2.3.4 Saponins

The following solutions for the colour development reagents were made: (A) 50 ml of conc. Sulphuric acidic as

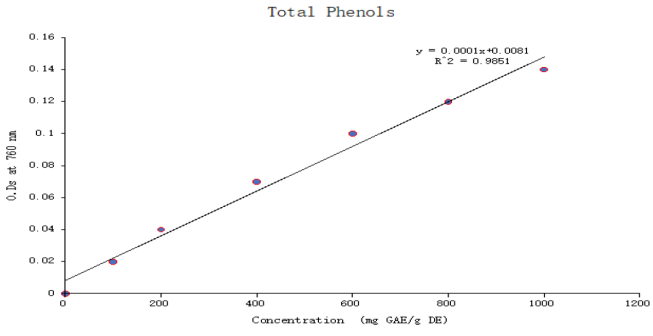


Figure 1. Standard Gallic acid curve for estimation of total phenols.

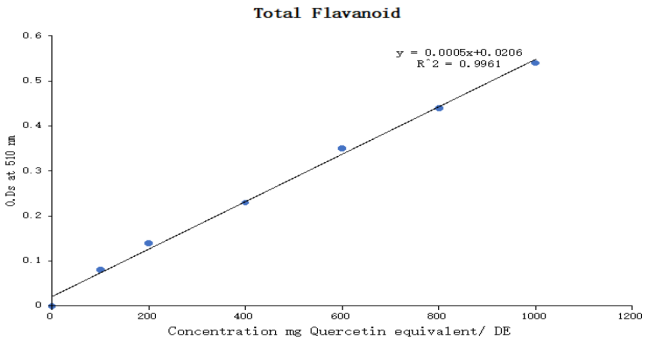


Figure 2. Standard flavanoid content of quercetin.

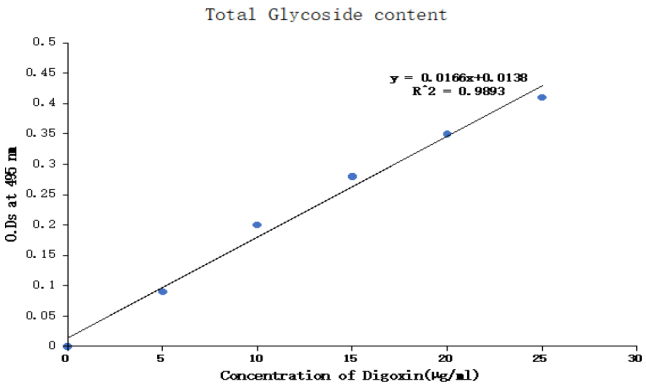


Figure 3. Standard digoxin curve for glycoside estimation.

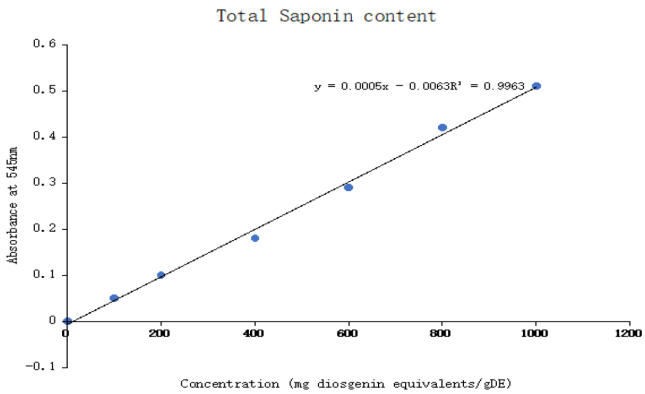


Figure 4. Total saponin content of *M. koenigii*.

