



Assessment of Chemoprotective Potential of Curcumin against DMBA-Croton Oil Induced Skin Cancer in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author RK has designed the study. Author SS has performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KKC and GKS have managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1130299

Editor(s):

- (1) Dr. Prem K. Ramasamy, Brandeis University, USA.
- (2) Dr. Sabyasachi Chatterjee, Burdwan University, India.
- (3) Prof. Marcello Iriti, University of Milan, Italy.

Reviewers:

- (1) A. Ranganadha Reddy, Vignan's Foundation for Science, Technology & Research (Deemed to be University), India.
- (2) Tejo Jayadi, Duta Wacana Christian University, Indonesia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/58948>

Original Research Article

Received 23 May 2020
Accepted 28 July 2020
Published 10 August 2020

ABSTRACT

Background: Skin cancer is the most aggressive form of cancer with a high mortality rate. Different medications of skin cancer are accessible, yet because of improvement of multi-drug resistance, flow and rising chemotherapies have a generally low achievement rate. This emphasize the importance of discovering new compounds that are both safe and effective against skin cancer. This study used and compared different routes of administration of a natural compound curcumin to investigate the anti-cancer effect.

In this investigation it has been resolved that the curcumin expands CAT and SOD levels alongside decline in the TBARS levels.

Methods: The hindrance of tumour rate Curcumin was assessed in mice on two phase procedure of skin carcinogenesis incited by single utilization of DMBA/7,12-Dimethylbenz[a]-anthracene (100

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µg/100 µl of CH₃)₂CO), and after 2 weeks advanced by rehashed use of croton oil (1% CH₃)₂CO/thrice in seven days) till the finish of the trial (i.e. 16 weeks). Oral administration of drug at a dose of 500 mg/kg body weight/day at the pre-initiation stage (i.e. 7 days prior and 7 days after DMBA application), promotional stage (for example from the time of croton oil application) and at both the stages (i.e. 7 days before DMBA application and proceeded till the finish of investigation) to the mice Treatment were started 1 week before the exposure to the carcinogen and continued till 25 weeks daily. At the end of experimental period all the animals were sacrificed and observed for various parameters.

Results: The following parameters (body weight, biochemical studies, haematological studies) were observed and data was collected time to time and calculated statistically to evaluate the anticancer effect of curcumin was found to effective against the skin cancer.

Keywords: Carcinogenesis; skin cancer; DMBA; croton oil; curcumin.

ABBREVIATIONS

DMBA : 7,12-Dimethylbenz[a]Anthracene

DNA : Deoxy Ribose Nucleic Acid

WBC : White Blood Cells

RBC : Red Blood Cells

Hb : Haemoglobin

OD : Optical Density

MDA : Malondialdehyde

H₂O₂ : Hydrogen Peroxide

TBARS : Thiobarbituric Acid Reactive Substances

CAT : Catalase

SOD : Superoxide Dismutase

ANOVA: Analysis of Variance

mismatching of bases and chromosome deletions and rearrangements. Hence, the antioxidants which can quench these free radicals could act as cancer chemo-preventive agents [3].

Skin cancer is the most common form of human cancer if melanoma, basal and squamous cell skin cancers are included. The yearly paces of all types of skin malignant growth are expanding every year, speaking to a developing open concern. Based on the Cancer Trends Progress Report by National Institute of Health of United States (NIH) [4], it is estimated that nearly half of all Americans who live to age 65 will develop skin cancer at least once [5].

1. INTRODUCTION

Now these days Cancer is a major public health burden in both developed and developing countries. It was evaluated that there were 10.9 million new cases, 6.7 million passings, and 24.6 million people living with malignancy around the globe in 2002. The National Cancer Institute gathered around 35,000 plant tests from 20 nations and has screened around 114,000 concentrates for anticancer action. Of the 92 anticancer medications monetarily accessible preceding 1983 in the US and among overall affirmed anticancer medications somewhere in the range of 1983 and 1994, 60% are of natural origin [1,2].

Oxygen free radicals are formed in tissue cells by many endogenous and exogenous influences like cellular metabolism, exposure to chemicals, carcinogens and ionizing radiation. Oxygen free radicals may assault all macromolecules in cell (proteins, lipids and DNA) offering ascend to a wide assortment of damaged products. Free radicals cause DNA double strand breaks,

Dangerous melanoma, the deadliest type of skin malignant growth, is one of the most quickly expanding diseases on the planet. This important issue has led researchers at non-profit academic organizations to reflect upon alternatives for cancer drug development [6,7].

