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MODULATING THE BCL2 TO BAX RATIO: TURMERIC'S POTENTIAL IN LEUKAEMIA THERAPY

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Abstract

Leukemia ranks among the ten most prevalent types of cancer in Malaysia, exhibiting a death rate of 4.4%. Therefore, the objective of this study was to assess the preventative effects of a high dose of dried *Curcuma longa* (*C. longa*) rhizomes supplementation in rats with N-Methyl-N-Nitrosourea (MNU)-induced leukemia. Two weeks were spent acclimatizing 64 mature male Sprague Dawley rats. At week 0, all rats were separated into A, B, C, and D groups. MNU was intraperitoneally given to Group C and D rats at 240 mg/kg. *C. longa* rhizomes were dried and given to Groups B and D rats at 5000 mg/kg. Group A rats were controls. The rats were killed at week 20. Blood samples were examined for the presence of leukaemic cells and underwent RNA extraction for quantitative real time polymerase chain reaction (RT-PCR). By blast cell appearance, all rats in Group C had 100% leukemia in the blood smear, while Group D had 88%. The ratio of Bcl-2 to Bax transcripts in blood was 3.3-fold (10.50±1.26) higher in Group C compared to the control rats (3.17±1.07), indicating high levels of anti-apoptotic cells caused to leukemia. Interestingly, the ratio of Bcl-2 to Bax transcripts in Group D rats (3.58±0.82) was similar to the control rats. A quantitative RT-PCR experiment found that adding dried *C. longa* rhizome to a meal significantly reduced Bcl2 to Bax ratio in leukemia rats, in which significantly reduced the incidence of leukaemia.

INTRODUCTION

The immune system is initially impacted by haematopoietic malignancies, such as lymphoma and leukaemia, which start in the bone marrow or lymph nodes [1]. Leukaemia is one of the top ten malignancies in the globe, according to data published in 2021 [2]. Children under 10 years old, both genders, are included in the age-standardized incidence rate. Consequently, leukaemia is one of the top 10 cancers in Malaysia with a 4.4% fatality rate. In order to explore haematopoietic malignancies in animals, researchers used the carcinogenic drug N-methyl-N-nitrosourea (MNU).

Da Silva Franchi and colleagues [3] carried out an experiment in which Wistar rats were exposed to high doses of

MNU (160 mg/kg and 240 mg/kg, i.p.) and effectively produced preneoplastic thymus and spleen at the end of the 20-week animal research. Accordingly, within 20 weeks of animal experiments, the administration of freshly produced MNU at a total dose of 240 mg/kg body weight administered i.p. caused 100% lymphoma and 30% leukaemia in male Sprague Dawley rats [4]. The approach proposed by Hutheyfa et al. [4] is employed in this study to generate leukaemia in the animal model, with MNU being selected as the inducing agent. According to the study, the high levels of Bcl-2 production that kept the cancer cells from dying were what caused the elevation of Bcl-2 (an anti-apoptotic gene) gene expression and the downregulation of Bax expression during the development of carcinoma. Additionally, it has been determined that the failure of the apoptotic pathway is strongly associated with the emergence of haematopoietic malignancy [5]. Bcl-2 gene expression is therefore justified to function as a biomarker in a wide range of human disorders, either alone or in combination with other indicators.

The active compound in *C. longa* L. named curcumin is commonly utilized as medication. Several cancer forms, including breast cancer [6 - 7], colon cancer [8], mammary tumour [7], tongue carcinoma [9], duodenal tumours [10], leukaemia–lymphoma [11], and mammary gland tumour [12], have been shown to respond favourably to *C. longa* L., either in vivo or in vitro. The research used various C. longa dosing methods and routes. It has been demonstrated that curcumin extract does not have the same anti-inflammatory effects as crude *C. longa* L. rhizomes supplementation [13]. To explore the herb's anti-cancer properties against MNU-induced mammary gland tumours in Sprague Dawley rats, one study used freshly powdered turmeric rhizomes [12]. They discovered that oral administration of turmeric as a pre-induction treatment increased anti-cancer action in a dose-dependent way. Limited research has been conducted to explore the potential of turmeric in the prevention of blood disorders, specifically leukaemia and lymphoma. Hence, it is imperative to conduct the current investigation in order to examine the potential preventive impact of turmeric consumption on leukaemia and lymphoma triggered by MNU in rats.

