RESEARCH

Hexane Extract of Rhizomes of Curcuma Longa , Zingiber Officinale and Curcumine Life Span Extension in Caenorhabditis Elegans By Reducing Fat in Intestine.

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ABSTRACT

Background: Curcumine is obtained from curcuma longa and we examined the effects of curcumin, hexane extracts of Curcuma longa and Zingiber officinale on the lifespan and aging in Caenorhabditis elegans and found that it responded to curcumin, hexane extracts of Curcuma longa and Zingiber officinale with an increased lifespan and reduced intracellular reactive oxygen species during aging.

Methods: MutantStrains, culture, Curcumin and Hexane extract of Rhizomes of Curcuma longa and Zingiber officinale treatment of C. Elegan.

Results: Curcumin and hexane extracts of Curcuma longa and Zingiber officinale increased the life span and life cycle of the N2 wild type and Zdls-5 worms. On comparison, curcumin was found to be the most effective followed by the hexane extracts of Curcuma longa and hexane extracts of Zingiber officinale. Hexane extracts of Zingiber officinale were found to be least effective. Sudan black staining exhibited that stored contents of fat in C.elegans decreased as the concentration of the drug increased.

Conclusions: Our study has established that curcumin and hexane extract of rhizomes of Curcuma longa and Zingiber officinale provides longevity and decreases the fat content in C.elegans.

Keywords Curcumin, Curcuma longa, Zingiber officinale, Lifespan, Aging, Caenorhabditis elegans

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INTRODUCTION

Curcumin, a yellow colouring agent present in the spice turmeric (Curcuma longa) and Zingiber officinale that belongs to the ginger (Zingiberaceae) familu is the pharmacologically active substance in turmeric. Traditional Indian medicine considers curcumin an effective drug for several disorders such as GI upset, flatulence, dysentery, ulcers, jaundice, arthritis, sprains, wounds, acne, skin and eye infections.[1] Currently, many lines of evidence indicate that curcumin exhibits anti-inflammatory, anticarcinogenic, antiaging and antioxidant properties.[2,3,4] Today, ginger root is widely used as a digestive aid for mild stomach upset and is commonly recommended by health care professionals to prevent or treat nausea

and vomiting associated with motion sickness, pregnancy, cancer chemotherapy and also used as an antiaging agent.[5,6,7] The nematode animal model, Caenorhabditis elegans (C. elegans) has been increasingly utilized for biological and medical studies and >65% of the genes relating to human diseases are conserved in C. Elegans.[8] Three hundred and five genes in C. elegans have been shown to be involved in reducing body fat and 112 genes are involved in increasing fat storage as demonstrated by RNAi and Sudan black staining, Nile Red staining.[9] C. elegans is well-suited to obesity studies because deposits of fat for energy storage can be found along its intestinal tract and the bodies of C. elegans are transparent.[10] Moreover, C. elegans and humans share similar aspects of ageing.[11] It is for these reasons that C. elegans is highly-valued for identifying compounds, genes, and mechanisms that may extend the longevity of humans.[12] To our best knowledge, the uptake of curcumin and other pharmacological active compounds or extracts of plant origin into the worms has not been studied yet. To investigate whether curcumin can delay aging and prolong the lifespan of a whole organism, we used the nematode C. elegans .

Herein, we study that the effect of curcumin, hexane extracts of Curcuma longa and Zingiber officinale treatment on resistance to oxidative stress and adult lifespan of C. elegans.

MATERIALS AND METHODS

Curcumin (Sigma–Aldrich) was dissolved in dimethyl sulfoxide (DMSO, Sigma–Aldrich). A final DMSO concentration of 0.1% (v/v) was maintained under all conditions.

Mutant Strains, culture, Curcumin and Hexane extract of Rhizomes of Curcuma longa and Zingiber officinale treatment of C. elegan

C. elegans two strains N2 Wild type and Zd Is 5 stain: sk4005 were collected from national centre for biological sciences Banglore.