Turmeric, the powdered rhizome of *Curcuma longa* (Family- Zingiberaceae), is extensively used as a spice in curries and mustards, is often responsible for their distinct colour (Synonym- Indian Saffron), and contributes a lot to their flavour because of the nearness of its oleoresins and essential oil. It is a spice that has long been used to enhance the flavour of foods in the form of "curry leaf or powder" [8].

Literature review revealed that curcumin (diferuloylmethane), the main yellow bioactive component of turmeric, possesses wide spectrum of biological properties including anti-inflammatory, antioxidant, anticarcinogenic and antimutagenic, antiulcer, and antiprotozoal, hypotensive and hypocholesteremic activities [9].

It is Perennial herb, cultivated extensively in south and southeast tropical Asia, prescribed abundantly for ailments in both traditional Chinese and Indian medicine [10].

1.1 Chemical Constitutes

Curcumin, demethoxycurcumin and bisdemethoxycurcumin collectively known as curcuminoids (3-6%) are major polyphenolic compounds in turmeric rhizomes [11]. Curcuminoids (mostly curcumin) and essential oils (primarily monoterpenes) are the major bioactive constituents showing different bioactivities. Calebin-A, vanillic acid, vanillin, quercetin, and other phenolic compounds have also previously been identified from turmeric [12].

The mechanism of action of natural as well as synthetic curcuminoids has been predicted through their antioxidant activity [13].

Their scavenging activities against a variety of reactive oxygen species including superoxide anion radicals and nitrogen dioxide radicals are predominant. They are also inhibitors of lipid peroxidation in different animal models [14].

A few reports say that Curcumin shows the antioxidant which is significant system in the treatment of cancer [15,16].

This rising interest in the anticancer and antioxidant activities of the existing natural drugs has led us to investigate the DMBA/Croton oil induced skin cancer study of extract of curcumin.



Fig. 1. Longa rhizomes (Fresh, dried and powder form)

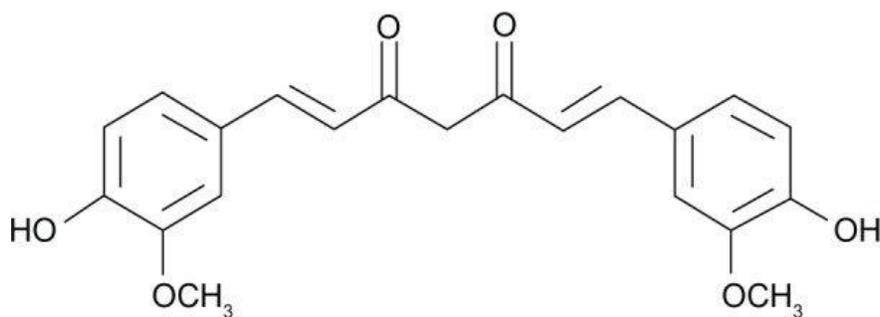


Fig. 2. Curcumin chemical structure

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Curcumin drug powder were purchased from Clinbio Pvt. Ltd. Bangalore, India and were identified by Dr. Raju Koneri, Dean & Head of the Department of Pharmacology. A few vouchers were submitted to the Herbarium for future references (voucher number- KCP/15-16/0554) was given. The powder was stored in an airtight container and protected from light.

2.2 Animals

Eight to ten weeks old Swiss albino mice having weight (25-30 gm) were purchased from Karnataka veterinary college, Hebbal, Bangalore, India. They were housed, six per poly propylene cage under standard laboratory conditions at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with 12 h light / dark cycle. The animals were provided with pellet chow and water ad libitum.

2.3 DMBA/Croton oil induced Skin Cancer Model (In-vivo model)

2.3.1 Chemicals

7,12-Dimethylbenz[a]-anthracene and croton oil were purchased from Sigma Chemical Pvt. Ltd. Bangalore India. WBC and RBC diluting fluids from Hi-Media Labs Mumbai, and 10% formalin from Karnataka chemicals Ltd.

2.3.2 Equipment

Neubauer Chamber, WBC pipette, cover slip, surgical needles, syringes, gloves.

Depilatory cream was applied to remove hair from the back (dorsal surface) of each mice and the mice were left untreated for two days. Mice having no hair development following two days were chosen for the examination.

Test drug treatment were started one week before to the exposure to carcinogen and continued for the 25 weeks daily.

A solitary topical use of 7,12-Dimethylbenz[a]-anthracene (100 $\mu\text{g}/100 \mu\text{l}$ of CH_3CO) followed 2 weeks after the fact by rehashed utilization of croton oil (1% in CH_3CO_3 times each week). The Mice were partitioned into six gatherings (each gathering of 6 mice), as follows:

Food and water were withheld 18 hours before sacrificing the animals. after 25 weeks, the animals in each group were sacrificed.