The goal of the current study was to determine if crude *C. longa* L. rhizomes can prevent MNU-induced leukaemia in male Sprague Dawley rats by measuring the ratio of Bcl-2 to Bax using a real-time PCR assay and blood smear. The motivation of the present study was to demonstrate the effect of crude *C. longa* L. rhizomes in slowing down the progression of MNU-induced leukaemia in male Sprague Dawley rats by upregulating the Bax transcript and downregulating the Bcl-2 transcript, which decreased the Bcl-2 to Bax ratio. This is by far was the first study done in leukaemia induced Sprague Dawley rats, therapeutic role of turmeric by modulating the expression of Bcl-2 to Bax ratio.

MATERIALS AND METHODS

2.1 Plant Preparation

Fresh rhizomes of turmeric were purchased from a wholesale market (Selangor, Malaysia). The debris and soils were removed. The rhizomes were chopped into slices and set on a tray for drying. The tray was placed into a hot air oven which was set at the optimal drying temperature of 50 - 60ºC [12] and dried overnight to eliminate any excess water and moisture. The dried rhizomes were then ground using a blender until yellow turmeric powder was produced and stored in an airtight container. The container was then placed in a 4ºC refrigerator until further use.

2.2 Ethical statement

All animals used in this experiment were subjected to approval by the Institutional Animal Care and Use Committee (IACUC) from the Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia.

2.3 Experimental Design

The first two weeks were allocated to acclimatise the rats to the new environment. The rats were housed in a colony room with two rats per cage, room temperature of 23 - 27ºC, humidity of 50 -80%, free access to water ad libitum and 12-h light/12-h dark at the Animal House of the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. A total number of 64 adult male Sprague Dawley rats (> 2.5 months old) with an average weight of 200-300 g were divided into four groups (A, B, C and D) using randomised complete block design (RCBD) analysis. All rats in Groups C and D were given a MNU administration by adapting a method from [4]. Groups B and D were treated with 5000 mg/kg of C. longa rhizomes by mixing in their standard diet intake. Group A was designated as the control group and was provided with a regular pellet diet for the duration of the study.. The experiment was conducted for a period of 20 weeks before all the rats were sacrificed.

2.4 N-Methyl-N-Nitrosourea Preparation

N-methyl-N-nitrosourea (MNU) powder was purchased from Sigma-Aldrich, USA. The powdered MNU was freshly dissolved by adding 40 mg into one mL of pH4 normal saline and mixing well by vortex. MNU solution is sensitive to light exposure and is reported to be carcinogenic to humans [14] (IARC, 1988). Thus, the bottle was wrapped with aluminium foil and handled with full care.

2.5 Blood Sampling

Blood samples were taken at week 0, 10 and 20 for blood smear analyses. For week 0 and 10, all the rats were anaesthetised with a combination of 50 mg/kg body weight ketamine and 5 mg/kg body weight xylazine via intramuscular (i.m.) injection. Prior to the presence of leukaemic cells, the blood smears were prepared and stained using Wright staining. The blood smears were covered with Wright stain and left for three minutes. An equal amount of buffer with pH 6.8 was distributed over the entire slide and left for seven minutes. The stain was washed out using tap water, followed by wiping from the back of slide and letting it dry overnight. The blood smears were further examined under a light microscope at 100x, 200x, 400x, 600x and 1000x magnifications. The presences of leukaemic cells were counted for blastic cells absolute value.