Maintenance of C. elegans

Preparation of bacterial food source

C. elegans were grown in the laboratory using E.coli strain OP50 as a food source.[13] A limited bacterial lawn was made because it allows for easier observation and better mating of the worms. The LB media were stored at room temperature and inoculated to overnight at 37°C. The E. coli OP50 streak plate and liquid culture were stored at 4°C. The E.coli was taken into NGM media for feeding worms.[14]

Method for preparation of NGM Petri plates.[15] NaCl 3g, 17 g agar, and 2.5 g peptone was mixed in a 2 litre Erlenmeyer flask. Water 975 ml was added. The mouth of flask was covered with aluminium foil and autoclaved for 50 min. The flask was cooled in 55°C water bath for 15 min. $CaCl_2$ 1 ml of 1 M, 1 ml in 5 mg/ml cholesterol in ethanol, 1 ml of 1 M $MgSO_4$ and 25 ml of 1 M KPO4 buffer was mixed. Swirling was done to mix well. Using sterile procedures, the NGM solution was dispensed into Petri plates using a peristaltic pump. Plates were filled with 2/3 full of agar. Plates were left at room temperature for 2-3 days before use to allow for detection of contaminants and to allow excess moisture to evaporate. Plates were stored in an air-tight container at room temperature.

Seeding NGM plates

Using sterile technique approximately 0.05 ml of E. coli OP50 liquid culture in small or medium NGM plates or 0.1 ml to large NGM plates using a pipette. Spreading will create a larger lawn which can aid in visualizing the worms. The worms tend to spend most of the time in the bacteria. E. coli OP50 lawn was allowed to grow overnight at room temperature or at 37°C for 8 hours (cool plates to room temperature before adding worms). Seeded plates were stored in an air-tight container which could be used for 2-3 weeks.

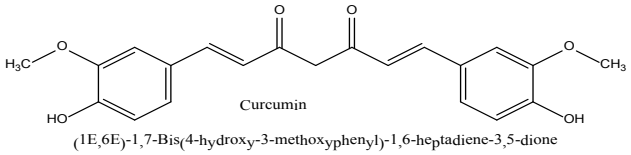
Culturing C. elegans on petri plates

C. elegans, single worms between the fourth larvae and adult stages: for synchronization maintained on NGM were picked with a platinum wire (heated in a flame red hot and cooled) and transferred to new NGM plates.

Assays

Experiments to test the tolerance of C.elegans to Curcumin (10, 20, 50, 100 and 500 µg/ml in 0.1% DMSO) was conducted in the absence and presence of E.coli in liquid NGM. Single colonies

of E.coli growing on LB plates were transferred to test tubes containing 3ml of liquid NGM medium. Ten worms growing on NGM plates were transferred to these tubes. The worms were observed periodically after shaking the test tubes. The time taken for the worms to become immobile was noted. The sensitivity of these worms to touch after transfer to solid NGM-media was monitored.



(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione

Life span of C.elegans in solid medium

Synchronized worms N2 wild, zdis-5 were incubated for 24 hrs on NGM/OP50 plates[16] several plates were seeded with ten worms each. The plates were then maintained at 20^0C, 25^0C or at 37^0C. The adult worms were picked daily and transferred to new plates for determination of life span. The ability of the worms to respond to touch was monitored daily. A worm was considered dead if it did not respond to touch and lay eggs. Experiment life span was determined in the absence of E.coli too. Experiments were carried out with the incorporation of 0.5, 1.0, 1.5 and 2.0 µg/ml of curcumin and the hexane extracts from Curcuma longa and Zingiber officinale (dissolved in 0.1% DMSO).

Sudan black staining

Preparation of Sudan black: Sudan black 0.7g was dissolved in 100ml propylene glycol slowly. The solution was heated up to 100^0C, cooled and filtered through Whattman filter paper. The stock was diluted 1:250. Diluted solution was used to stain the worms.

Staining procedure: The worms treated by Curcumin and hexane extract of Curcuma longa and Zingiber officinale (0.5, 1.0, 1.5 and 2.0 µg/ml) at 20^0C were washed with M9 solution[17] and then stained with the solution of Sudan black and photographed[18] to determine the difference in fat content in worms.