The anticancer activity of curcumin was screened with respect to the following parameters:

3. PARAMETERS FOR ESTIMATION OF ACTIVITY

3.1 Body Weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

3.2 Effect on Haematological Parameters

At the end of the experimental period, 6 mice of each group from whom Blood was collected by Retro-orbital route the next day after an overnight fastened used for the estimation Haemoglobin (Hb%) content, red blood cell count (RBC) [17] and white blood cell count (WBC) [18].

3.3 Biochemical Estimation

3.3.1 Lipid peroxidation assay

Levels of lipid peroxides were evaluated utilizing the technique for Ohkawa et al. [19]. Quickly, thiobarbituric acid (0.8%), sodium dodecyl sulfate (0.1%) and acetic acid (20%) were added to 100 ml of the tissue homogenate (10%) arranged as portrayed previously. This blend was warmed for 30 min, cooled, extricated with N-butanol-pyridine, and the OD of MDA (Malondialdehyde) recorded at 532 nm. The substance of MDA is communicated as nmol/mg protein.

3.3.2 Catalase (CAT) assay

This was assayed by the method of Aebi[20]. The change in absorbance was followed spectrophotometrically at 240 nm after the addition of H_2O_2 (30 mM) to 100 ml of the supernatant (of 10% tissue homogenate obtained as described above) in 50 mM phosphate buffer (pH 7). The activity of the enzyme is expressed as U/mg of protein, where 1 U is equivalent to 1 mol of $\text{H}_2\text{O}_2/\text{mg}/\text{min}/\text{mg}$ protein.

3.3.3 SOD assay

This was determined by the method of Misra and Fridovich [21] based upon the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH. An aliquot of 40 ml of the supernatant of 10% tissue homogenate, obtained as described above, was taken in 0.1 M carbonate buffer (pH 10.2). After mixing

epinephrine, the expansion in absorbance was estimated at 480 nm. The activity of the enzyme is expressed as U/mg of protein, where 1 U of the enzyme is defined as the amount of enzyme required to inhibit the rate of epinephrine auto-oxidation by 50% under the conditions of the assay.

3.4 Statistical Analysis

Results have been reported as mean value \pm SEM.

The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Graph pad prism 6.0).

4. RESULTS

In vivo data in DMBA/Croton oil induced skin cancer model

4.1 Effect on Body Weight

The underlying normal body weight of the typical control was approx.25.50 gm and ailment control were approx. 21.20 gm which has expanded up to 29.80 gm. what's more, 34.30 gm as conclusive normal body weight individually because of development of tumors. In all the treatment bunches change in normal body loads shows that curcumin is powerful as tumor silencer in different courses of administration patterns.

4.2 Effect on Haematological Parameters

In normal control the normal complete WBC check was $9.41 \text{ cells/ml} \times 10^3$, normal all out RBC tally $11.30 \text{ cells/ml} \times 10^6$ and normal Hb 15.30 gm/dl, in sick control these boundaries were $23.10 \text{ cells/ml} \times 10^3$, $3.01 \text{ cells/ml} \times 10^6$ and 5.79 gm/dl individually. In all the treatment bunches change in haematological boundaries are toward recuperation.

4.3 Effects on Biochemical Parameters

The degrees of the diverse biochemical boundaries as normal TBARS, SOD and CAT in normal control the 4.96 m mol/mg, 9.09 u/mg and 55.60 u/mg separately while diseased control show indistinguishable boundaries from 10.80.96 m mol/mg, 2.63 u/mg and 40.55 u/mg. different gatherings shows the information close to typical.

4.4 Graphical Presentation of Data

Graph. 4.4.1 (A & B). Shows deviation of initial & final body weight and Graph. 4.4.2 (A, B & C). Shows deviation of haematological parameter (RBC, WBC & Hb) of the animal. Graph. 4.4.3 (A, B & C). Shows deviation of biochemical parameter (TBARS, SOD & CAT) of DMBA/Croton oil induced skin cancer model.

4.5 Pictographically Representation

Fig. 1 shows a Pictograph indicating impacts of medication on creatures of the considerable number of gatherings which was taken in each multi week.

5. DISCUSSION

In numerous malignant growths investigates, it has been discovered that the biochemical boundary like TBARS increments and CAT, SOD, GPx, GSH diminishes in disease prompted creatures. In the above investigation, curcumin rewarded creature information diminished TBARS and expanded other cancer prevention agent levels.

The body weight of creatures in sick benchmark group will increment because of tumor arrangement and in this analysis the body weight of DMBA/Croton oil rewarded creatures was found to expanded. curcumin rewarded diminished the body weight close to typical qualities, oral + skin curcumin organization created all the more better outcomes contrast with just oral and skin, anyway just oral delivered preferred outcomes over just skin sedate organization.