2.6 Quantitative RT-PCR in Blood Samples

The total RNA extraction was done using the QIAamp® RNA

Blood Mini Kit (Qiagen, USA). The total RNA was reverse transcribed into cDNA by using the GeneAmp RNA PCR Core Kit (Applied Biosystem, USA). Two house-keeping genes and two target-genes were used in this study, including glyceraldehydes 3-phosphate dehydrogenase (GAPDH), Beta actin, Bax and Bcl-2 [15 – 18]**.** Quantitative real time PCR (qRT-PCR) assay was done by using a real-time RT-PCR machine (Biorad® CFX96, Biorad, USA). Gene expression analysis was done by using the CFX96 ManagerTMSoftware, Version 1.6 (Biorad Laboratories, Inc., Hercules, CA) provided by the manufacturer (Biorad® CFX96, Biorad, USA). In order to conduct the gene expression analysis, the determination of the efficiency of references genes (GAPDH and Beta actin) and genes of interest (Bcl-2 and Bax) must be determined by development of standard curve of each gene. The efficiency of primers were 90.7%, 91.3%, 104.6% and 90.5% for GAPDH, Beta actin, Bcl-2 and Bax, respectively [18]. These efficiencies were later used to analyse the gene transcription. The transcription level of gene interest (Bcl-2 and Bax) in all groups were normalised to the transcription level of reference genes (GAPDH and Beta actin). The normalisation factor was calculated automatically by using the CFX ManagerTM Software (Biorad Laboratories, Inc., Hercules, CA). The formula was as follow:

$$\textbf{Normalisation factor}_{\textbf{sample (GOI)}} = (RQ_{\textbf{sample (ref 1)}} \times RQ_{\textbf{sample (ref 2)}} \times \dots RQ_{\textbf{sample (ref n)}})$$

Note: RQ = Relative quantity, n = number of reference targets, GOI = Gene of interest, Bcl-2 and Bax

The transcription levels of gene interest (Bcl-2 and Bax) were normalised to the transcription levels of a control sample which is contained no treatment (Group A). This normalisation also was calculated automatically by using the CFX ManagerTM Software (Biorad Laboratories, Inc., Hercules, CA). The formula was as follow:

$$\textbf{Normalisation expression}_{\textbf{sample (GOI)}} = \frac{RQ_{sample\,(GOI)}}{(RQ_{sample\,(ref\,1)} \times RQ_{sample\,(ref\,2)} \times \dots RQ_{sample\,(ref\,n)})^{\frac{1}{n}}}$$

Note: RQ = Relative quantity of sample, Ref = Reference target in the experiment (GAPDH and Beta actin), GOI = Gene of interest (Bcl-2 and Bax)

The relative gene expression of Bcl-2 and Bax in the control group was set as 1.0 and the relative gene expression of these interest genes were expressed as fold changes compare to the control group. The ratios of Bcl-2 to Bax in all groups were calculated manually by using the Microsoft Excel (Microsoft, USA). Finally, all results were analysed using SPSS 19.0 statistical software (IBM, USA) and the differences at $p<0.05$ were considered as significant.

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2.7 Statistical analysis

The haematological and gene expression results were presented as mean±SEM and analyzed using a one-way ANOVA with IBM SPSS Statistic 19.0 software.