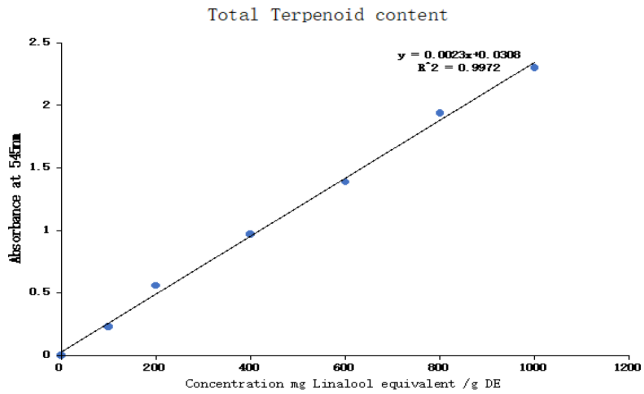


Figure 5. Total terpenoid content derived from a standard curve of linalool.

well as 50 ml of ethyl acetate; (B) 0.5 ml of p-anisaldehyde as well as 99.5 ml of ethyl acetate. A 10 ml test tube had a 2 ml solution of diluted saponins. The test tube was then closed sac with a glass stopper after 1 ml of all the reagent solutions (A) and (B) were add-on. The test tubes were stirred and then permitted to develop colour for 10 min at 60°C in a water bath. It was then permitted to cool for 10 min at room temperature in another water bath. At 430 nm, the color-developed solution's absorbance was measured. The absorption was determined utilizing ethyl acetate as a control. 2 ml of ethyl acetate was added to a test tube as a reagent blank, and it performed similarly to the saponin solution. As standard saponins, diosgenin dissolved in ethyl acetate[21]. The concentration of Saponin content was derived from the standard curve of Diosgenin (Figure 4).

$$Saponins = \frac{Conc.\ of\ \ diosgenin \times amount\ \ of\ \ saponins\ \ extracted}{Weight\ \ of\ \ crude\ \ powder} \times 100$$

2.3.5 Terpenoids

Total terpenoids were calculated utilizing the Ghorai *et al.* approach. In summary, 200µl of dissolved methanol was introduced to each 2 ml micro-centrifuge tube along with 1.5 ml of chloroform and a known amount of Murraya extract (20 mg). After fully vortexing the sample mixture, let it sit for three min. Each 2-ml micro-centrifuge tube should contain 100 µl of concentrated sulfuric acid (H_2SO_4). After that, the assay tubes were incubated for 1.5 to 2 hr in a dark environment at room temperature (30°C). Each assay micro- centrifuge tube will have a reddish-brown acceleration in it at the completion of the incubation period. The reaction mixture liquid's supernatant was then cautiously and gently decanted without causing any disruption to the precipitation. After adding 1.5 ml of 95% (v/v) methanol, the composite was eagerly vortexed until the residue was entirely dissolved. The sample was moved from the assay tube to a colorimetric cuvette, and a blank of 95% (v/v) methanol was utilized to calculate the absorption at 538 nm[22]. A standard curve was established using Linalool as standard (Figure 5).

$$Terpenoids = \frac{Conc.\ of\ \ linalool \times amount\ \ of\ \ terpenoids\ \ extracted}{Weight\ \ of\ \ crury\ \ leaf\ \ powder} \times 100$$

2.3.6 Total Tannin content

The tannin content of the plant extract was determined using the methodology of Siddhuraj and Manian. 500 µl of extracts with a certain concentration were placed in separate test tubes and treated with 100 mg of polyvinyl polypyrrolidone and 500 µl of distilled water. The solution was incubated at 4°C for 4 hrs. The sample was centrifuged at 5,000 rpm for 5 min, after which the supernatant

was collected. This supernatant contains just simple phenolics devoid of tannins since the tannins would have precipitated with the polyvinyl polypyrrolidone. The phenolic content of the supernatant was quantified at 725 nm, represented as free phenolics on a dry matter basis in terms of Gallic Acid Equivalent (GAE). The tannin content was quantified using the following calculations[23].

Tannins = Total phenol – Free phenol

2.4 Experimental Animals

Female adult Wistar rats were procured from Lacsmi Bio farms, Pune. The Institutional Animal Ethics Committee (IAEC) of Goa University (Zoology), Goa, India, authorized the experimental procedure with the protocol number (Approval no: GUZ/IAEC/23-24/N1, Project code no. IAEC/23-24/N1). The animals were kept in a room with proper ventilation, maintained at a temperature of 22±3°C and a relative humidity of 40-65%. They followed a 12-hr light/dark cycle with artificial lighting. The rodents were fed with rodent feed from M/s Provimi Animal Nutrition, India, and had unlimited access to purified water.