Table 1. DMBA/Croton oil induced skin cancer Model

Group I	Normal control (healthy animals)
Group II	Disease control (croton oil -1% in acetone 3 times a week).
Group III	Treated with test drug (500 mg/kg - oral administration with croton oil)
Group IV	Treated with test drug (500 mg/kg - topical application with croton oil)
Group V	Treated with test drug (500 mg/kg -oral+ topical application with croton oil)
Group VI	Only DMBA/Croton oil for 1 month– than treatment started with test drug (500 mg/kg – oral + topical application with DMBA)

Table 2. Effect of curcumin (500 mg/kg/day/25 weeks) on body weight of skin cancer induced by DMBA/Croton oil in mice

Sl. no.	Group	Avg initial body weight in (gm)	Avg final body weight in (gm)
1.	Normal control	25.50 ± 0.18	29.80 ± 0.20
2.	Disease control (only DMBA/Croton oil)	21.00 ± 0.20***	34.30 ± 0.22***
3.	DMBA/Croton oil+ Curcumin (oral- 500 mg/kg)	19.40 ± 0.12***	29.54 ± 0.22***
4.	DMBA/ Croton oil + Curcumin (topical-500 mg/kg)	23.31 ± 0.09***	31.43 ± 0.19***
5.	DMBA/ Croton oil + Curcumin (oral+topical-500 mg/kg)	22.54 ± 0.06***	27.70 ± 0.19***
6.	Only DMBA/ Croton oil for 1 month–than treatment started with test drug (500 mg/kg – oral + topical application with DMBA)	24.20±0.12***	31.56 ± 0.14***

Values are expressed as Mean ± SEM, (n= 6 mice in each groups). Significant difference in each group versus the control were observed: ***P<0.05

Table 3. Effect of curcumin (500 mg/kg/day/25weeks); on various Haematological parameters of skin cancer induced by DMBA/Croton oil in mice

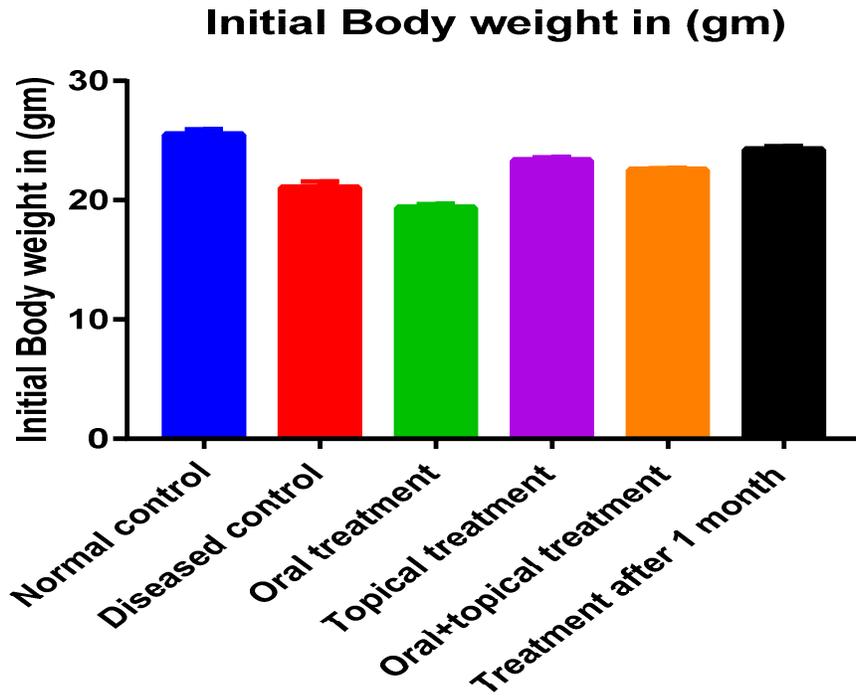
Sl no.	Group	Avg Total WBC count in (cells/ml×10 ³)	Avg RBC Count in (cells/ml×10 ⁶)	Avg Hb content (gm/dl)
1.	Normal control group	9.41 ± 0.13	11.30 ± 0.12	15.30 ± 0.16
2.	Disease control (only DMBA/Croton oil)	23.10 ± 0.29***	3.01 ± 0.07***	5.79 ± 0.07***
3.	DMBA/Croton oil + Curcumin (oral-500 mg/kg)	14.20 ± 0.18***	7.17 ± 0.07***	11.54± 0.11***
4.	DMBA/Croton oil + Curcumin (topical-500 mg/kg)	19.51 ± 0.17***	6.09 ± 0.08***	8.84 ± 0.13***
5.	DMBA/Croton oil +Curcumin (oral+topical-500 mg/kg)	12.70 ± 0.12***	8.71 ± 0.08***	13.00± 0.09***
6.	Only DMBA/ Croton oil for 1 month–than treatment started with test drug (500 mg/kg – oral + topical application with DMBA)	15.67 ± 0.19***	7.02 ± 0.07***	10.66 ± 0.14***