3.1 MNU-Induced Leukaemia in Rats

The morphology of the leukaemic cells observed in the MNU-induced leukaemic rats is characterised as large round cells with dense nuclear chromatin, multiple nucleoli and scanty basophilic cytoplasm (**Fig. 1A and 1B**). Based on microscopic observation, it was noticeable that there were a high percentage of leukaemic cells in all MNU-treated rats (Groups C and D) at 20 weeks post administration of MNU. **Table 1.** provides information on blastic cell absolute value in those MNU-induced leukaemic rats at Week 0, 10 and 20. Increasing trend of absolute value of leukaemic cells was observed in Group C rats from (0.16±0.07 x 10^9/L) at Week 10 to (0.24±0.05 x 10^9/L) at Week 20. Smaller absolute value was counted in Group D rats, where it gave (0.04±0.02 x 10^9/L) and (0.20±0.04 x 10^9/L), respectively at Week 10 and 20. Potentially due to supplementation of 5000 mg/kg dried *C. longa*. Interestingly, no leukaemic cells were observed in the positive and negative control rats (Group A and B). Therefore, a total dose of 240 mg/kg MNU successfully triggered the proliferation of blast cells which correlated with development of leukaemia in those rats. Those absolute value in Group C and D, respectively were statistically different as compared to control rats where (Group A and B) $p<0.05$.

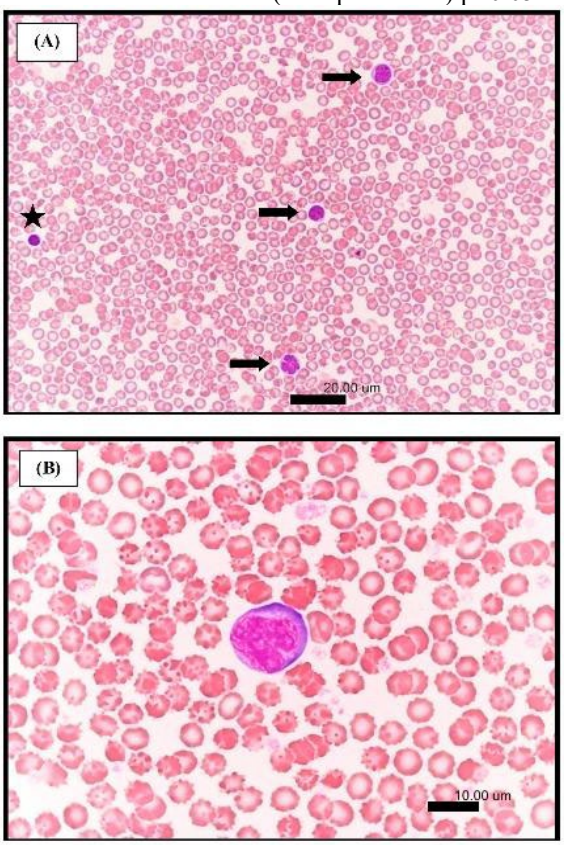

Fig. 1A and 1B. (A) Photomicrograph of leukaemic cells (20µm) in Group C. The star depicts a normal lymphocyte and arrows depict a few large leukaemic cells. (B) Higher magnification of (A) shows one of the leukaemic cells (10µm) with larger size and scanty basophilic cytoplasm.

RESULTS AND DISCUSSION

Table 1. Leukaemic cell count in blood smears at weeks 0, 10 and 20.

Group	Blast cell count (10^9/L)		
	Week 0	Week 10	Week 20
A	0	0	0
B	0	0	0
C	0	0.16±0.07[b]	0.24±0.05[b]
D	0	0.04±0.02[a]	0.20±0.04[b]

Means±SEM were observed by week and different superscript at the same column were significantly different at $p<0.05$.

By employing a technique identical to that used by Hutheyfa et al. [4], the present investigation successfully caused 100% leukaemia in the 16 rats in Group C. Blast cells are present in most leukaemia cases including lymphoblasts and myeloblasts. In a different investigation, Ross et al. [19] tested the expression of the breast cancer resistance protein in blast cells from acute leukaemia patients. As a result, the blast cell is suited for use as a biomarker for the prognosis of blood diseases. The blast cells found in Group C in the current study were described as big cells with cytoplasmic azurophilic granules and a thick chromatin nucleus. Other investigations that described the blast cells as having a range of 12 to 16 µm in diameter, a spherical nucleus with coarsely clumped chromatin, abundant sky-blue cytoplasm, and frequently including a few fine azurophilic granules by Rozenberg G.[20] which complement in the present study findings.