2.5 Study Design

OECD guideline 423 was followed to perform a limit test on female rats[24]. The rats weighing 250-350 gm were segregated into 3 groups of 3 rats each. Group 1 representing controls received Normal saline (10ml/kg bw). The other two groups, G2 and G3 received single acute dosages of 200 and 2000 mg/kg bw respectively. The LD50 determination was evaluated based on the mortality rate of the animals under observation for 14 days. The rats' overall behaviour and indications of toxicity were continuously monitored for one hour, followed by intermittent observation for 4 h, and subsequently over a span of 24 h. The rats were attentively tracked every day for 14 days to detect any indications of toxicity or mortality[25]. The weight of the subjects was measured before administration and subsequently on a weekly basis until the experiment was concluded.

2.6 Biochemical and Haematological Parameters

A blood sample was drawn for biochemical and haematological investigations. Blood was collected using the cardiac puncture method under anaesthesia. Each blood sample was split into 3 distinct tubes, one containing the anticoagulant Ethylenediamine Tetraacetate (EDTA), the other with fluoride and, lastly, without any anticoagulant. The blood samples in the tubes lacking the anticoagulant were left to clot for 2 hours. Subsequently, the tubes were centrifuged at 5000 rpm for 10 mins. The analysis of various biochemical parameters including fasting blood sugar, Liver enzymes like Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetate Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Renal function tests including Urea and Creatinine were performed. All biochemical parameters were analysed by standard methods (Table 1) using a biochemistry auto analyser (Coralyser Mini, Tulip Diagnostics, India). The EDTA sample was tested for haematology parameters, Haemoglobin (Hb), White Blood Cells (WBC), Differential count (lymphocytes, neutrophils, eosinophils), Red Blood Cells (RBC), Packed Cell Volume (PCV) and Platelets using an automatic cell counter (Mindray-BC 5150).

2.7 Histopathological Examination

At the conclusion of the experiment, significant pathological alterations were also noted. The Liver and kidneys were surgically excised and subsequently weighed and sent for histopathological examination. Relative Organ Weight (ROW) of every experimental rat was computed using formula[25].

$$ROW(\%) = (\text{Organ weight/body weight}) \times 100$$

The fragments of the liver and kidney were fixed in formalin 10% solution, and then embedded in paraffin

Table 1. Standard kit methods for estimation of Glucose, Urea, Creatinine, SGOT, SGPT and ALP

Parameters	Method (Spectrophotometric)	Reference
Blood Glucose	Glucose Kit using Glucose oxidase Peroxidase (GOD-POD) method	[26]
Blood Urea	Urea Kit using the Modified Berthelot method	[27]
Serum Creatinine	Creatinine Kit using Modified Jaffe's Kinetic method	[28]
SGOT	SGOT Kit using Modified IFCC method	[29]
SGPT	SGPT Kit using Modified IFCC method	[30]
ALP	ALP Kit using pNPP kinetic DEA method	[31]

wax, cut into sections, and stained with hematoxylin and eosin to be visualized under an optical microscope, pictures were captured by camera microscope with × 100x magnification. The sections were observed for tissue integrity, necrosis, degeneration, fatty liver, oedema, and infiltration of leukocytes indicating toxicity.

2.8 Statistical Analysis

Data analysis was done using the Statistical Package for Social Studies version 25. Descriptive statistics were presented as mean±SD. The significance of variance was assessed through ANOVA followed by Dunnet's multiple comparison test. A p-value below 0.05 ($p<0.05$) was considered indicative of significant differences between experimental conditions.

3. Results

3.1 Phytochemical Analysis of Chloroformic Extract of *M. koenigii*

Freshly prepared chloroformic extracts of *M. koenigii* were subjected to phytochemical analysis and the presence of flavonoids, phenols, saponins, anthraquinones, glycosides, tannins and terpenoids was reported in a previous study[8]. Phytochemical quantification of the plant extracts under this study revealed the presence of tannins, terpenoids, phenols and saponins in high concentration as depicted in Table 2. However, the extract was devoid of alkaloids and steroids. Results from another study also reported the absence of alkaloids in the *M. koenigii* extract[32]. The observed phytochemical compounds in the chloroformic extract have been reported to possess psychological activities and medicinal importance. The results of quantitative estimation of phytochemicals are presented in Table 2.

