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Evidences of in vivo cytotoxic and apoptotic potential of anthelmintic phytochemical kaempferol derivative isolated from *Lysimachia ramosa* (Wall. ex Duby) in Wistar rats

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Abstract

Background *Lysimachia ramosa* (Wall. ex Duby) is a traditionally used medicinal plant in Meghalaya, a northeastern state of India. The people use the leaves of the plant to cure gastrointestinal worm infection. Kaempferol derivative, isolated from the leaves of the plant *L. ramosa*, was found to be the active anthelmintic compound in previous studies. The phytochemical present in the leaves causes alterations in the ultrastructure of the tegument of cestode, its glycogen content and tegumental enzymes. However, it has been found in the review of the literature that phytoproduct should be consumed in a limited dose during longtime treatment period to avoid any potential ill effects on the health of the consumers as many plant-based products are found to be toxic beyond a safe dose limit. Thereby, it becomes necessary to check the potential toxic effects of the active compound taking an animal model. Hence, the present study had been carried out to evaluate the toxicity of the active component on Wistar rats to find out the safe dose of the test compound for oral consumption through observations, if any, on chromosomal abnormalities, ultrastructural changes in bone marrow cells and apoptosis in liver and kidney.

Results The result shows kaempferol derivative causes a significant rise in chromosomal abnormalities (2.83 ± 0.19%, 3.83 ± 0.11%) and a significant decline in mitotic indices (3.17 ± 0.02%, 3.10 ± 0.01%) upon treatment with 500 mg and 1000 mg/kg b.w. dosages. At this level, the number of micronucleated erythrocytes increases significantly in the animals (2.33 ± 0.33%, 3.15 ± 0.24%). Intercellular integrity and eosinophil in the bone marrow are observed to be deformed and damaged on treatment with 1000 mg compound/kg b.w. of animals. Apoptotic indices are seen to be increased significantly (3.12 ± 0.18%, 6.17 ± 0.17%) and (2.43 ± 0.13%, 4.17 ± 0.17%) for the liver and kidney, respectively, in the animals of higher-dose treatment groups.

Conclusions The compound, kaempferol derivative in the present study, has been found to have cytotoxic and pro-apoptotic effects on the test animals beyond a certain dose, if consumed for longer period of time. Therefore, it can be concluded that kaempferol derivative extracted from *Lysimachia ramosa* should be tested for future trials on animals/humans at dose below 250 mg/kg body weight.

Keywords Kaempferol derivative, Wistar rat, Chromosomal abnormality, Micronucleus, Bone marrow, Apoptosis

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Background

Plants are very useful against various diseases that occur in humans. India is the home of thousands species of plants and is one of the largest producers of medicinal plants. Among these, many medicinal plants are mentioned in ancient texts (Kamboj, 2000). According to the WHO, the use of herbal remedies worldwide exceeds two to three times that of conventional medicines (Pal & Shukla, 2003). The use of traditional medicinal plants to cure various ailments is being practiced from time immemorial and is passing from generation to generation without any experimental tests of the drugs on the consumers. In a similar fashion, many traditional medicine practitioners are working in the Indian subcontinent who are not only streaming their knowledge without any scientific validation but treating patients with severe illnesses also, so it requires proper scientific approach and documentation of the plants and plant products in treatment of diseases. Many studies have reported that not all plants and their products are safe for human consumption and they may have adverse toxic effects in the body of consumers (Wink, 2018). Ames, (1983) studied various species of plants and their metabolites and observed mutagenic activities of 16 mutagens of plant origin, such as coumarins, eugenols, hydrazine, phorbol esters and plant extracts of *Vicia faba*, alfalfa and others, using a bacterial test. In another study, aqueous extracts of three plant species (*Achyrocline satureoides*, *Bachharis anomala* and *Luchea diyarticata*) used in Brazilian folk medicine in the treatment of inflammation, diuresis and arthritis showed positive mutagenic activity in the Ames test with microsomal activation (Vargas et al., 1991). Similarly, capsaicin which is an alkaloid of chili pepper extract has been found to induce mutations in *Salmonella typhimurium* through alteration of histidine related to metabolic activity (Nagabhushan & Bhide, 1985).