3.2 The Effect of Dried C. Longa on Blood Smears

Based on blast cell incidence, the percentages of leukaemic rats of MNU-treated groups (Groups C and D) were determined and shown in **Fig. 2**. A total of sixteen rats (100%) in Group C were diagnosed with leukaemia due to abundant incidence of blast cells in each rat. Whereas there was a slight reduction in the number of leukaemic rats in Group D, which were given 5000 mg/kg of dried *C. longa* supplementation. It also can be seen in **Fig. 2** that a small percentage of rats in Group D (12 % out 16 rats) successfully escaped from getting leukaemia. Those high percentages of leukaemic rats in Group C and D respectively, were shown huge differences between Group A (positive control) and Group B (negative control), where none of blast cells were observed in those control rats. Thus provide the evidence of MNU carcinogenic properties to induce leukaemia in rats.

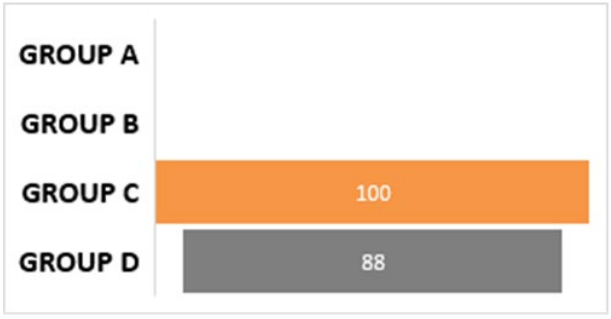

Fig. 2. The percentage of leukaemic rats in MNU-treated model (Group C and D) at week 20 via the presence of blast cells in those rats.

C. longa is safe to use for medical purposes. According to previous study, giving rats dried C. longa rhizomes at a dose of 500 mg/kg daily for 28 days had a hepatoprotective effect against lead-induced toxicity by Baxla et al. [21]. In the current investigation, rats were administered dried C. longa rhizomes at a dose of 5000 mg/kg body weight daily, and the serum biochemistry and histopathology revealed no harmful effects (data not shown). Additionally, leukaemia occurrences in Group D rats (88%) were lower than those in Group C (100%) after receiving daily supplements of dried C. longa rhizomes. As a result, although the incidence of leukaemia in rats was only marginally decreased, daily supplementation of dried C. longa rhizomes at 5000 mg/kg body weight dramatically decreased the incidence of the disease.

3.3 The Effect of Dried C. Longa on the Expression of Bcl-2 to Bax Ratio via qRT-PCR Assay

The bar chart provides information on the expression of Bcl-2 to Bax ratio, as shown in **Fig. 3**. In this study, the expression of Bcl2 to Bax ratio in Group A was assigned as positive control (3.17±1.07) which was fixed to 1-fold. By comparison, there was a significant increase in the expression of Bcl-2 to Bax ratio in MNU-induced leukaemic rats (Group C) compared to Group A. This represents the highest expression of Bcl-2 to Bax ratio among the groups, as it increased to 3.3-fold (10.50±1.26) in the blood of those rats in Group C. It is also noticeable that the expression of Bcl-2 to Bax ratio in Group D rats was insignificantly different from the rats in Group A. There was only a slight 1-fold (3.58±0.82) increase of the expression of Bcl-2 to Bax ratio compared to the control rats in Group A. Most importantly, the expression of Bcl-2 to Bax ratio in Group D was significantly lower than Group C which suggested that *C. longa* plays a role in inhibiting the proliferation of circulating leukaemic cells through downregulation of the Bcl-2 to Bax ratio.