RESULTS

Extraction of rhizomes of Curcuma longa and Zingiber officinale

The yield of hexane extract of Curcuma longa obtained by reflux was found to be 13gm and the yield of hexane extract of Zingiber officinale was found to be 15gm. Extracts were obtained by silica gel column chromatography of Curcuma longa and Zingiber officinale, using hexane, Chloroform : hexane (1:1), chloroform, chloroform : Methanol (1:1) as a solvent. The total yield of material and % of hexane extract of these fractions were found to be 13, 0.404, 0.888, 0.338, 2.574, 0.690, 0.497, 0.388, 1.005gm and 100, 3.1, 6.8, 2.6, 19.8, 5.3, 3.8, 2.9, 7.7 % for Curcuma longa and 15, 0.348, 0.215,0.294, 1.921, 0.560, 0.785, 0.299, 1.464 gm and 100, 2.3, 1.4, 1.9, 12.8, 3.7, 5.2, 1.9, 9.7% for Zingiber officinale.[19]

Tolerance of C.elegans to Curcumin

Tolerance of C. elegans to Curcumin at different concentration (10, 20, 50,100 and 500 µg/ml in 0.1% DMSO) was conducted in the absence and presence of E.coli in liquid NGM. All the worms were found dead on the third day of the experiment.

Life span in solid medium

Life span in solid medium without E.coli at 20^0C, 25^0C for N2 wild type and zdls-5 worms was performed in all the concentrations (0.5, 1.0, 1.5 and 2.0 µg/ml). Worms went into the dauer stage including control on the second day of the experiment and at 35^0C worms including control were found to be dead in all the concentrations.

Life span in solid medium with E.coli at 20^0C, 25^0C for N2 wild type and zdls-5 worms was performed, worms went into the dauer stage in all the concentrations with curcumin, hexane extract of Curcuma longa and Zingiber officinale and at 35^0C both the strains of worm were found dead within 20 mins..

Life span in liquid medium

Life span in liquid medium with E.coli at 20^0C and 25^0C N2 wild type and zdls-5 was done, worms went into the dauer stage in all the concentrations with curcumin, hexane extract of Curcuma longa and Zingiber officinale after particular hours and then further transferred to a new fresh plate their life span was seen it was found same as solid medium. At 35^0C both the strains of worms were found dead within 20 mins.

Table 1 Results at 20^{0}C life cycle of worms with curcumin in solid mediu

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	12hrs	14hrs	15hrs	17hrs	18hrs	21hrs	21hrs	21hrs	22hrs	24hrs
Eggs-L1	15hrs	16 hrs	17hrs	19 hrs	21hrs	23 hrs	25 hrs	27hrs	27hrs	29 hrs
L1-L2	16hrs	16 hrs	18hrs	19 hrs	20hrs	21 hrs	23 hrs	23hrs	25hrs	28 hrs
L2-L3	12hrs	13 hrs	13hrs	14 hrs	17hrs	16 hrs	18 hrs	20hrs	20hrs	25 hrs
L3-L4	12hrs	12 hrs	14hrs	15 hrs	15hrs	17 hrs	17 hrs	19hrs	19hrs	21 hrs
L4-Adult	17hrs	15 hrs	20hrs	25 hrs	22hrs	25 hrs	24 hrs	28hrs	29hrs	31 hrs

Table 2 Results at 25^{0}C life cycle of worms with curcumin in solid medium

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	8hrs	10hrs	10hrs	12hrs	14hrs	15hrs	17hrs	18hrs	20hrs	21hrs
Eggs-L1	10hrs	12 hrs	12hrs	15 hrs	16hrs	17 hrs	22 hrs	20hrs	21hrs	24 hrs
L1-L2	12hrs	13 hrs	13hrs	14 hrs	16hrs	17 hrs	20 hrs	21hrs	23hrs	26 hrs
L2-L3	10hrs	11 hrs	13hrs	14 hrs	15hrs	15 hrs	16 hrs	19hrs	16hrs	21 hrs
L3-L4	10hrs	11 hrs	12hrs	13 hrs	15hrs	15 hrs	15 hrs	17hrs	17hrs	20 hrs
L4-Adult	13hrs	14 hrs	17hrs	19hrs	19hrs	21 hrs	21 hrs	24hrs	22hrs	27 hrs