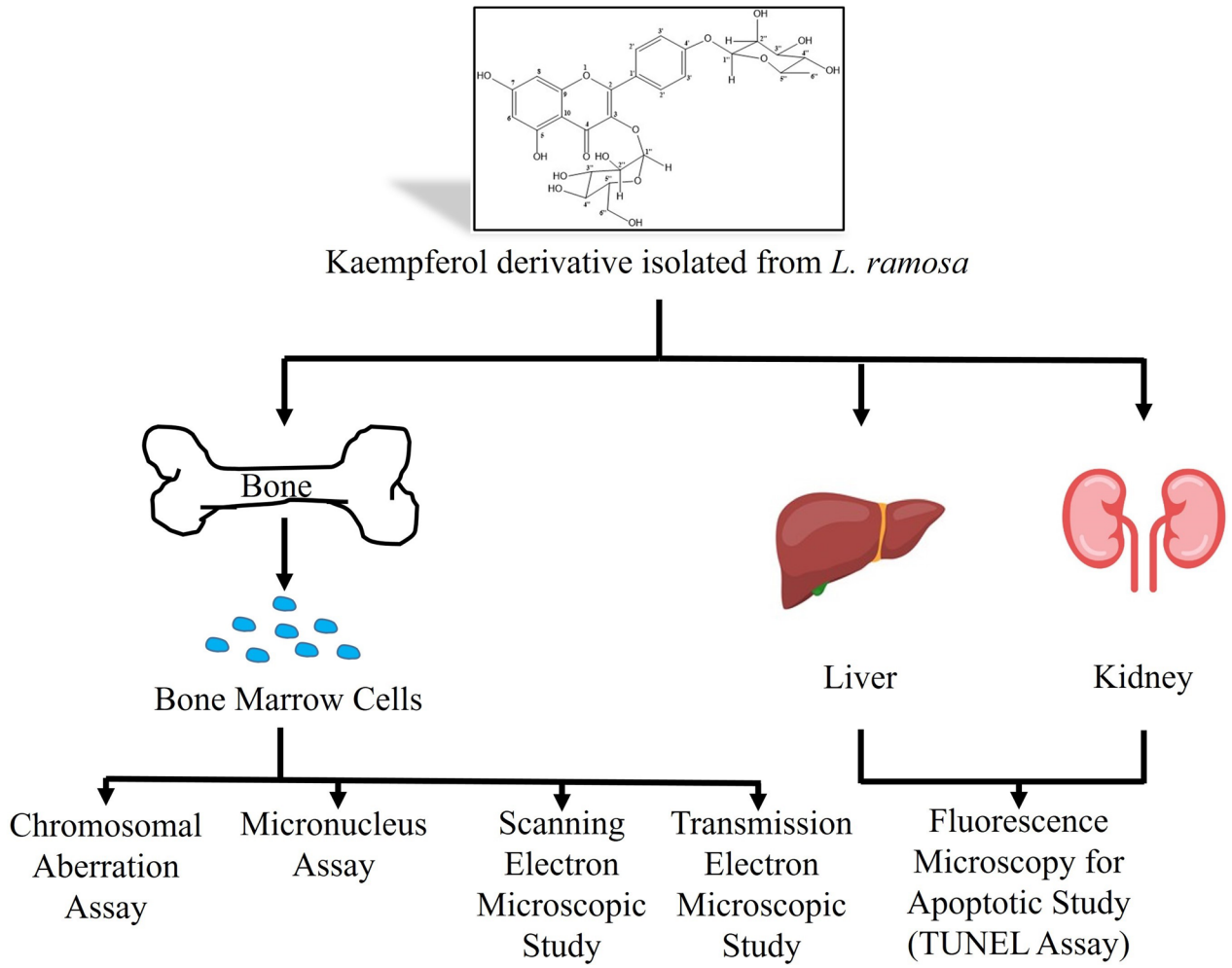

Fig. 1 Diagrammatic representation of the proposed study

The plant *Lysimachia ramosa* (Wall. ex Duby) is found in the East Khasi Hills of Meghalaya. Local tribes consume aqueous extract of it to cure intestinal helminthiases. Crude leaf extract of this plant has been found to cause deformities on worm surfaces (Challam et al., 2010). Dey and Roy, (2018, 2020) noted that the n-butanol fraction of crude leaf extract of the plant altered the glycogen content and many other tegumental enzymes in the parasite *Raillietina echinobothrida*. Recently, Dey et al., (2021) have showed that kaempferol derivative, an active principle of the leaves extract of the plant, is responsible for anthelmintic activity. Lately, Sarkar and Roy, (2022a, 2022b) observed that kaempferol derivative of the plant *Lysimachia ramosa* had some negative impacts on the male reproductive system, and hematological and biochemical parameters of Wistar rats. Therefore, this study has been carried out to observed cytotoxic effects of the kaempferol derivative through observations on chromosomal abnormalities, ultrastructural changes in bone marrow cells and apoptosis in liver and kidney (Fig. 1).

Methods

Collection of plant material and isolation of kaempferol derivative

The plants *Lysimachia ramosa* (Wall. ex Duby) were collected from Jowai region of Meghalaya, India. The locals cultivate the plants and consume as vegetables. Taxonomical identity of the plant was confirmed by Dr. N. Odyuo, Scientist-E and H.O.O. at Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, India (Accession No. 98577). Leaves were detached and air-dried. Dry leaves were grounded into fine powder and processed for preparation of crude methanolic extract. The active n-butanol fraction was obtained from the crude methanolic extract using the fractional distillation method (Simon et al., 2012). From the active n-butanol fraction, the effective anthelmintic compound was isolated and identified as a kaempferol derivative using column chromatography, thin-layer chromatography and nuclear magnetic resonance as described earlier (Dey et al., 2021; Sarkar & Roy, 2022a, 2022b). The kaempferol derivative was later considered for in vivo toxicological study.

Experimental animals

The investigation was carried out on Wistar rats ensuing the animal ethics committee's guidelines for laboratory animal use and care. Permission to conduct experiments on the animals was obtained from Institutional Ethics Committee (Animal models), North-Eastern Hill University (Sanction No.: NEC/IEC/2018/003). Animals received human care in compliance with the guideline principles of CPCSEA (1998). The animals were put up in a grilled enclosure at a steady temperature of 22–25 °C. Proper food and water as per requirement were provided to the animals. All the animals were conformed to the environment 14 days before starting of the experiment. All the rats were divided into four groups comprising three rats each. The first group, designated as control group, was provided with a vehicle (PBS + 1% DMSO). The other three groups were given oral dosages of 250 mg, 500 mg and 1000 mg of kaempferol derivative/kg body weight (b.w.) of rat continuously for 28 days following OECD (2008) guideline.

Chromosome aberration assay

To study any changes in the structure of chromosomes and mitotic index, the rats were administered with 0.2 cc of 0.5% cholchicine (0.5 g colchicine in 100 ml sterile distilled water) intraperitoneally (ip), at least 1 h before killing the rats (Alimba et al., 2006; Preston et al., 1987). After killing the rats, femur(s) and tibia(s) were removed from the hind limb. The bone heads of the bones were cut off so that a 23 gauge needle can easily be inserted into the bones to flush out the bone marrow cells with freshly prepared 1 cc of 0.56% (0.075 M) KCl. The cells in the tubes were then incubated at 37 °C for 25–30 min. Later, centrifugation was done at 2000 rpm for 5 min. The supernatant was discarded. 0.5 ml of Carnoy's fixative (mixture of methanol and glacial acetic acid in the ratio of 3:1) was added to each tube without disturbing the pellet. The fixative was aspirated after 3–4 s and again freshly added. The cells in the tubes were left incubated at room temperature for 30 min. After that, the tubes were centrifuged again to remove the supernatant. The addition of fresh fixative was done twice more. The last addition of the fixative was made such that a thin cell suspension was formed.