Table 2. Amount of Flavonoids, Glycosides, Phenols, Saponins, Terpenoids and Tannins in the Chloroformic extract of *M. koenigii*

S. No.	Assay	Unit	Chloroform extract
1	Total Flavanoids Content	mg Quercetin equivalent/DE	369 ±1.73
2	Total Glycosides content	mg digoxin equivalent/DE	244 ±3.4
3	Total Phenol content	mg Gallic acid equivalent/g DE	422 ±0.40
4	Total Saponin content	mg Diosgenin equivalent/g DE	301.6 ±0.32
5	Total Tannin content	mg Gallic acid equivalent/g DE	773.5 ±0.5
6	Total Terpenoid content	mg Linalool equivalent/g DE	428.6 ±1.38

Results are expressed as mean±S.E. of three determinations.

3.2 Acute Toxicity of Chloroformic Extract of *M. koenigii* in Rats

Table 3 shows the effect of MK chloroformic extract on behavioural observations in rats after acute oral administration. No treatment-related deaths, abnormal behaviour, or indications of toxicity were observed in all the experimental animals. Hypoactivity and Anorexia were observed in the group that received 2000 mg/kg/bw. LD50 of the test drug can be considered to be more than 2000 mg/kg bw as it was observed to be safe when given once orally to fasting female Wistar rats.

3.3 Effect of Acute Dose on Body Weight of Female Rats

Data presented in Figure 6 shows that there were no notable alterations observed in the weight of the treated groups in comparison to the control group ($p>0.05$) The baseline body weight of all the rats included in the acute toxicity study group was in the range of 220-320 g. On day 7 and at the end of the experimental period, the body weight of the three groups of rats was significantly reduced in contrast to the control group of rats (Table 4). Although the control and treated rats appeared healthy at the end of the experiment, there was anorexia observed in the rats treated with a maximum dose of 2000 mg/kg bw on day 1 post dosage. According to the data obtained, there was no weight loss in any of the groups. The percentage changes in body weight of the chloroformic extract-treated groups were not substantially different compared to the control rats ($p>0.05$).

3.4 Liver and Kidney Weights

Table 5 displays the mean absolute and relative weights of the liver and kidneys of rats treated for 14 days. There were no notable variances in the absolute and relative weights of the liver and kidneys of G2 and G3 in comparison to controls. There was no statistically significant difference

Table 3. General findings and observations of Acute toxicity study for both control and treatment groups

Observation	G1 (NS-10 mg/kg bw)			G2 (2000 mg/kg/bw)			G3 (200 mg/kg/bw)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Ataxia/convulsions/ tremors	×	×	×	×	×	×	×	×	×
Temperature	×	×	×	×	×	×	×	×	×
Anorexia	×	×	×	✓	×	×	×	×	×
Urination	×	×	×	×	×	×	×	×	×
Rate of respiration	×	×	×	×	×	×	×	×	×
Change in skin/eye colour.	×	×	×	×	×	×	×	×	×
Hypoactivity	×	×	×	✓	×	×	×	×	×
Diarrhea	×	×	×	×	×	×	×	×	×
General health-nasal discharge, salivation, dyspnea	×	×	×	×	×	×	×	×	×
Death	×	×	×	×	×	×	×	×	×

✓ - Present; X – Absent

Table 4. Body weights of rats of the acute toxicity study groups

GRP	Treatment	Body weight (g)		
		Day 1	Day 7	Day 14
I	Control (N.S.)	287.33±43.14	298.33±47.24	310.66±54.37
II	MK Chloroformic extract (2000 mg/kg b.wt.)	271.66±37.5	278±36.36	290±34.64
III	MK Chloroformic extract (200 mg/kg b.wt.)	238.66±10.01	246±11.53	254.33±14.97

