Research Article

Pre-clinical Evaluation of Anti-cancer Activity of *Citrullus colocynthis Linn.* Fruit Extract

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ABSTRACT

The present study was performed to evaluate the anti cancer activity of *Citrullus colocynthis Linn.* fruit extract. Cancer was induced in experimental Swiss albino mice by introduction of Dalton ascites lymphoma (DAL) cells intraperitonealy (i.p). The above cancerous agent brought abnormalities in levels of RBC (red blood cells), WBC (white blood cells), SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), GSH (glutathione), CAT (catalase), SOD (superoxide dismutase) and tumor volume. These parameters were statistically improved by our extract. Might be because of the presence of numerous phytoconstituents present in the extract, it is showing anti cancer activity by virtue of antioxidant, free radical scavenging and tumor cytotoxicity activities.

Keywords: Anti-cancer, mice, DAL, *Citrullus colocynthis.*

INTRODUCTION

Cancer is a group of diseases in which there is an abnormal growth of cells with capability to spread in the remaining areas of the body. In 2015 there were around 90.5 million people who had cancer. In India in the year 2014 around 28 lakh people had cancer.[1] Current allopathic drugs and therapies which are used in the treatment of cancer are not fully effective and safe. Allopathic drugs (methotrexate, carmustine, flurouracil, mercaptopurine etc.) and radiation therapy have various side effects such as low bioavailability, alopecia, hepatotoxicity, compromised immunity, decreased cell counts, photosensitivity, seizures, kidney damage etc.[2] So there is a need to develop new, alternative anti cancer drugs which are more safe and effective. One important strategy to achieve this is to look at natural sources. Herbals are safe and have less or no side effects. Anti cancer drugs from plants and their derivatives have been shown to be effective in cancer treatment.[3] Till now, no scientific work has been done on anti-cancer potential of *Citrullus colocynthis Linn.* fruit extract. Hence the present study is aimed to investigate the anti-cancer activity of *Citrullus colocynthis Linn.* fruit extract.

MATERIALS AND METHOD

Chemicals

All chemicals, solvents used for this study were of the analytical grade obtained from Merck Specialities Private Limited- Mumbai, RFCL Limited- New Delhi, Finar Chemicals Limited- Ahmadabad, India. Biochemical estimation kits were procured from Robonik India Pvt. Ltd. India. Dalton ascites lymphoma (DAL) cells were procured from a reputed university in Karnataka.

Animals

Healthy adult Swiss albino mice weighing 20-25g were used for the study. They were housed in polypropylene cage under 12 hours dark-light cycle with temperature between 22±1°C and relative humidity 50±5%. The animals were allowed free access to standard laboratory pellet diet and drinking water *ad libitium*. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol (SDCP/IAEC-13/2012-13). All procedures involving animals in the present research study were done according to Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Plant material and extraction[4]

The fruits of *Citrullus colocynthis Linn.* were collected from Tirupati, Andhra Pradesh, India. Fruits were dried in the shade and pulverized. Uniform moderately coarse powder (#44) was obtained. The sieved powder was stored in airtight high density polyethylene container. The powder was packed into soxhlet extractor and was subjected to successive extraction with petroleum ether, hydro-ethanolic and then distilled water to get aqueous extract. After the residual extraction, solvent was distilled off and removed. The extract was concentrated under the vacuum then placed into desiccator.

Dose selection[5]

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) Guideline 423. Here 2 groups were taken i.e, 300 mg/kg and 2000 mg/kg each consisted of 3 animals (swiss albino mice). Test drug, *Citrullus colocynthis* was administered for 14 days. During this period changes in body weight, toxicity, time of death,

skin, fur, lacrimation, salivation, diarrhea, respiration, lethargy, sleep, convulsion, stereotype, tremors, catalepsy, hallucination, retropulsion, stupor, excitement were observed. After 14 days of study the drug failed to show any signs of toxicity as described above. Depending upon this study the higher and lower doses for carrying out further acute and chronic study was fixed at 200 mg/kg and 400 mg/kg.

DAL induced tumor model [6-10]

Swiss albino mice were used. They were divided into 5 groups consisting of 6 animals each. Group 1 served as normal. Group 2 served as negative control toxic group. Group 3 served as low dose (200 mg/kg p.o) *Citrullus colocynthis Linn.* fruit extract (LDCCFE) treatment group. Group 4 served as high dose (400 mg/kg p.o) *Citrullus colocynthis Linn.* fruit extract (HDCCFE) treatment group. Group 5 served as standard drug 5-fluorouracil (20 mg/kg) treatment group (5-FU). Mice of groups 2, 3, 4 and 5 were treated with $1x10^6$ Dalton ascites lymphoma (DAL) cells intraperitonealy (i.p). On 2nd day groups 3, 4, 5 animals were treated with LDCCFE, HDCCFE and 5-FU respectively. This treatment division went on for next 14 days. On 15th day the animals were sacrificed. Blood and serum were collected accordingly. From blood, RBC (red blood cells) and WBC (white blood cells) were measured (counting chamber-haemocytometer). From serum, SGPT (serum glutamic pyruvic transaminase) and SGOT (serum glutamic oxaloacetic transaminase) were measured (biochemical kits-semi autoanalyser). From liver homogenate, GSH[11] (glutathione), SOD[12] (superoxide dismutase) and CAT[13] (catalase) were measured. Tumor volume was also measured.