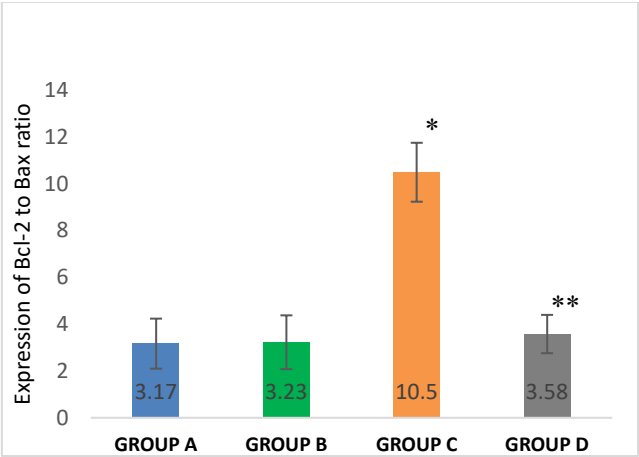

Fig. 3. The ratio of Bcl-2 to Bax transcription levels in the blood of rats in each group at week 20 post MNU administration. Means±SEM were observed by group.
*Bar chart with the asterisk was significantly different at $p\leq0.05$ compared to Group A.
** Bar chart with the two asterisk was significantly different reduced at $p\leq0.05$ compared to Group C

Additionally, the outcomes of this study's RT-PCR assay supported the outcomes from the blood smear, which revealed the incidence of leukaemia. In order to observe the regulation of anti-apoptotic genes over the pro-apoptotic genes in cancer investigations, the expression of the Bcl-2 to Bax ratio is calculated. High levels of Bcl-2 have been hypothesized to support the growth of cancer cells by inhibiting the apoptotic pathway from previous studies by Shabana et al., Karademir et al., Zhou et al., Valko et al., Adamo et al. [22 – 26]. This is consistent with the study's molecular findings, which showed that the expression of the Bcl-2 to Bax ratio was significantly higher in the blood of leukemic rats (Group C) than in the control group (3.17 to 1.07), increasing to 3.3-fold (10.50 to 1.26).

Numerous studies have used the expression of the Bcl2 to Bax ratio as a treatment response. Apoptosis caused by chlorambucil was utilized in one study by Zhou et al. [24] to predict how untreated chronic lymphocytic leukaemia (CLL) patients would react to cytotoxic treatment. Del Poeta et al. [27] also tested the expression of the Bax to Bcl-2 ratio to identify patients with de novo acute myeloid leukaemia (AML) who would spontaneously undergo apoptosis. In order to forecast the chemo-resistance response, the ratio represents a possible indication of clinical outcome and targets in pro-apoptotic molecules was followed by Pepper et al. [28]. Additionally, other research has shown that the activation of the apoptotic pathway in human myeloid leukaemia cells in vitro and myasthenia gravis patients can make the Bax to Bcl-2 ratio a predictive marker for therapeutic responses by Liu etal., Salakou et al. [29 -30]. In the current investigation, rats in Group D fed with 5000 mg/kg dried C. longa rhizome exhibited significantly lower blood levels of the Bcl2 to Bax ratio than rats in Group C ($p<0.05$). Furthermore, there was no discernible difference between the rats in Group D and the control group in terms of the expression of Bcl-2 to Bax ratio transcripts, which had only increased by a factor of one (3.58±0.82).

According to reports, the chemical ingredient in C. longa, curcumin, which can block Bcl-2 at high levels while simultaneously promoting the production of Bax to initiate the apoptotic pathway, is what gives the plant its anti-proliferative properties. This result was consistent with a study by William et al. [31], which demonstrated curcumin's anti-proliferative effects on leukemic cells expressing wild-type or T315I-BCR-ABL by inducing apoptosis and increasing the survival of mice with acute lymphoblastic leukaemia (ALL). The ar-turmerone extract from C. longa's stimulation of apoptosis in human leukemia cells line (Molt 4B and HL-60) was also confirmed by a different investigation from Aratanechemuge et al. [32]. Additionally, Chendil et al. [33] suggested the C. longa extract as an independent factor in the prosurvival of gene expression in the prostate cancer line PC-3 and highlighted the expression of the Bcl-2 to Bax ratio as a radio sensitizing indication. The expression of Bcl-2 to Bax ratio may change due to the lowering of Bax transcription, which may also block the apoptotic pathway. The qRT-PCR test has a good sensitivity for detecting Bcl-2 expression in rats with leukaemia brought on by MNU. Bcl-2 levels in patients with chronic lymphocytic leukaemia (CLL) were previously identified by Kaluni et al. [34]. By considerably extending survival in mice and causing a rapid loss of leukaemia cells, Bcl-2 has been explicitly validated as a reasonable target for cancer therapy based on Salavatipour et al. [35].