Values expressed in mean±SD; n=3

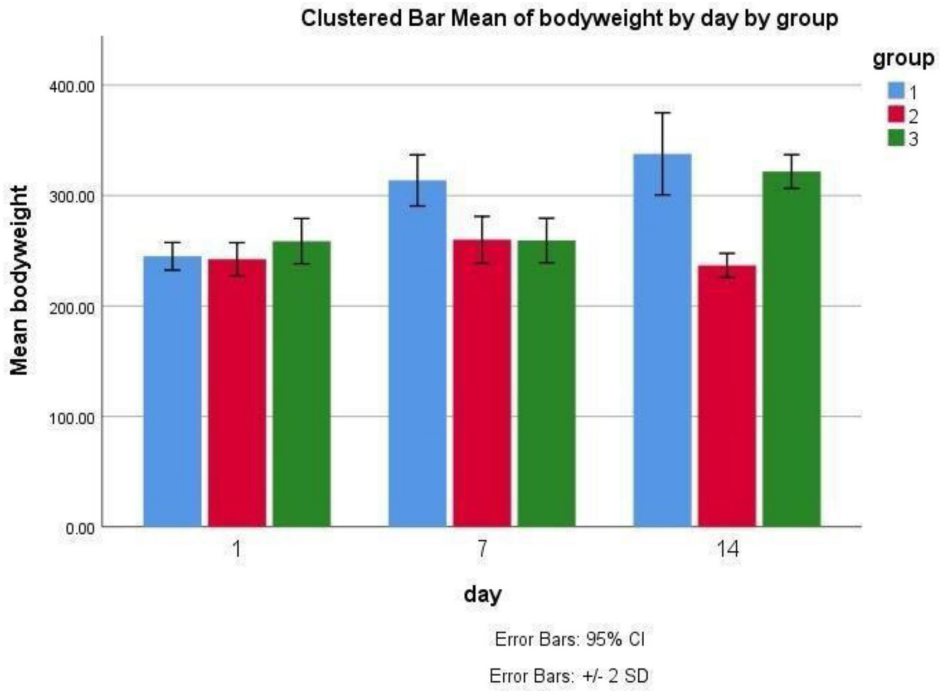


Figure 6. Body weight of the rats treated with chloroformic extract of *M. koenigii* in a 14-day acute toxicity study.

in ROW between the control and chloroformic extract-treated test groups at p>0.05.

3.5 Haematological Parameters

Blood parameters which include Total white blood cell count, Differential count of Neutrophils, Lymphocytes and Eosinophils, Hemoglobin, Platelet count, Red Blood Cell (RBC) count and Packed Cell Volume (PCV) in rats treated with acute MK dose of 2000 mg/kg bw and 200 mg/kg bw along with control group (N.S.) were analysed and results are shown in Table 6. There was no significant difference in haematological parameters between groups (p>0.05). The results showed that all haematological indicators, including haemoglobin (HB) and total white blood cell count, were within normal limits in both the control and MK-treated groups. In the ANOVA test, there is no significant correlation between the groups in the acute test (p>0.05). At a dose of 2000 mg/kg bw, the chloroformic extract of *M. koenigii* shows a slight increase in total WBC count. However, there was no other noteworthy changes in the blood routine tests were observed.

3.6 Biochemical Parameters

Table 7 presents the outcome of the treatment of rats with acute doses of *M. koenigii* chloroform extract on serum fasting glucose, liver enzymes, urea and creatinine. The results of the biochemical parameters measured indicate that there were no significant differences between the two groups that received acute doses of 2000 mg/kg bw and 200 mg/kg bw in contrast to the control group.

3.7 Histopathological Examination of Liver and Kidneys

The control group exhibited typical histology that is found in a normal rat liver, as shown in Figure 7(a), while the group administered with 200 mg/kg dose of CEMK also displayed normal liver histology (Figure 7(c)). However, the rats that received a 2000 mg/kg dose of CEMK (Figure 7(b)) showed slight abnormalities in the liver, such as infiltration of lymphocytes and congested blood vessels.

Histology of the Kidney sections of rats in the control group exhibited a typical and healthy structure (Figure

Table 5. Absolute and relative organ weights of rats treated orally with chloroform extract of *M. koenigii*. values expressed as mean±SEM; n=3

Parameters		G1 (Control)	G2 (2000 mg/kg/bw)	G3 (200 mg/kg/bw)
Absolute organ weights	Liver (g)	10.7 ±0.40	10.5 ±0.3	10.5 ±0.05
	RT Kidney (g)	0.94 ±0.04	0.89 ±0.02	0.89 ±0.01
Relative organ weights	Body WT (g)	310.66±54.37	290±34.64	261±10.5
	Liver (g)	3.51 ±0.60	3.59 ±0.28	4.03 ±0.15
	RT Kidney (g)	0.30 ±0.04	0.30 ±0.03	0.34 ±0.02