Clean slides were taken for the study. Before starting the experiment, the slides were kept in ice because chilled slide was necessary for this work. The "bomb method" was applied to burst the cells. For that, with the help of a syringe cell suspension was dropped from a height of nearly 1 feet on the chilled slides. Then immediately, the chilled slides with the drops of cell suspension were heated on a hot plate to give the cells on the slides the "heat-shock treatment." After that, the slides were left to dry and then brought to get stained with 5% Giemsa (v/v, Giemsa stain stock/distilled water). Finally the slides were viewed under a compound microscope. The mitotic index was calculated by counting cells in metaphase from approximately 3000 cells and expressed as percentage. Chromosomal aberrations were assessed blind to treatment, and at least 100 well-distributed metaphasic cells were analyzed.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100\%$$

Micronucleus test

For the micronucleus test, bone marrow cells from the femur(s) were obtained and processed following Schmid, (1975). Briefly, the rats had been anesthetized. The femur(s) had been eliminated from every rat. The proximal and distal epiphyses were cut, and their medullary canal was gently flushed from the bones with bovine serum albumen (BSA). Bone marrow cells had been centrifuged at 2000 rpm for 5 min, and after discarding the supernatant, the pellets were taken on slides and smears of bone marrow cells were prepared. The slides were stained with 5% Giemsa stains. At least a thousand cells had been scored for micronuclei in polychromatic erythrocytes.

Scanning electron microscopic study of bone marrow cells

For scanning electron microscopic study, tissue samples were taken after the rats were killed and then washed thoroughly in phosphate-buffered saline (PBS). Proper cleaning was done to remove the debris attached to the tissue surface. Fixations of the tissues were done in 10% neutral buffered formalin (NBF) having pH 7.0. The samples were then passed through different concentrations of acetone in ascending order (30%, 50%, 70%, 90%, 95% and 100%) for the dehydration of the samples. After that, the samples were immersed in tetramethylsilane [TMS-$(CCH_3)_4Si$, boiling point 26.3 °C, surface tension 10.3 dynes/cm at 20 °C] for 10 min and then brought to room temperature to dry (Dey et al., 1989 modified by Roy & Tandon, 1991). Later the samples were mounted on brass stubs (10 mm in diameter). The metal coating of the samples was done with gold in a fine coat Ion Sputter JFC-1100 (JEOL) by establishing a low vacuum (10^{-3} Torr) in the sputter chamber. Finally the samples on stub were viewed under scanning electron microscope (SEM) JSM 6360 (JEOL Ltd, Tokyo, Japan) using the secondary electron emission mode at electron accelerating voltages ranging between 10 and 20 kV.

Transmission electron microscopic study of bone marrow cells

To view the ultrastructure of the bone marrow cells, at first a small mass of bone marrow cells from the medullary cavity was taken and then fixed in the modified Karnovsky's fixative for 4 h. The fixative, 1% osmium tetraoxide (OsO_4) was used for postfixation of the samples for another 1 h at 4 °C. After washing in buffer, the samples were then passed through ascending grades of acetone with two changes of 15 min each for dehydration. To remove excess acetone from the samples, propylene oxide was used for 30 min. Infiltrations were carried out gradually in different proportions of propylene oxide and embedding medium (Araldite CY212, 10 ml; dodecenyl succinic anhydride, 10 ml; DMP-30 {tri-(di-methylaminomethyl) phenol}, 0.4 ml; and dibutyl phthalate, 1 ml). Tissue embedding was performed in araldite using BEEM capsules. The embedded blocks were heated at 50 °C in oven for 24 h. Later temperature was raised to 60 °C and kept for 48 h for complete polymerization. Ultra-thin sections (600–800 Å) of the samples were prepared with the help of a RMC ultra-microtome (MT-X, USA) having a diamond knife. The sections were then collected on a copper grid and stained with uranyl acetate, an alcoholic saturated solution for 10 min at room temperature at dark followed by lead citrate for 5 min (Reynolds, 1963). Finally the sections were examined in a JEM 100CXII (JEOL) TEM at an accelerating voltage of 120 kilovolt.

In situ DNA fragmentation assay (TUNEL assay)

To detect DNA fragmentation at single-cell level, terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling assay (APO-BrdU™ TUNEL Assay Kit) was used (Moore et al., 2021). At first, the fresh liver and kidney tissues of both control and active compound-treated rats were needed to be dissociated to prepare the single-cell suspensions. In a sterile dish containing phosphate-buffered saline [PBS (dissociating medium)], small pieces of the tissues were minced. A mesh strainer (70 or 100 micron) was placed on a sterile 50-ml conical tube. The dissociated tissues along with the media were transferred to the strainer and gently passed through the strainer. The tissues and cells were washed in the strainer by gently adding few milliliters of PBS. The step was repeated, and the leftover tissues were discarded. The tubes having cells in PBS were centrifuged at 3000 rpm for 5 min. The supernatants were later discarded. To the pellets of each tube, 0.5 ml of PBS was added. For fixation of the cells, 5 ml of 1% (w/v) paraformaldehyde was added to the sample. Centrifugation was done at 3000 rpm for 5 min and supernatant was discarded. The cells at 5 ml of PBS were washed with centrifugation. The steps were repeated again. The cells were re-suspended in 0.5 ml of PBS. 5 ml of ice-cold 70% (v/v) ethanol was added to the tube and kept for 30 min. The cells can be stored in this condition at − 20 °C freezer for several days before use.

After proper fixation of the cells, 1 ml aliquots was taken in a tube and it was centrifuged at 3000 rpm for 5 min; later, the supernatant was discarded. The cells were re-suspended in 1 ml of wash buffer, and again, centrifugation was done to remove the supernatant. 50 µl of DNA-labeling solution [10 µl of reaction buffer, 0.75 µl of Terminal deoxynucleotidyl transferase enzyme

(TdT), 8 µl of 5-Bromo-2′-deoxyuridine 5′-triphosphate (BrdUTP) and 31.25 µl of distilled water were mixed] was prepared and added to each sample tube. The cells were incubated in the DNA-labeling solution at 37 °C for 60 min. After the incubation, the cells were rinsed in 1 ml of rinse buffer and centrifuged to remove the supernatant. This step was repeated again for proper rinsing. The cell pellets were suspended in 100 µl of antibody staining solution (prepared by mixing 5 µl of Alexa Fluor™ 488 dye-labeled anti-BrdU antibody and 95 µl of rinse buffer) and incubated for 30 min at room temperature. The samples were protected from light during incubation. Finally the cell suspension from each tube was deposited onto slides after the antibody staining step. The cells were covered with a cover slip on the slides and then viewed under a fluorescence microscope (EVOS, USA). The fluorescence excitation and emission maxima were 495 nm and 519 nm, respectively. The damaged and irreparable fragmented DNA, trafficked to the cytosol (Hacohen & Lan, 2019), had been observed as bright green spots in the cellular periphery.

$$\text{Apoptotic Index (AI)} = \frac{\text{Number of apoptotic cells}}{\text{Total number of cells}} \times 100\%$$

Statistical analysis

Comparison of the mean values of the experimental data and respective controls was made using Student's *t-test*, and $p \leq 0.05$ was taken as the threshold of significance. The data are represented as mean ± SEM.