Values are expressed as Mean ± SEM, (n= 6 mice in each groups). Significant difference in each group versus the control were observed: ***P<0.05

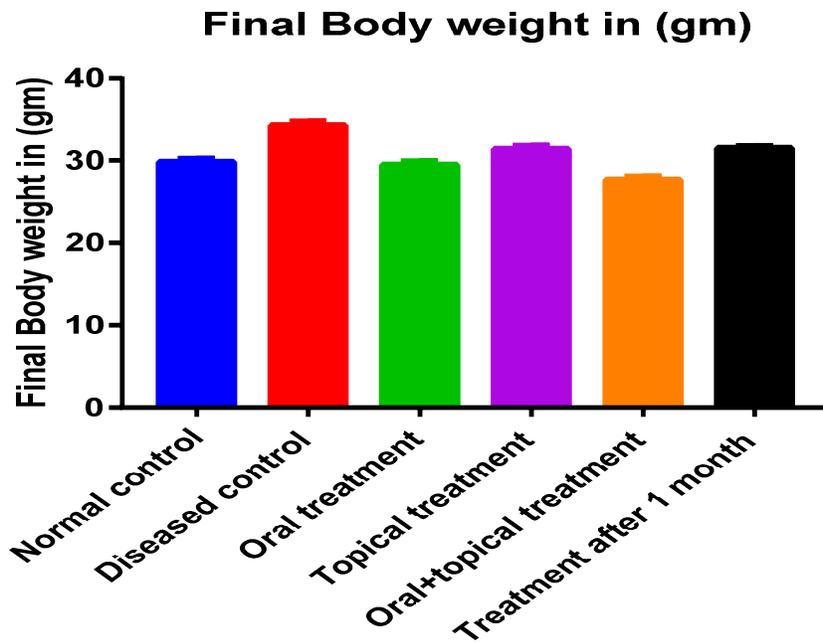
Table 4. Effect of curcumin (500 mg/kg/day/25weeks); on various Levels of TBARS, SOD and CAT antioxidants of skin cancer induced by DMBA/Croton oil in mice

SI No	GROUP	TBARS Avg (m mol/mg)	SOD Avg (u/mg)	CAT Avg (u/mg)
1.	Normal control group	4.96±0.25	9.09±0.10	55.60±0.07
2.	Disease control (only DMBA/Croton oil)	10.80±0.23 ^{###}	2.63±1.10 ^{###}	40.55±0.0 ^{###}
3.	DMBA/Croton oil +Curcumin (oral- 500 mg/kg)	8.17±0.08 ^{***}	5.52±0.07 ^{***}	50.00±0.03 ^{***}
4.	DMBA/Croton oil + Curcumin (topical- 500mg/kg)	9.18±0.15 ^{***}	4.82±0.06 ^{***}	44.10±0.03 ^{***}
5.	DMBA/Croton oi l+ Curcumin (oral+topical-500 mg/kg)	7.28±0.30 ^{***}	7.93±0.01 ^{***}	51.50±0.06 ^{***}
6.	Only DMBA/Croton oil for 1 month–than treatment started with test drug (500 mg/kg–oral+topical application with DMBA)	8.63±0.20 ^{***}	5.83±0.12 ^{***}	49.70±0.13 ^{***}

Values are expressed as Mean ± SEM, (n= 6 mice in each groups). Significant difference in each group versus the control were observed: ***P<0.05