Table 3 Results at 20^{0}C life cycle of worms with hexane extract of Curcuma longa in solid medium

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	12hrs	13hrs	14hrs	15hrs	16hrs	19hrs	18hrs	20hrs	20hrs	21hrs
Eggs-L1	13hrs	15 hrs	15hrs	17 hrs	19hrs	21 hrs	22 hrs	25hrs	24hrs	26 hrs
L1-L2	14hrs	15 hrs	15hrs	17 hrs	17hrs	19 hrs	20 hrs	21hrs	21hrs	24 hrs
L2-L3	11hrs	12 hrs	13hrs	13 hrs	14hrs	15 hrs	16 hrs	17hrs	18hrs	21 hrs
L3-L4	10hrs	12 hrs	12hrs	13 hrs	14hrs	15 hrs	15 hrs	15hrs	15hrs	18 hrs
L4-Adult	15hrs	16 hrs	18hrs	22 hrs	19hrs	21 hrs	21 hrs	23hrs	25hrs	27 hrs

Table 4 Results at 25^{0}C life cycle of worms with hexane extract of Curcuma longa in solid medium

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	8hrs	10hrs	9hrs	11hrs	12hrs	14hrs	15hrs	16hrs	17hrs	19hrs
Eggs-L1	10hrs	12 hrs	11hrs	13 hrs	14hrs	15 hrs	16 hrs	17hrs	19hrs	20 hrs
L1-L2	12hrs	12 hrs	13hrs	13 hrs	14hrs	16 hrs	14 hrs	17rs	21hrs	23 hrs
L2-L3	10hrs	11 hrs	12hrs	12 hrs	13hrs	14 hrs	15 hrs	17hrs	15hrs	19 hrs
L3-L4	12hrs	11 hrs	12hrs	12 hrs	13hrs	14 hrs	15 hrs	16hrs	15hrs	17 hrs
L4-Adult	14hrs	14 hrs	16hrs	16 hrs	18hrs	17 hrs	19 hrs	19hrs	20hrs	21 hrs

Table 5 Results at 20^{0}C life cycle of worms with Zingiber officinale in solid medium

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	11hrs	13hrs	13hrs	14hrs	14hrs	17hrs	16hrs	19hrs	18hrs	19hrs
Eggs-L1	13hrs	15 hrs	15hrs	17 hrs	17hrs	19 hrs	20 hrs	21hrs	20hrs	23 hrs
L1-L2	14hrs	15 hrs	15hrs	17 hrs	15hrs	17 hrs	17 hrs	19hrs	19hrs	21 hrs
L2-L3	11hrs	12 hrs	13hrs	13 hrs	13hrs	13 hrs	13 hrs	14hrs	16hrs	18 hrs
L3-L4	10hrs	11 hrs	11hrs	13 hrs	13hrs	14 hrs	12 hrs	12hrs	13hrs	15 hrs
L4-Adult	17hrs	16 hrs	18hrs	18 hrs	18hrs	18 hrs	19 hrs	20hrs	21hrs	24 hrs

Table 6 Results at 25^{0}C life cycle of worms with Zingiber officinale in solid medium

Stages	Control		0.5µg/ml		1.0µg/ml		1.5µg/ml		2.0µg/ml	
	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5	N2	Zdls-5
Adult-eggs	8hrs	10hrs	9hrs	11hrs	10hrs	13hrs	13hrs	15hrs	15hrs	17hrs
Eggs-L1	10hrs	10 hrs	11hrs	13 hrs	13hrs	13 hrs	14 hrs	14hrs	17hrs	19 hrs
L1-L2	12hrs	14 hrs	13hrs	13 hrs	13hrs	14 hrs	13 hrs	14hrs	17hrs	19 hrs
L2-L3	10hrs	12 hrs	12hrs	12 hrs	12hrs	12 hrs	13 hrs	15hrs	13hrs	17 hrs
L3-L4	12hrs	11 hrs	12hrs	14 hrs	13hrs	14 hrs	14 hrs	15hrs	14hrs	15 hrs
L4-Adult	13hrs	12 hrs	14hrs	15 hrs	14hrs	17 hrs	16 hrs	17hrs	19hrs	21 hrs