Curcumin modulates the process of apoptosis, which refers to programmed cell death, through the regulation of specific proteins within the BCL-2 family based on Abdel-Hakeem et al. [36]. BCL-2 and BAX are two crucial constituents of this gene family, exhibiting contrasting functions in the determination of cellular destiny. The downregulation of the anti-apoptotic protein BCL-2 by curcumin has been shown to decrease its inhibitory impact on cell death. Concurrently, curcumin enhances the upregulation of the pro-apoptotic protein BAX, hence amplifying its involvement in facilitating cellular apoptosis. The simultaneous regulatory effect shown here leads to a modification in the equilibrium between BCL-2 and BAX, favouring apoptosis and so promoting the activation of the programmed cell death pathway. The molecular alterations mentioned highlight the potential of curcumin as a therapeutic intervention in the treatment of cancer, specifically in cases of leukemia. In this context, it is essential to maintain a proper equilibrium of these proteins to effectively regulate aberrant cell growth and viability.

It has been suggested that it has a role in the initiation of caspase activation, a crucial mechanism involved in programmed cell death, also known as apoptosis. Caspases, a group of protease enzymes, are extensively involved in the process of apoptosis by facilitating the degradation of cellular constituents. Research findings indicate that curcumin has the ability to induce the activation of caspases by Hu et al. [37], specifically caspase-3 and caspase-9, which play crucial roles in the apoptotic pathway. The initiation of these caspases triggers a sequence of enzymatic processes that culminate in the systematic breakdown of cellular components, ultimately leading to the regulated demise of the cell. Curcumin facilitates the activation of caspases, thereby playing a role in the coordination of apoptotic pathways. This mechanistic understanding sheds light on its anti-cancer attributes and its potential as a therapeutic intervention in contexts where the control of programmed cell death is of utmost importance, such as in the treatment of leukemia.

Memarzia et al. [38] summarized *C.longa* extracts functioning as an anti-inflammatory drug, regulates multiple molecular pathways by suppressing the function of pro-inflammatory mediators and transcription factors. This encompasses the suppression of cytokines, enzymes, and adhesion molecules that are implicated in the inflammatory response. Moreover, curcumin has robust antioxidant characteristics through the process of scavenging free radicals and augmenting the efficacy of natural antioxidant enzymes, reviewed by Cheng et al. [39]. Curcumin plays a role in the prevention of chronic inflammatory disorders and the subsequent cellular damage by reducing inflammation and oxidative stress. The simultaneous impact on inflammation and oxidative stress holds significant importance within the realm of cancer, since chronic inflammation and heightened oxidative stress play a role in both the onset and advancement of the disease.

Curcumin disrupts the delicate balance between pro- and anti-apoptotic proteins in mitochondria, which regulate apoptosis. By causing mitochondrial malfunction, curcumin can release pro-apoptotic substances like cytochrome c into the

cytoplasm as discussed by Zheng et al. [40]. The caspase cascade is activated by this release. Curcumin may also inhibit mitochondrial membrane anti-apoptotic proteins, causing apoptosis. This mitochondrial imbalance favors programmed cell death, making it a key mechanism by which curcumin fights cancer.