Table 6. Effect of an acute dose of chloroformic extract *M. koenigii* on Blood routine parameters

Parameters	G1 (NS 10 mL/ kg)	G2 (2000mg/ kg/bw)	G3 (200mg/ kg/bw)
Total WBC (109/L)	10.6 ±1.03	16.5±8.38	9.2 ±1.24
Neutrophils (%)	13.9 ±1.84	17.3±2.08	27.8 ±2.25
Lymphocytes (%)	85.9 ±1.81	81.9 ±2.58	71.3 ±2.25
Eosinophils (%)	0.16±0.05	0.7 ±0.52	0.86 ±0.23
Hemoglobin (gm%)	13.9±0.15	13.7 ±0.49	14.7 ±1.21
Platelets (109/L)	9.0 ±0.63	8.0±0.54	9.0±1.39
RBC (1012/L)	8.13 ±0.18	8.25 ±0.61	8.59±0.98
PCV(%)	41.6 ±0.52	41.4 ±1.15	45.0±3.86

Values expressed as mean±SEM; n=3

Table 7. Effect of an acute dose of chloroformic extract *M. koenigii* on Blood glucose, Kidney Function markers like urea, creatinine, and liver enzymes like SGOT, SGPT, ALP

Para meters	Units	G1 (NS 10 mL/ kg)	G2 (2000mg/ kg)	G3 (200 mg/kg)
Glucose	mg/dl	98 ± 2.51	106± 11.06	96± 7.0
Urea	mg/dl	32 ±7.57	43.66± 4.04	36± 5.2
Creatinine	mg/dl	0.5 ±0.06	0.48± 0.06	0.46± 0.11
SGOT (AST)	U/L	163 ±36.9	191± 6.02	187± 9.29
SGPT (ALT)	U/L	72 ±4.04	98.66± 12.50	68.6± 7.23
ALP	U/L	141 ±28.5	131.3± 8.02	156± 20.4

Values expressed as mean±SEM; n=3

8(a)). Rats treated acutely with 200 mg/kg bw of CEMK did not have a negative impact on the histological structure of the kidneys (Figure 8(c)). In contrast, there was slight oedema observed in the sections of the kidneys from the CEMK-treated group (2000mg/kg) but no significant alteration in the architecture (Figure 8(b)).

4. Discussion

The findings of this research underscore the significant impact of extraction methods and environmental conditions on optimizing the yields of bioactive compounds from *M. koenigii* extracts. Elements such as photoperiod, light intensity, temperature, soil composition, and water availability play a crucial role in the synthesis of phytochemicals[11]. The selection of a solvent for the extraction of bioactive compounds should prioritize low toxicity, the ability to evaporate at relatively low temperatures, and optimal physiological permeability. Additionally, the solvent should not cause dissociation of the extract, should enhance the stability of crude metabolites, and must not facilitate their degradation[33]. Solvent Chloroform could reap the best amounts of phytochemicals that can be used in pharmacological industries.

The use of medicinal plants is an old practice dating back to ancient times. ''However, plants can be toxic depending on their nature and origin." The phytochemical content of the *M. koenigii* leaves is the basis for its pharmacological activity. Preliminary phytochemical analyses of the leaf extracts from *M. koenigii* have identified the presence of saponins, flavonoids, coumarins, tannins, terpenoids, glycosides, and phenols. The well-studied pharmacology and photochemistry of *M. koenigii* and the therapeutic potential of this plant need to be revised as the plant leaf in our investigation