DISCUSSION

Curcumine has wide range of beneficial effects i.e anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic activities. The pleiotropic activities of curcumin are likely linked to its ability to influence multiple signalling pathways. 20 Many lines of evidence indicated that curcumin can modulate several different transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes.[21] In addition, studies have shown that curcumine delayed the process of senescence as well as the onset and progression of many age-related diseases.[22-24] Recent studies have implied that curcumin extended the lifespan of two different strains of Drosophila melanogaster.[25-26] Relatively little is known about whether curcumin is able to delay aging and prolong lifespan in whole animals due to cost and duration of the study.

Dauer stages with curcumin in solid medium (Fig.1 and Table-1), when increase in concentration (0.5-2µg/ml) and with time 78-84 Hrs at 20^{0}C. in N2 wild type and in Zdls-5 Dauer stages also increases from 72-81 Hrs. at 20^{0}C. Dauer stages were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control. Dauer stages were slightly increases when taken hexane extract of Curcuma longa and Zingiber officinale in solid medium as compared with Curcumin. Dauer stages were found to be 80-88 Hrs with hexane extract of Curcuma longa and 80-88 Hrs with hexane extract of Zingiber officinale at 20^{0}C. Dauer satges were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control.[27]

Dauer stages with curcumin in liquid medium (Fig.2), when increase in concentration (0.5-2µg/ml). Dauer stages were increases from 70-76 Hrs in N2 wild type and in Zdls-5 Dauer stages also increases from 69-73 Hrs. at 20^{0}C. Dauer stages were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control.

Dauer stages were slightly decreases when taken hexane extract of Curcuma longa and Zingiber officinale in liquid medium (Fig.2) as compared with solid medium. Dauer stages were found to be 72-82 Hrs with hexane extract of Curcuma longa and 74-85 hrs with hexane extract of Zingiber officinale at 20^{0}C. Dauer satges were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control.

At 20^{0}C with curcumin, total life span of worms (N2wild & Zdls-5 type) were increases, when increases in concentration (0.5-2µg/ml). In case of N2 wild type, the life spans were found to be 5.3-6.5 weeks and 5-6 weeks, in case of Zdls-5 as compared with control (4.8 & 4.5 weeks). Life spans were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control (Fig.3).

At 20^{0}C with hexane extract of Curcuma longa, total life span of worms (N2 wild & Zdls-5 type) were slightly decreases, when increases in concentration (0.5-2µg/ml) as compared with curcumin. In case of N2 wild type, the life spans were found to be 5.4-6 weeks and 5.2-5.6 weeks, in case of Zdls-5 as compared with control (4.8 & 4.4 weeks). Life spans were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control (Fig.3).

At 20^{0}C with hexane extract of Zingiber officinale, total life span of worms (N2 wild & Zdls-5 type) were slightly decreases, when increases in concentration (0.5-2µg/ml) as compared with curcumin. In case of N2 wild type, the life spans were found to be 5.2-5.6 weeks and 5.0-5.3 weeks, in case of Zdls-5 as compare with control (4.6 & 4.4 weeks). Life spans were slightly decreases when increases in temperature 25^{0}C. But all worms were found to be dead at 35^{0}C in 20 minutes including control (Fig.3).

At 20^{0}C life cycle of worms with curcumin in solid medium were increases when increases in concentrations. At 0.5 µg/ml concentration, life cycle of worms were completed in 20-25 Hrs but at 2.0 µg/ml concentration, life cycle of worms were completed in 29-31 Hrs (Table 1).

At 20^{0}C life cycle of worms with curcumin in liquid medium were increases when increases in concentrations. At 0.5 µg/ml concentration, life cycle of worms were completed in 18-18 Hrs but at 2.0 µg/ml concentration, life cycle of worms were completed in 21-24 Hrs (Table 2).

At 20^{0}C life cycle of worms with hexane extract of Curcuma longa in solid medium were slightly decreases when increases in concentrations as compare with curcumin in solid medium. At 0.5 µg/ml concentration, life cycle of worms were completed in 18-22 Hrs but at 2.0 µg/ml concentration, life cycle of worms were completed in 25-27 Hrs (Table 3).