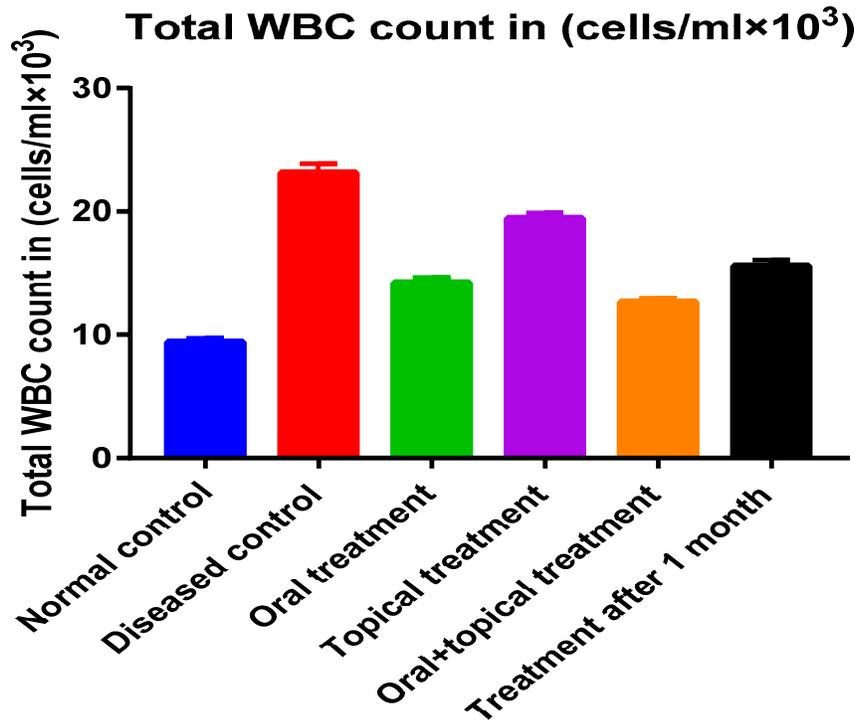


Graph 4.4.1A

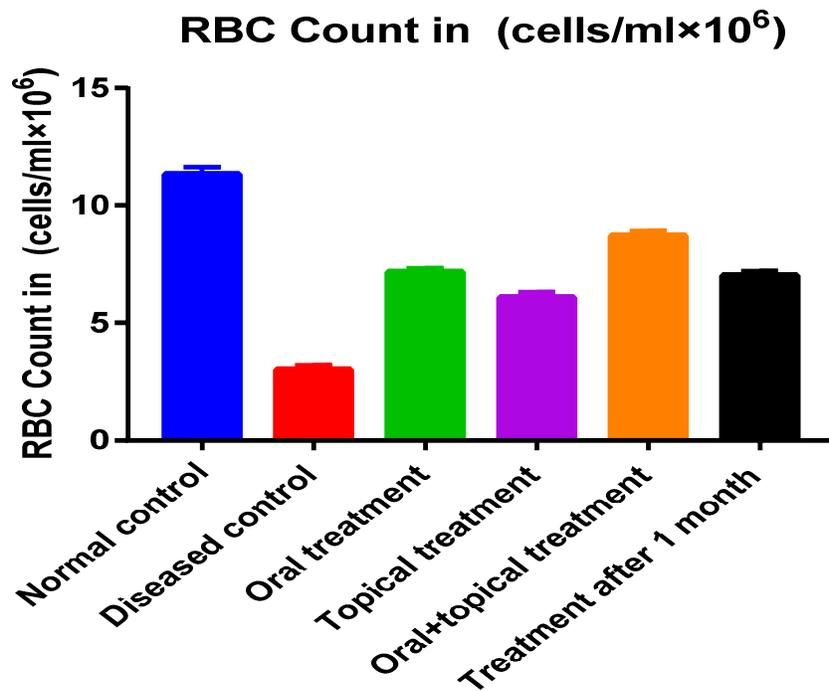


Graph 4.4.1B

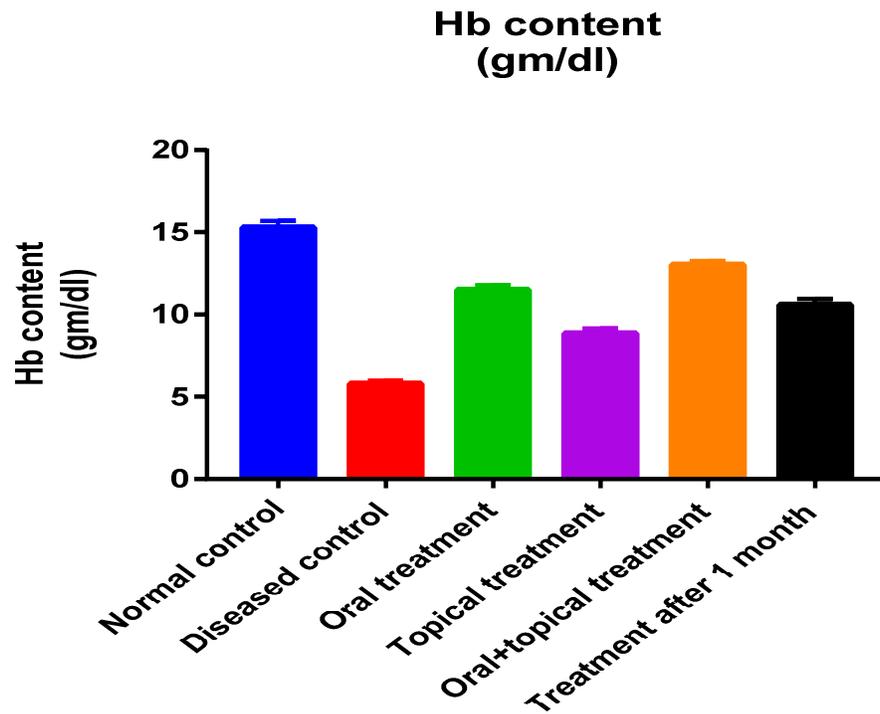
Graph. 4.4.1(A & B). Shows deviation of initial & final body weight of DMBA/Croton oil induced skin cancer model