Results

Study of occurrences of abnormal chromosome induced by kaempferol derivative

When the rats were treated with 250 mg of kaempferol derivative/kg b.w., the average value of mitotic indices was noticed to be decreased; however, upon exposing the rats to 500 mg and 1000 mg/kg b.w. of the isolated compound, the significant drops in the mean values of mitotic indices in the rats were recorded as compared to the control values of the untreated group animals as shown in Table 1.

In the treatment group of 250 mg/kg b.w. kaempferol derivative, the frequency of chromosomal aberrations was detected to be increased slightly. But treatment with 500 mg and 1000 mg of active compound/kg b.w. rats caused a significant rise in the mean value of frequencies of chromosomal aberrations in the bone marrow cells of the test animals (Table 1).

A total of four types of chromosomal aberrations were observed in the study of bone marrow cells of the rats, and they were chromatid exchange (CE), chromatid break (CB), ring chromosome (RC) and dicentric chromosome (DC) as shown in Fig. 2. The CE type was the most commonly occurring chromosomal aberration in all the treatment groups of rats including the control animals. The average values of this chromosomal aberration in the control rats and 250 mg active compound/kg b.w.-treated rats were found to be statistically similar. But treatment with the higher doses of 500 mg and 1000 mg/kg b.w. of the compound caused significant rises in the rats of the respective test groups (Table 1).

The occurrence frequencies of CE, CB and DC significantly increased in the animals exposed to 500 mg and 1000 mg/kg b.w. of kaempferol derivative; however, the significantly higher number of RC was observed in rats treated with 1000 mg of isolated compound/kg b.w (Table 1).

Study of effect of kaempferol derivative on micronucleated bone marrow cells

Three types of cells were observed in the bone marrow smear preparation namely polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE) and polychromatic erythrocytes with micronucleus (MnE) shown in Fig. 3. On treatment with 250 mg kaempferol derivative/kg b.w., the percentage of micronucleated

Table 1 Effect of kaempferol derivative on mitotic index and frequency of chromosomal aberrations

Group	Mitotic index (%)	Frequency of chromosomal aberrations (%)	CE (%)	CB (%)	RC (%)	DC (%)
Control	3.26 ± 0.01	1.88 ± 0.12	1.17 ± 0.05	0.17 ± 0.03	0.33 ± 0.06	0.17 ± 0.04
250 mg/kg	3.19 ± 0.02	2.00 ± 0.13	1.17 ± 0.05	0.19 ± 0.04	0.33 ± 0.06	0.17 ± 0.04
500 mg/kg	3.17 ± 0.01*	2.83 ± 0.19*	1.67 ± 0.04*	0.50 ± 0.03*	0.33 ± 0.06	0.33 ± 0.05*
1000 mg/kg	3.10 ± 0.01*	3.83 ± 0.11*	2.17 ± 0.03*	0.83 ± 0.02*	0.50 ± 0.04*	0.33 ± 0.03*

Effect of kaempferol derivative isolated from *L. ramosa* on the mitotic index and frequency of chromosomal aberrations including chromatid exchange (CE), chromatid break (CB), ring chromosome (RC) and dicentric chromosome (DC). Values are expressed as mean ± SEM, **P* value is significant at ≤ 0.05

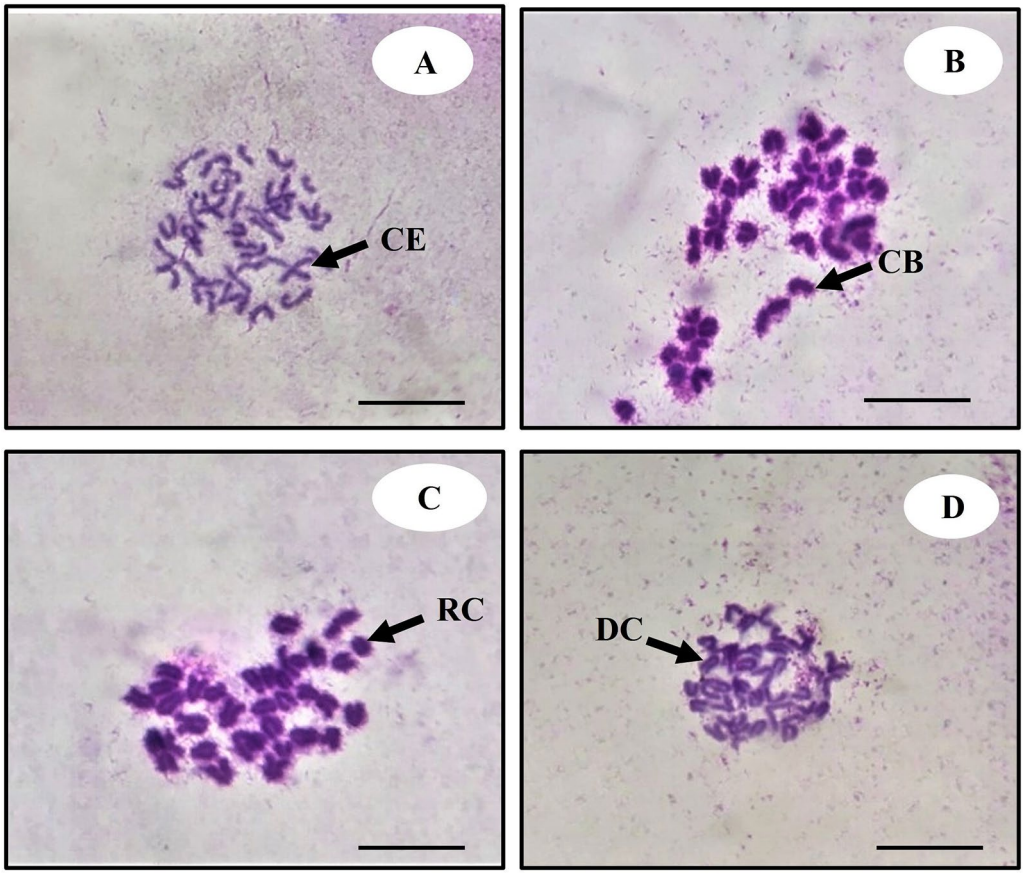

Fig. 2 Different types of chromosomal aberrations. **A** Chromatid exchange (CE). **B** Chromatid break (CB). **C** Ring chromosome (RC). **D** Dicentric chromosome (DC) (scale bar: 200 µm)