At 25^{0}C life cycle of worms with curcumin, hexane extract of Curcuma longa were found to be less as compared to 20^{0}C life cycle of worms with curcumin, hexane extract of Curcuma longa (Table 4, 5, 6).

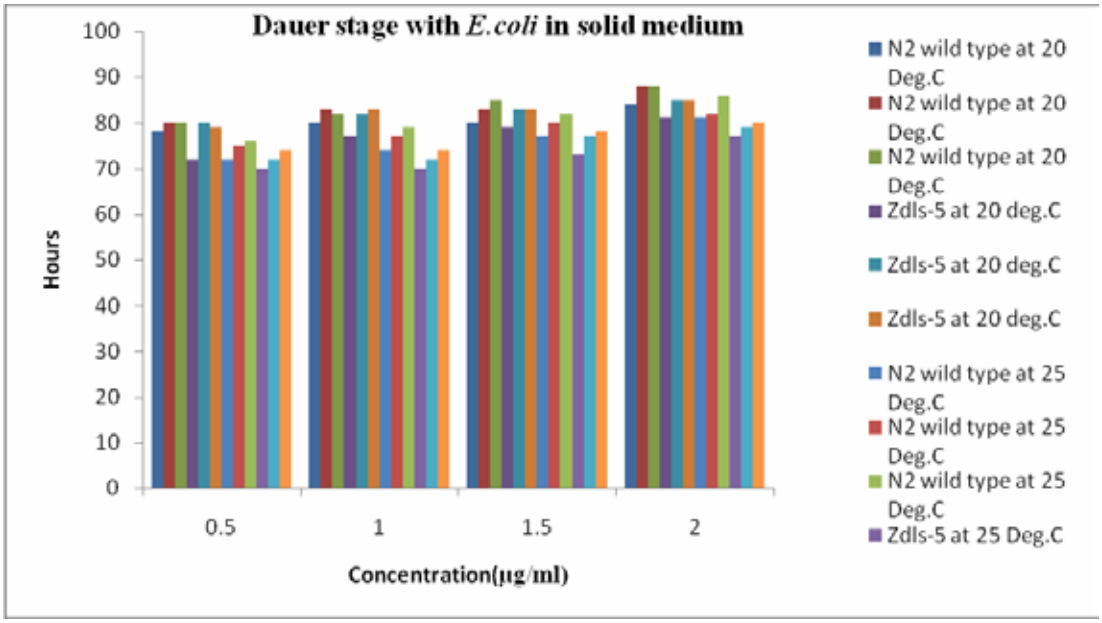


Fig 1: curcumin, hexane extract of Curcuma longa and Zingiber officinale in solid medium