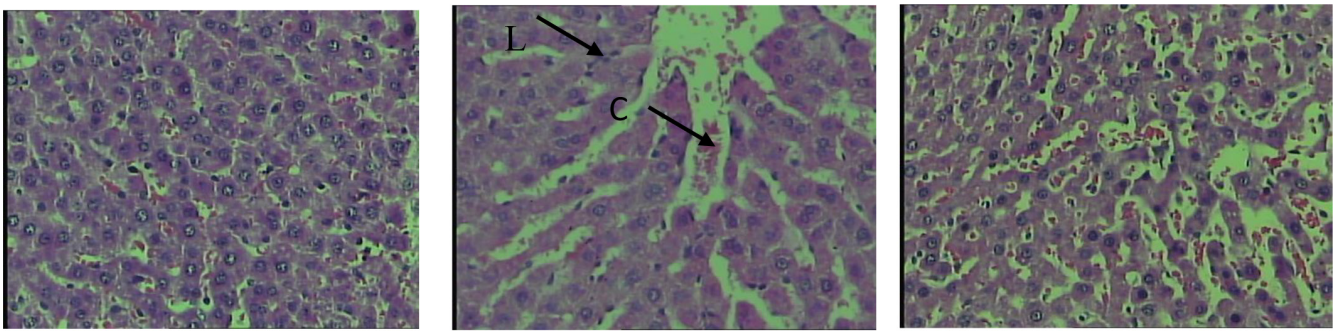


Figure 7. Effect of an acute oral dose of chloroformic extract of *M. koenigii* on the liver. The numbers on the images represent the treatment groups. **(a)** Control rats, **(b)** Rats treated with 2000 mg/kg bw, and **(c)** Rats treated with 200 mg/kg bw. Arrow pointing lymphocytic infiltration.

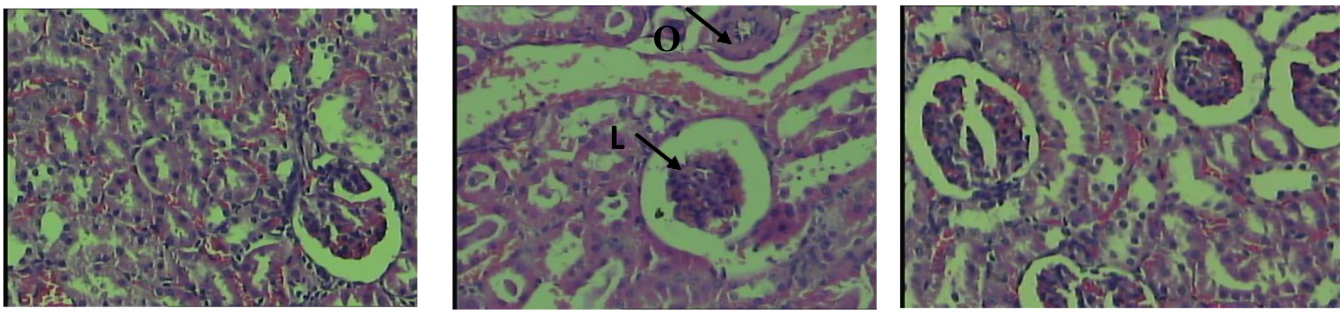


Figure 8. Effect of acute oral dose of chloroformic extract of Murraya Koenigii on Kidney. The numbers on the images represent the treatment groups. **(a)** Control rats, **(b)** Rats treated with 2000 mg/kg bw, and **(c)** Rats treated with 200 mg/kg bw. Arrow points at lymphocytic infiltration and oedema.

showed the absence of Alkaloids. Although, alkaloids are the main bioactive components responsible for the plant's antidiabetic activity[34]. Previous studies have reported high levels of alkaloids and saponins, followed by tannins and phenolics in the *M. koenigii* plant[35]. Similarly, the result of the phytochemical screening by Igara *et al.*[36] revealed the presence of the bioactive constituents comprising the highest amount of flavonoids and phenols, followed by saponins.

Phenolic compounds are acknowledged for their potential chemopreventive properties, particularly due to their antioxidant activity[37] and their role in enhancing cardiovascular health[38]. The Total Phenolic Content (TPC) and antioxidant activities of methanol extracts from *M. koenigii* and *M. minutum* demonstrate a hierarchy with *M. koenigii* exhibiting greater activity than *M. minutum* at a specified antioxidant concentration suggesting the influence on the overall antioxidant activity[39]. Cai *et al.*[40] demonstrated that the incorporation of trace elements, including Co, Ag, and Cd, significantly enhanced the synthesis of phenolic acids.

Flavonoids constitute a diverse group of phytonutrients found in a wide array of plant-derived foods, recognized for their notable antioxidant, anti-inflammatory, and health-promoting properties. The unique chemical structure of flavonoids, characterized by two benzene rings connected by a heterocyclic pyran ring, is essential for their classification and biological activities[41]. Numerous research studies have shown that flavonoids and phenolic compounds can inhibit the pancreatic lipase enzyme in pigs by interacting with the enzyme-substrate complex, which subsequently decreases lipid absorption[42]. These compounds derived from plants may act as effective antioxidants that can reduce LDL oxidation[43].