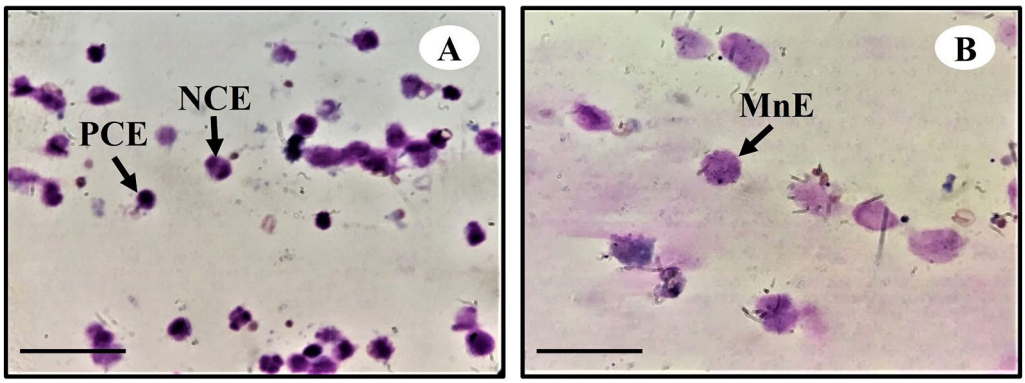

Fig. 3 Types of cells observed in a bone marrow smear. **A** Image showing polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). **B** Image showing a polychromatic erythrocyte having a micronucleus (MnE) (scale bar: 200 µm)

erythrocytes was observed to be normal. In rats upon exposure to higher dosages of kaempferol derivative, the number of MnE was found to be increased significantly shown in Fig. 4.

Study of bone marrow cells through scanning electron microscope

Scanning electron microscopic study of bone marrow depicted a normal arrangement of bone marrow cells. The mesenchymal stromal cells were connected

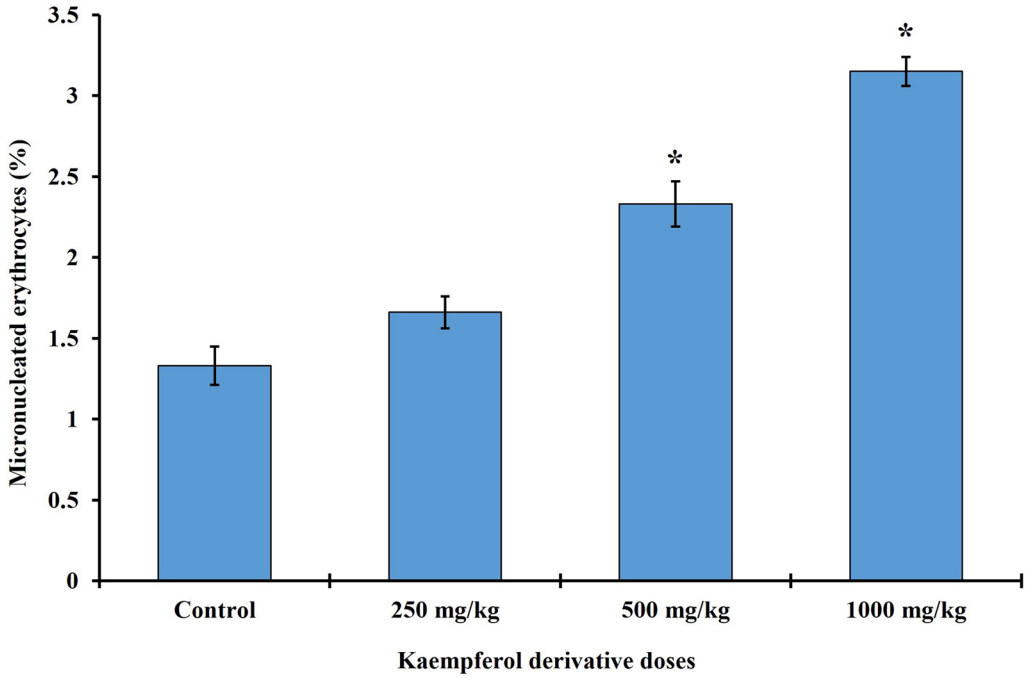

Fig. 4 Effect of kaempferol derivative on occurrences of micronucleated erythrocytes. Graphical representation of effect of kaempferol derivative isolated from *L. ramosa* on occurrences of micronucleated erythrocytes in the bone marrow of the treated Wistar rats. Values are expressed as mean ± SEM, **P* value is significant at ≤ 0.05

intercellularly by cellular processes as shown in Fig. 5A. The cells had convulsions on their surfaces. The extravascular space was also normal in shape and size. Minor changes in shape, size of the stromal cells and extravascular space were observed in the bone marrow of 250 mg and 500 mg of kaempferol-treated animals compared to the control shown in Fig. 5B,C; however, in animals which were exposed to 1000 mg kaempferol derivative/kg b.w., the normal bone marrow cellular structure was found to be changed drastically. The regular shape and size of the stromal cells were noticed to be deformed and decreased. The extravascular space has been observed to be enlarged in size (Fig. 5D).

Study of bone marrow cells through transmission electron microscope

Eosinophil cells of bone marrow of rats from control group showed typical bilobed nucleus (N). The cytoplasm was found to be rich in secondary granulocytes (Gr). The secondary granulocytes were characterized by the presence of crystalloid bodies (crystalline core) inside them. Mitochondrial (M) structures were normal. Abundant lipid bodies (LB) were observed to be distributed in the cytoplasm and their shapes were distinctly normal. Membrane-bound endoplasmic reticulum (ER) were noticed to be closely situated parallel to the nucleus shown in Fig. 6A. The ultra-thin sections of bone marrow of the rats exposed to 250 mg and 500 mg of kaempferol derivative/kg b.w. showed the presence eosinophils with peripheral changes shown in Fig. 6B, C. On treating the rats with 1000 mg of the compound/kg b.w., the bone marrow sections showed shrinkage in the cytoplasmic content and deformed shape and size of a regular eosinophil shown in Fig. 6D.