Graph 4.4.2A

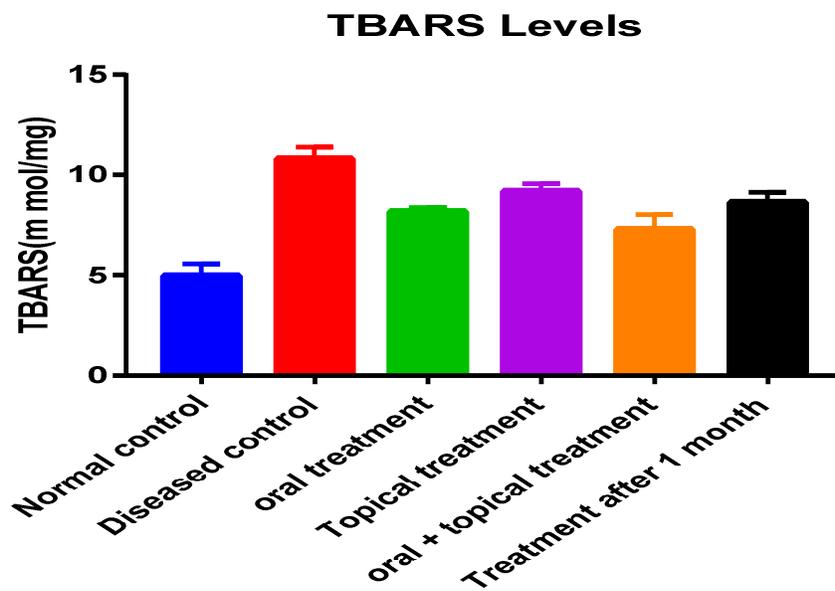


Graph 4.4.2B

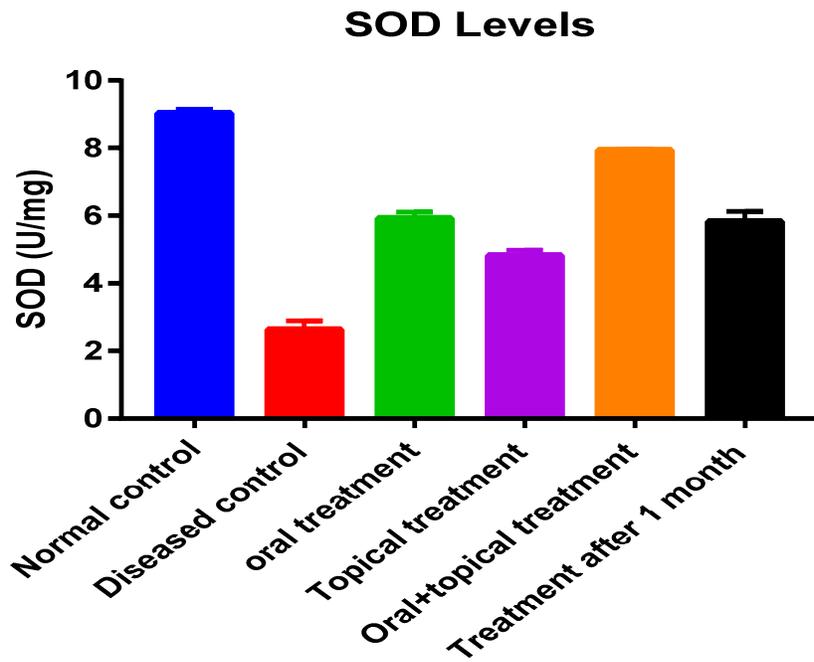


Graph 4.4.2C

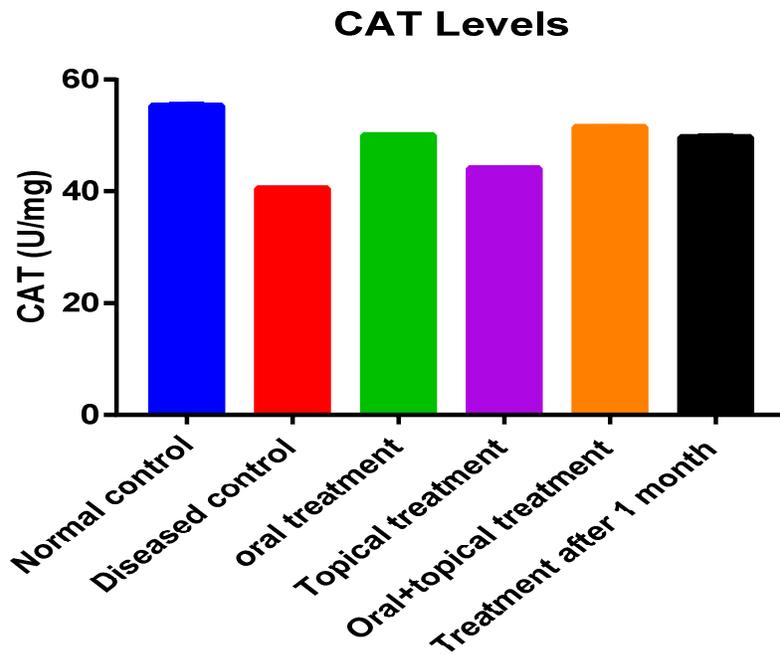
Graph. 4.4.2(A,B & C). Shows deviation of haematological parameter of DMBA/Croton oil induced skin cancer model



Graph 4.4.3A



Graph 4.4.3B



Graph 4.4.3C

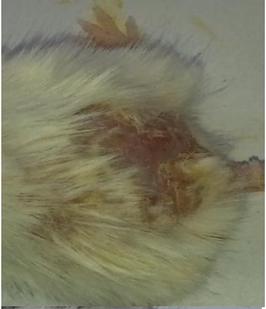
Graph. 4.4.3(A,B & C). Shows deviation of biochemical parameter of DMBA/Croton oil induced skin cancer model

Groups

GP-I
Normal



GP-II
DMBA/
Croton oil



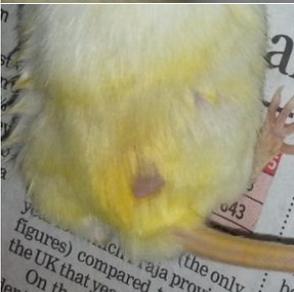
GP-III
Oral
treated



GP-IV
Topical
treated



GP-V
Oral+
topical



GP-VI
1 month
after
treated

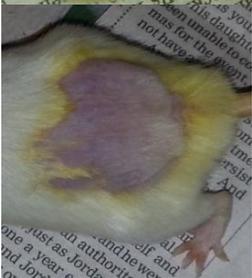


Fig. 1. Pictograph showing effects of drug

In typical control skin was watched ordinary and smooth with hide yet in sick control nearness of papilloma on skin and skin become hard.

Normally, in malignancy chemotherapy the serious issues that are being experienced are of myelo concealment and iron deficiency because of decrease in RBC or Hemoglobin rate. In the above investigation in DMBA/Croton oil rewarded bunch the all out WBC check was found to increment with a decrease in the hemoglobin content and the RBC tally. Curcumin treatment changed these adjusted boundaries to recuperate close to typical qualities in a portion subordinate way.

In all the different boundary estimation nearly oral + skin curcumin organization delivered all the more better outcomes contrast with just oral and skin, just oral created preferable outcomes over just skin sedate organization. Following multi month of disease acceptance treatment with oral + skin curcumin organization additionally demonstrated huge outcomes.

6. CONCLUSION

The requirement for elective and less harmful treatments for skin is clear. As a characteristic item, curcumin is both non-harmful just as broadened in its inhibitory consequences for a large number of pathways engaged with carcinogenesis and tumor arrangement.

In the current examination we showed that the dietary segment curcumin has given some encouraging outcomes as an enemy of malignant growth operator by adjusting the different haematological (RBC, WBC, Hb) and biochemical (TBARS, SOD, CAT) boundaries.

Further examinations and clinical preliminaries in humans are expected to approve curcumin as a successful anticancer specialist.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal husbandry procedures and experimental protocol were in accord with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Before beginning the experiment, ethical clearance was taken (reg. no. KCP/IAEC/16/004) from Institutional Animal Ethics Committee (IAEC).

ACKNOWLEDGMENTS

Authors are grateful to the department of the pharmacology of Karnataka College of pharmacy, Bangalore-64.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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