Saponins are typically composed of a sapogenin core linked to a sugar moiety[44]. A high concentration of saponins is known to reduce lipid absorption[45]. Naturally occurring saponins in plant extracts can inhibit lipid accumulation in liver parenchymal cells and effectively regulate fat accumulation in adipose tissue[43]. Research comparing the total saponin content in herbs cultivated in urban versus coastal regions revealed a higher concentration in the coastal area[21]. This report ascertains our investigation in establishing the significance of environmental factors in influencing the variability of phytochemical composition.

Many plants produce a thick resin that is largely composed of volatile terpenes[46]. The levels of terpenoids can differ markedly between leaf and bark tissues, and they may also fluctuate in reaction to various environmental and biological stressors[22]. In a comparative analysis of various solvents, including ethanol, methanol, acetone, DMSO, and water, it was determined that the hydrolysable tannin concentration in *M. koenigii* leaf extracts was highest when using isopropanol for both mature and young leaves, with measurements of 46.3 mg and 46.7 mg GAE/g, respectively[47-49].

Glycosides are composed of a sugar moiety, termed the glycone, and a non-sugar moiety known as the aglycone or genin. Glycosides play a vital role in the treatment of cardiac failure and arrhythmias, which is why they are classified as cardiac glycosides[50]. Currently, 66 functional C-glycosyltransferases (CGTs) have been identified in plants, which provide sustainable and efficient approaches for the synthesis of C- glycosides[51].

In accordance with OECD guideline 423, the outcomes of this test facilitate the grading and classification of the substance using the Globally Harmonised System of Classification and Labelling of Chemicals. Consequently, the *M. koenigii* chloroformic extract is categorized as category 5, indicating a low risk of acute toxicity, which represents the lowest toxicity classification[24].

In our investigation, the treated rats with an acute dose of 2000mg/kg bw Chloroformic extract of *M. koenigii* developed an increase in the total White Blood Cell count along with infiltration of lymphocytes in the liver but statistically insignificant as the WBC count may vary and the normal reference range is so vast[52]. Histological examination of kidneys treated acutely with 2000 mg/kg bw showed slight oedema in the kidney, although the normal architecture was maintained. Mild hypo activity was also noted with 2000 mg/kg bw of the extract. However, there was no mortality observed at any dose level. These results are not attributed to any toxicological significance[25], as the liver enzymes (SGPT, SGOT, and ALP) and Kidney function tests, including Urea and Creatinine, performed had all the parameters in normal ranges described for rats[52].

Our results can be compared with results that evaluate the methanol extract of *M. koenigii* leaf exhibiting moderate toxicity in rats causing liver inflammation at higher doses[53]. Other studies have shown that crude leaf powder and methanolic leaf extract are safe in up to 9,000 mg/kg doses in rats[54]. Another study confirms the consumption at a dose of 500 mg/kg is safe and does not cause structural organ damage[5]. Furthermore, acute

administration of the ethanolic extract of *M. koenigii* has been shown to be safe at doses up to 1000 mg/kg[55].

The present study was designed to examine the acute toxicity of the chloroform extract in rats, initiating with a dose of 2000 mg/kg and decreasing the dose by 1/10th, *i.e.*, 200 mg/kg bw, which is considered effective for evaluating toxic effects in this investigation. The limit test, described in OECD guideline 401 [8], is a restricted version of an acute toxicity test and is used after a range-finding study or a literature search has made it likely that the chemical is of low toxicity[56]. The findings of this study indicated that the chloroformic extract of *M. koenigii* can be utilized for evaluating medicinal properties as it does not induce acute toxicological effects, and no fatalities occurred among the rats. Therefore, it can be concluded that the

M. koenigii extract is tolerated at a dose of 2000 mg/kg body weight when administered as a single dose.

5. Conclusion

Limit study of chloroformic extract of *M. koenigii* using a single acute dose showed mild toxic effects at the studied dose level of 2000mg/kg bw but is safe when administered at a dosage level of 200 mg/kg bw as it does not pose any toxicological risks. More work on the subacute toxicity of the chloroformic extract is required which could provide remarkable data on the toxic profile of the chloroformic extract of *M. koenigii*.

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