In situ DNA fragmentation assay (TUNEL assay)

In the control animals, the numbers of apoptotic cells in the vital organs such as the liver and kidney were observed to be very low shown in Fig. 7A–C. But when the Wistar rats were exposed to different concentrations of dosages of kaempferol derivative, the numbers of apoptotic cells changed shown in Fig. 7B–D and they became significantly greater in liver and kidney of animals exposed to 500 mg and 1000 mg of kaempferol derivative/kg b.w shown in Table 2.

Discussion

In the present study, it has been observed that the mitotic index dropped significantly when the animals were treated with higher doses of kaempferol derivative isolated from the plant, *L. ramosa*. Lower values of mitotic indices indicate the decreased rates of cell division. This indicates the cytotoxic potential of the phytoproduct at higher dosages. The isolated compound may have this

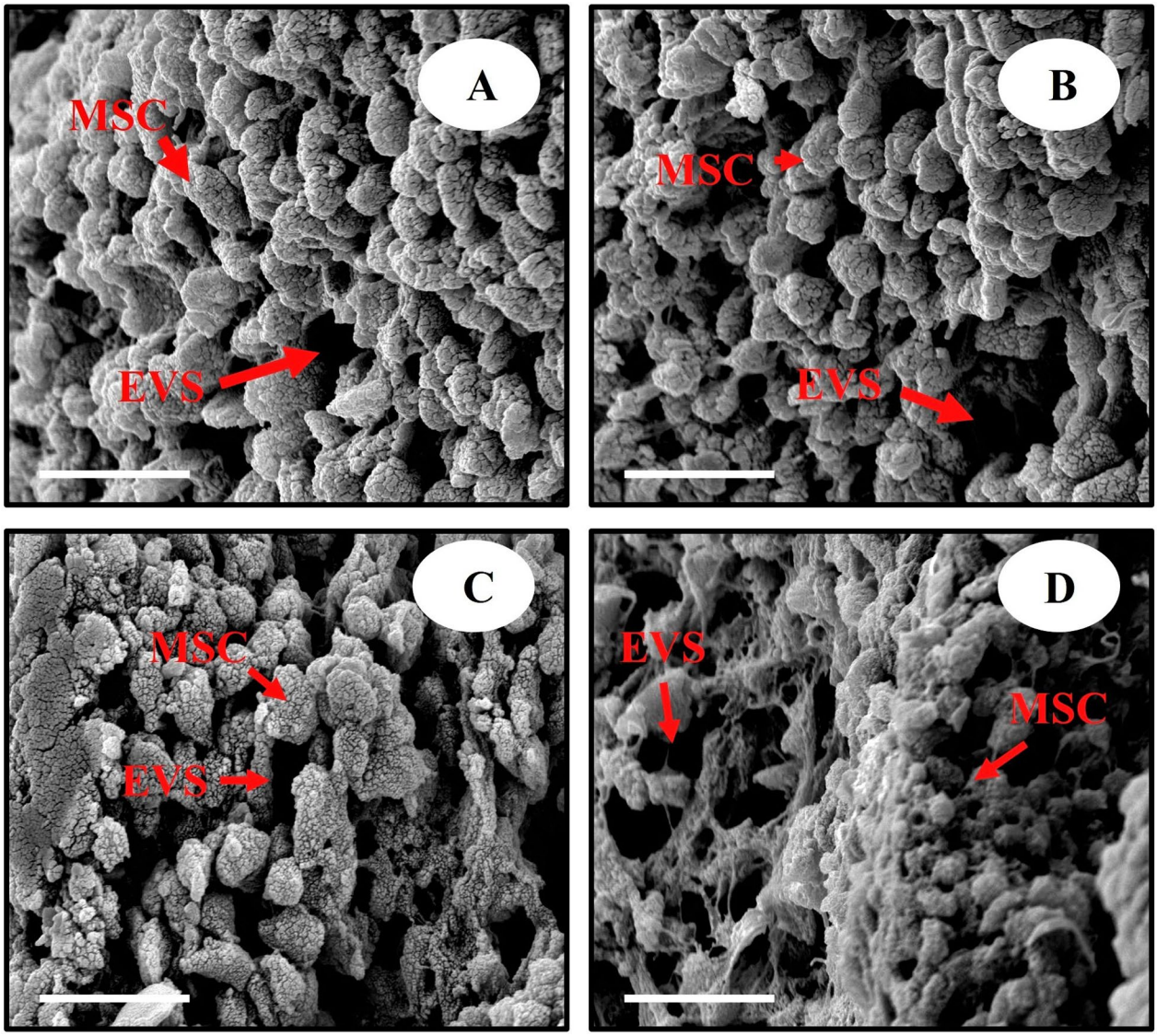

Fig. 5 Scanning electron micrograph of bone marrow of Wistar rats. **A** Normal bone marrow having mesenchymal stromal cells (MSC) and extravascular space (EVS). **B** 250 mg kaempferol derivative/kg b.w.-treated bone marrow cells. **C** 500 mg kaempferol derivative/kg b.w.-treated bone marrow cells. **D** 1000 mg kaempferol derivative/kg b.w.-treated bone marrow cells (scale bar: 10 µm)

cytotoxic potential due to its ability to cause microtubular/spindle disturbances in the cells, which leads to the inhibition of cell division (Carbonell et al., 1989). It has been reported that there are many drugs that produce differential dose-dependent effects to inhibit microtubule-mediated functions (Yang et al., 2010). Like our observations, the mitotic index decreased upon oral administration of deltamethrin in rats (Agarwal et al., 1994).