In this work, the researchers utilized dried rhizomes of *C. longa* and administered a dosage of 5000 mg/kg to rats with MNU-induced lymphoma and leukaemia. This dosage corresponds to a curcumin content of 0.61% - 1.45% by Garg et al. [41]. The objective was to initiate the apoptotic pathway. Regarding the body weight and feed intake within the specified groups, it was observed that the rats exhibited acceptance and consumption of the mixed meal including dried *C. longa* rhizomes. In this investigation, a range of curcumin content spanning from 365 mg to 1050 mg was utilized. Significantly, no harmful effects were found in Group B rats with a dose of 5000 mg/kg dried *C. longa* rhizomes, as evidenced by the absence of mortality. The rats in Group B exhibited typical behaviour and demonstrated appropriate weight increase over the course of the 20-week experiment.

In comparison to rats given MNU to generate leukaemia, rats fed with dried *C. longa* rhizome had a somewhat lower percentage of leukaemic rats, according to blood smear analysis. Furthermore, it is noteworthy that the observed reduction in the presence of blast cells in Group D, when compared to Group C at week 20, albeit not significantly different. Additionally, the quantitative RT-PCR assay revealed a significant decrease in the expression of the Bcl2 to Bax ratio in rats with leukaemia when a diet supplemented with dried *C. longa* rhizome was administered. Based on these findings, we can conclude that the daily supplementation of dried *C. longa* for a period of 20 weeks effectively decelerated the progression of carcinogenesis in rat models of leukaemia.

Curcumin, which is the main ingredient in turmeric, shows promise as an extra or alternative treatment for cancer patients. Curcumin may help fight cancer by changing important molecular processes that control tumor growth, cell death, and inflammation, according to many studies. Turmeric is a powerful cancer-fighting substance because it can change the BCL-2 to BAX ratio, activate caspases, control mitochondrial activity, and reduce inflammation and free radicals. Although it's important to remember that turmeric is not a cure-all for cancer, it is interesting to think about how it could be used in addition to other treatments. Turmeric is generally safe, and it has been used in traditional medicine for a long time. These reasons make it worth thinking about as an extra way to help cancer patients get better. But strong clinical trials and more study are needed to find the best doses and treatment plans, as well as to prove that it works and is safe for many types of cancer, including leukemia. Using turmeric as part of a full cancer treatment plan might help with a more complete way of treating cancer. Before adding turmeric or curcumin supplements to their cancer treatment plan, patients should talk to their doctors to make sure they will work with their general treatment plan.

FUTURE RECOMMENDATION

While curcumin has intriguing anti-cancer capabilities, its limitations limit its clinical usefulness. Curcumin has low bioavailability due to poor absorption and quick metabolism. For optimal systemic availability, nanoformulations or co-administration with absorption enhancers are essential. Curcumin may also affect normal cells because it doesn't target cancer cells. Developing targeted delivery techniques could reduce off-target impacts. Clinical translation of curcumin's preclinical success requires defining optimal dosage, treatment duration, and long-term safety in varied patient populations. To determine curcumin's efficacy across leukaemia's many subtypes, subtype-specific studies are needed. To maximize curcumin's leukaemia treatment potential, these restrictions must be addressed.

Curcumin research should address many issues to enhance its medicinal effects. Explore nanoparticle formulations or innovative delivery technologies to boost curcumin's bioavailability for greater absorption and sustained release. Meanwhile, focused delivery strategies can improve specificity and reduce off-target effects on healthy cells. To prove curcumin's efficacy and safety in varied leukaemia patient populations, rigorous clinical trials must address dosage, treatment duration, and side effects. Combining curcumin with other anti-cancer drugs may improve therapeutic efficacy. Finally, more mechanistic investigations are needed to determine curcumin's anti-leukemic molecular pathways and design more targeted and effective treatments. This thorough research method will help curcumin cure leukemia to its full potential.

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CONFLICT OF INTEREST

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

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