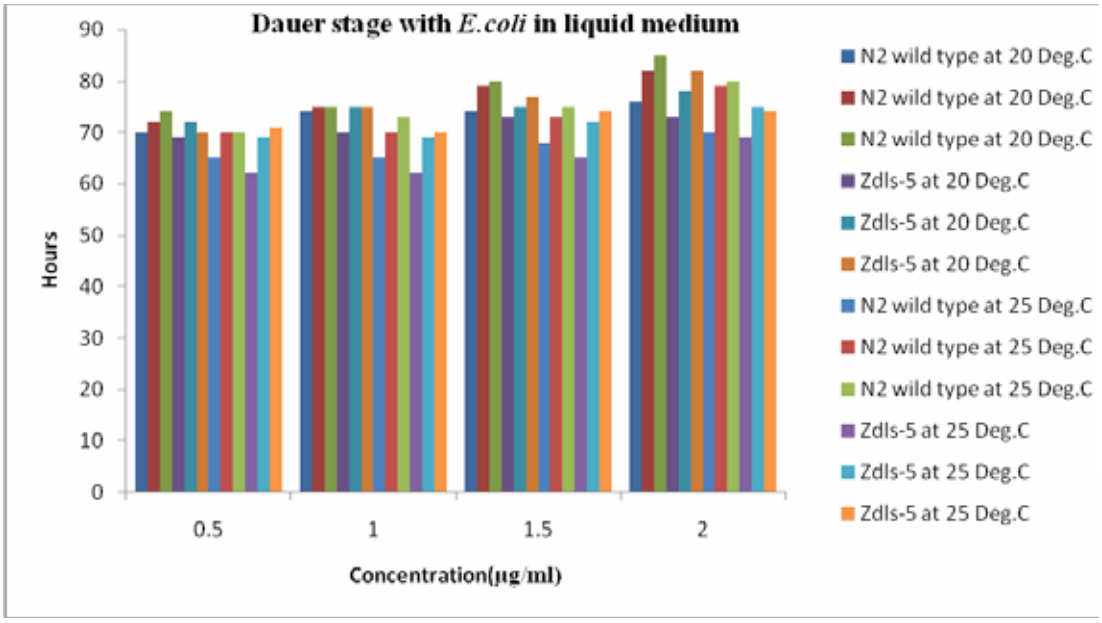


Fig 2: curcumin, hexane extract of Curcuma longa and Zingiber officinale in liquid medium

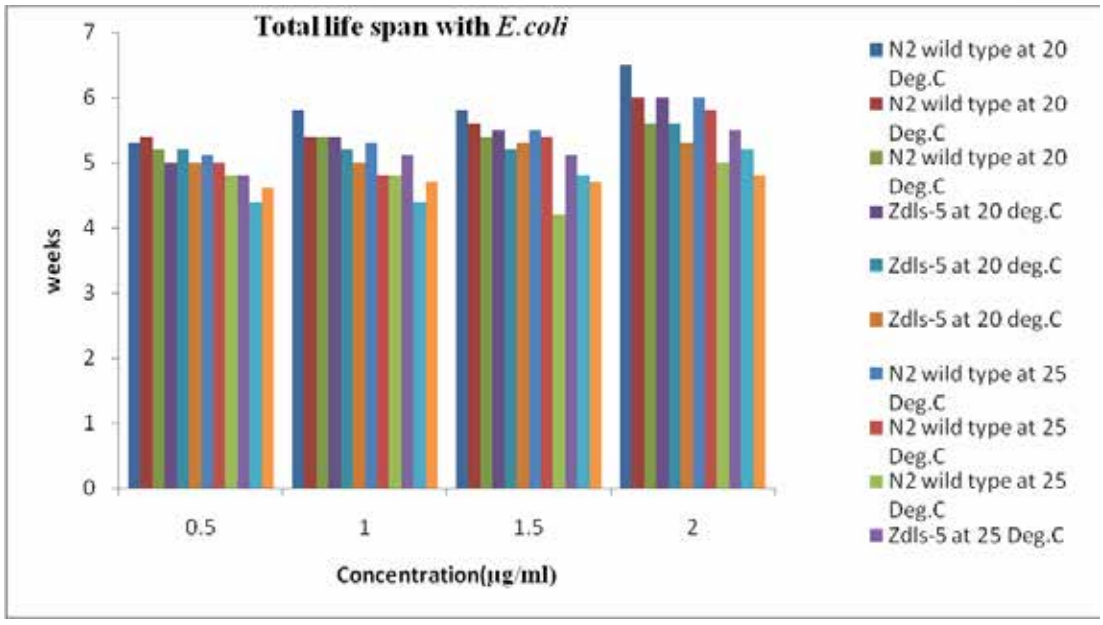


Fig 3: curcumin, hexane extract of Curcuma longa and Zingiber officinale in solid medium

CONCLUSION

we found that curcumin and hexane extracts of Curcuma longa and Zingiber officinale increased the life span and life cycle of the N2 wild type and Zdls-5 worms. On comparative basis curcumin was found to be the most effective followed by hexane extracts of Curcuma longa. The hexane extracts of Zingiber officinale were found to be least effective. Sudan black staining showed that stored contents of fat in C.elegans decreased as the concentration of the drug increased. Hence, curcumin and hexane extract of rhizomes of Curcuma longa and Zingiber officinale provides longevity and decreases the fat content in C.elegans. However further studies are needed in future to elucidate the mechanism behind delaying aging by curcumin.

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