Various chemical and physical factors such as toxic gaseous substances in air, harmful substances in food and water, and radiations are there in our surroundings that may affect mitotic index, which in turn develop into micronucleated cells. Micronuclei are tiny extranuclear bodies originate from chromosome fragments. Celik, (2012) reported that increased occurrences of chromosomal aberrations and micronucleus can be considered as an index of clastogenicity of plants and their ingredients. In our investigation also, occurrences of a significant number of micronucleus in the bone marrow cells of animals that were exposed to higher dosages of the kaempferol derivative were observed. At higher doses of treatment, incidences of chromosomal aberrations have also been noticed to be significantly increased. Negligible amount of chromosomal aberrations and micronucleus induction have been observed in control animals

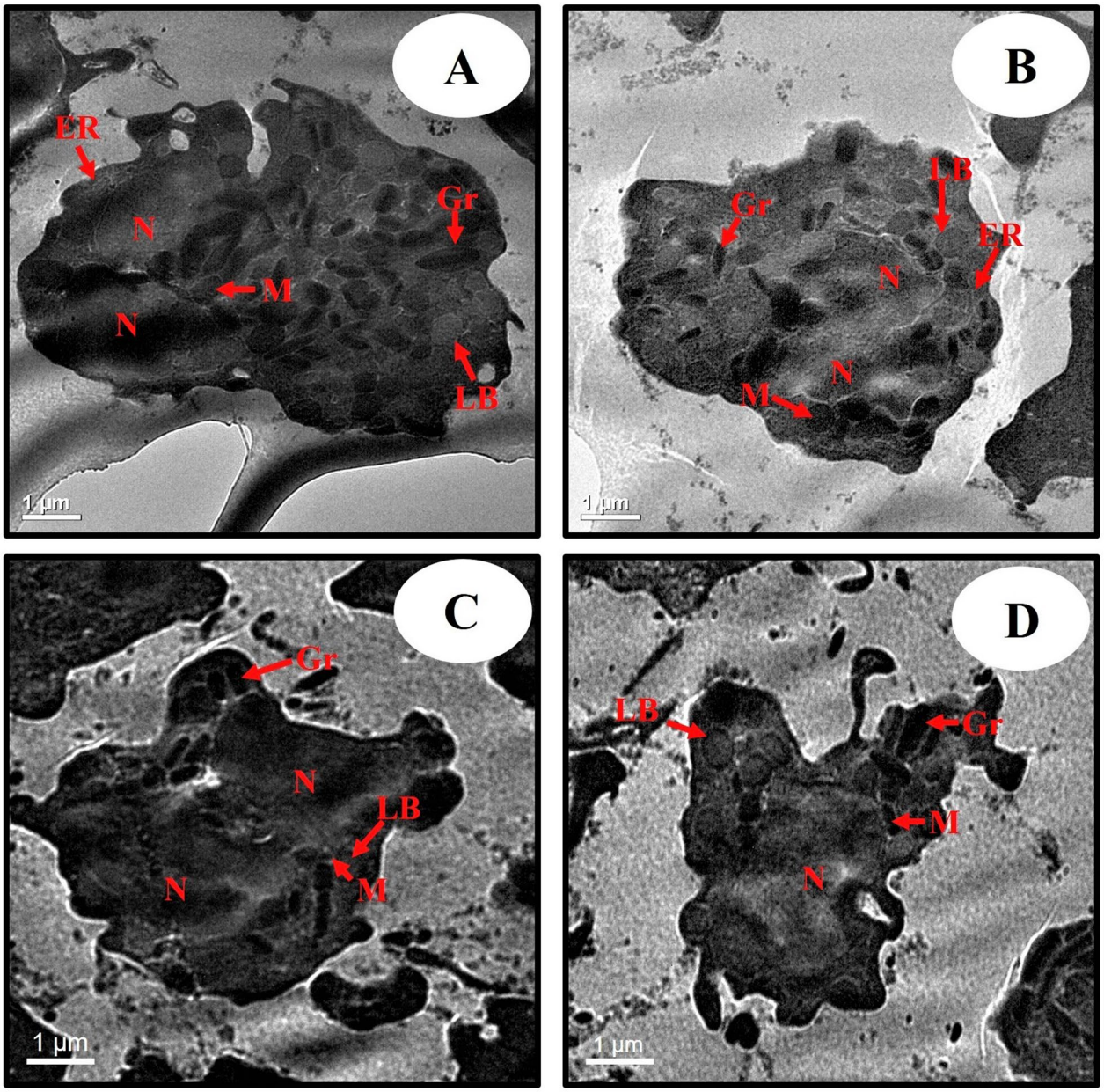

Fig. 6 Transmission electron microscopy of bone marrow of Wistar rats. **A** Eosinophil of control bone marrow showing bilobed nucleus (N), mitochondria (M), lipid bodies (LB), secondary granulocytes (Gr), endoplasmic reticulum (ER). **B** Eosinophil of 250 mg kaempferol derivative/kg b.w.-treated bone marrow. **C** Eosinophil of 500 mg kaempferol derivative/kg b.w.-treated bone marrow. **D** Eosinophil of 1000 mg kaempferol derivative/kg b.w.-treated bone marrow (scale bar: 1 µm)

which may be due to the experimental conditions that might cause oxidative stress in rats. Administration of colchicine to arrest cells in metaphase may also be responsible for the same as colchicine affects cell division and genetic material both (Jordan & Wilson, 1999; Vallarino & Morales, 2001). Similar kinds of observations of chromosomal aberrations and micronucleus inductions in human lymphocytes were also observed due to the presence of higher concentrations of kaempferol isolated from fresh *Lilium candidum* bulbs as reported by Jovtchev et al., (2014). In an *in vivo* toxicological assessment, seed extract of *Annona squamosa* was found to cause chromosomal aberrations in rats (Grover et al., 2009).

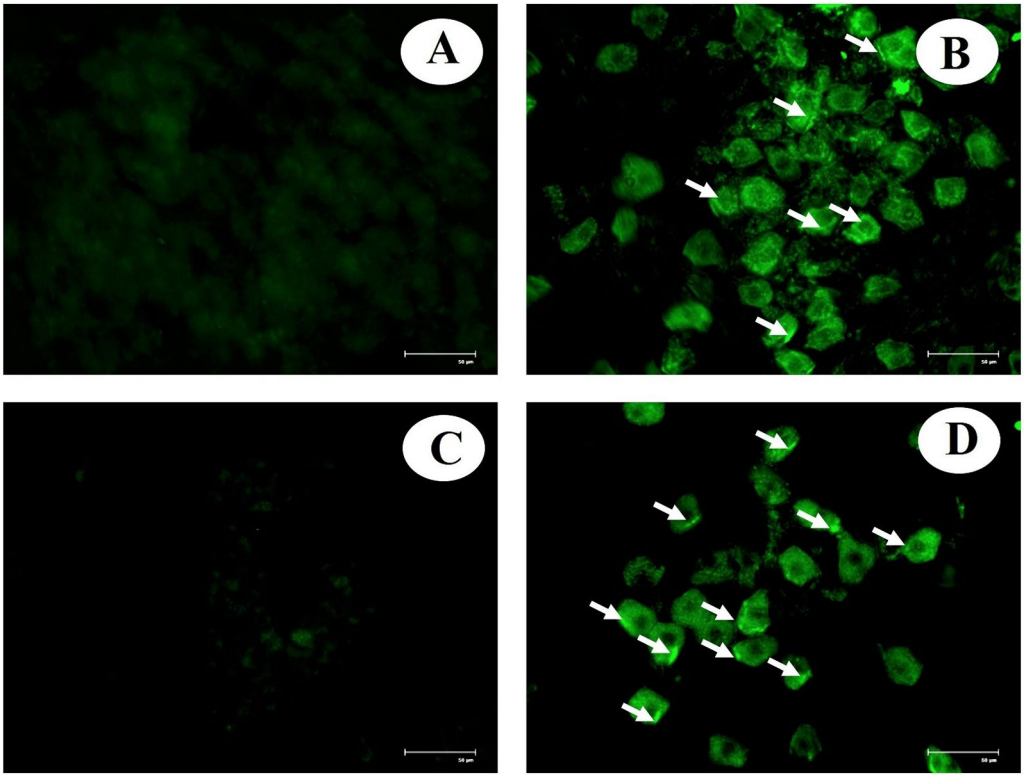

Fig. 7 Fluorescence image of liver and kidney cells of Wistar rats. **A** TUNEL stained control cells of the liver. **B** Treated cells of liver. **C** Control kidney cells. **D** Treated cells of the kidney (scale bar: 50 µm)

Bone marrow is a semisolid part that remains inside the spongy area of a bone. The stroma of the marrow has mesenchymal stromal cells that differentiate into various tissues (Rubin et al., 2008). This is sometimes referred to as the bone marrow microenvironment or stem cell niche (Curry et al., 1967; Schofield, 1978). Scanning electron microscopic study reveals that when the animals were treated with 1000 mg kaempferol derivative/kg b.w. of rats, the normal structures and shapes of the stromal cells have been seen to be deformed along with enlarged extravascular spaces that surround the cells filled with interstitial fluid. The devitalized effect of the compound on bone marrow cells suppresses the differentiation of stromal cells into blood cells, thereby lowering "hemopoiesis." If this condition persists in long term, it may develop into aplastic anemia (Marsh et al., 1991; Philpott et al., 1995). Disturbance in the process of "hemopoiesis" in the 1000 mg kaempferol derivative/kg b.w.-treated rats has been witnessed in transmission electron microscopic observation of bone marrow. The granulocytes and eosinophils have been observed to be shrunk along with condensed protoplasmic material in the bone marrow. Similar kinds of damage have been observed in the bone marrow cells in mice suffering from aplastic anemia (Chatterjee et al., 2010). The present finding supports the fact that the test substance kaempferol derivative which is a phenolic compound has some negative effects on bone marrow cells when treated with high concentrations for longer time. Lang et al., (1991) also observed the increased occurrences of necrotic bone marrow cells on increasing level of phenolic compounds in the surrounding.

Table 2 Effect of kaempferol derivative on apoptotic index in liver and kidney cells

Group	AI in the liver (%)	AI in the kidney (%)
Control	2.00 ± 0.19	1.17 ± 0.17
250 mg/kg	2.17 ± 0.14	1.67 ± 0.14
500 mg/kg	3.12 ± 0.18*	2.43 ± 0.13*
1000 mg/kg	6.17 ± 0.17*	4.17 ± 0.17*

Effect of kaempferol derivative isolated from *L. ramosa* on the apoptotic index (AI) in the liver and kidney of Wistar rats. Values are expressed as mean ± SEM. **P* value is significant at ≤ 0.05

There are various biomolecules in the animal body, but the DNA is very special not only as genetic material but also as the only biomolecule that can be repaired and can be synthesized after damage until terminal DNA fragmentation occurs. Therefore, terminal DNA

fragmentation is considered as the cause of natural cell death or apoptosis (Basnakian et al., 2017; Hengartner, 2001). Apoptosis is a process by which the body eliminates unwanted or dead cells from the system. In our present study, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay has been performed to detect any terminal DNA fragmentations in the cells of the liver and kidney. The results from the present investigation reveal a significant increase in the apoptotic index in the organs of the rats that were exposed to 500 mg and 1000 mg kaempferol derivative/kg b.w. This higher frequency of cells having fragmented DNA upon treatment with high doses of the phenolic compound may be due to the result of activation of the caspases triggered by the compound (Kumar et al., 2016). DNA fragmentation caused by the plant extracts in the helminthes had been observed by TUNEL assay as observed by Giri et al., (2013). Likewise, T-2 toxin often found in maize and other cereal crops including barley is reported to cause injury and DNA damage in liver cells (Atroshi et al., 1997). It is also reported that extract of areca nut, a common chewing nut, causes oxidative DNA damage by generation of hydrogen peroxide (Liu et al., 1996).

Conclusions

From this study, it can be concluded that kaempferol derivative, an active anthelmintic principle, isolated from *Lysimachia ramosa* did not show any significant harm at 250 mg/kg b.w. but has some cytotoxic potential at higher dosages and induces apoptosis in vital organs such as the liver and kidney at 500 mg/kg b.w. and more. Therefore, this compound should be considered for future studies on animals or humans preferably below 250 mg/kg b.w.

Abbreviation

b.w. Body weight

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Author contributions

BR and AS planned the outline of the research work. BR supervised the work. AS and DD performed the work. AS analyzed the data. AS and DD contributed in paper writing. BR and AS prepared the final draft. All authors have read and approved the final draft.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

To perform the experiments on Wistar rats, ethical permission was obtained from Institutional Ethic Committee, North-Eastern Hill University (Sanction No.: NEC/IEC/2018/003). Consent to participate is not applicable.

Consent for publication

N/A.

Competing interests

The authors declare that they have no competing interests.

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