Statistical analysis

Statistical analysis was done using Graph Pad Prism version 4 software (Graph Pad Inc., USA). ANOVA followed by Dunnett's Multiple Comparison test was applied. Data presented as MEAN±SEM. Confidence level was taken at 95%.

RESULTS

Induction of DAL cells brought tumor volume. Treatment with low and high doses of the extract brought an extremely significant decrease in tumor volume in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 1.

Induction of DAL cells brought an extremely significant decrease in RBC count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in RBC count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 1.

Induction of DAL cells brought an extremely significant decrease in WBC count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in WBC count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 1.

Table 1:

Groups	Tumor VOL (ml)	RBC (10^6/cu.mm)	WBC (10^3/cu.mm)
Normal	-	10.26±0.31	6.58±0.17
Diseased	11.7±0.2	4.62±0.25***	3.74±0.23***
Low dose CCFE	5.2±0.5$^{\#\#\#}$	7.31±0.47$^{\#\#\#}$	5.11±0.15$^{\#\#\#}$
High dose CCFE	4.1±0.2$^{\#\#\#}$	8.94±0.29$^{\#\#\#}$	5.87±0.19$^{\#\#\#}$
Standard	2.8±0.3$^{\#\#\#}$	9.57±0.38$^{\#\#\#}$	6.13±0.27$^{\#\#\#}$

All values are MEAN±SEM. N=6. *p<0.05, **p<0.01, ***p<0.001 when compared with normal. $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 when compared with diseased.

Table 2:

Groups	GSH (mcg/g)	CAT (g protein/ml)	SOD (units/ml)
Normal	109.12±0.26	3.1±0.4	86.82±0.48
Diseased	29.90±0.30***	0.3±0.5***	12.96±0.17***
low dose CCFE	74.17±0.38$^{\#\#\#}$	2.6±0.3$^{\#\#}$	63.74±0.29$^{\#\#\#}$
High dose CCFE	86.39±0.13$^{\#\#\#}$	2.7±0.4$^{\#\#}$	71.35±0.24$^{\#\#\#}$
Standard	95.35±0.19$^{\#\#\#}$	2.9±0.2$^{\#\#\#}$	79.14±0.31$^{\#\#\#}$

All values are MEAN±SEM. N=6. *p<0.05, **p<0.01, ***p<0.001 when compared with normal. $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 when compared with diseased.

Table 3:

Groups	SGPT (U/L)	SGOT (U/L)
Normal	116.55±0.28	148.74±0.26
Diseased	21.74±0.37***	34.52±0.28***
Low dose CCFE	93.29±0.48$^{\#\#\#}$	115.63±0.57$^{\#\#\#}$
High dose CCFE	102.38±0.39$^{\#\#\#}$	127.95±0.16$^{\#\#\#}$
Standard	109.61±0.16$^{\#\#\#}$	135.71±0.39$^{\#\#\#}$

All values are MEAN±SEM. N=6. *p<0.05, **p<0.01, ***p<0.001 when compared with normal. $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 when compared with diseased.

Induction of DAL cells brought an extremely significant decrease in GSH count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in GSH count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 2.

Induction of DAL cells brought an extremely significant decrease in CAT count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought a moderately significant increase (improvement) in CAT count in the treatment animals when compared with negative control toxic

animals. Standard drug showed extremely significant observation. See table no.- 2.

Induction of DAL cells brought an extremely significant decrease in SOD count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in SOD count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 2.

Induction of DAL cells brought an extremely significant decrease in SGPT count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in SGPT count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 3.

Induction of DAL cells brought an extremely significant decrease in SGOT count in the experimental animals when compared with normal. Treatment with low and high doses of the extract brought an extremely significant increase (improvement) in SGOT count in the treatment animals when compared with negative control toxic animals. Standard drug also showed similar observation. See table no.- 3.

DISCUSSION

Tumor was induced in the experimental animals by the use of DAL cells. Dalton's lymphoma is a transplantable T cell lymphoma. Its origin is spontaneous in nature in the thymus. Host is murine.[14] DAL cells directly induce cancer. It also causes increase in number of cancer cells.[7] DAL cell induce inhibition of various immune responses. It also causes decrease in CD_4 cells and impairment of other T cells. DAL cells also cause rapid increase in ascites tumour volume. Tumour cells get its direct food source from ascites fluid.[15]

Citrullus colocynthis Linn. fruit extract contain many phytoconstituents such as glycosides, triones, alkaloids, flavonoids, saponins, phenols, diterpenes etc. [16,17] *Citrullus colocynthis Linn.* fruit extract have antioxidant principle. It also posses cytotoxicity towards tumour cell. It arrest tumour growth. *Citrullus colocynthis Linn.* fruit extract have protective action on haemopoetic system. It also posses free radical scavenging activity. Might be because of the above properties the extract is showing anti cancer activity.

CONCLUSION

Citrullus colocynthis Linn. fruit extract demonstrated anti cancer activity induced by DAL cells. The pharmacological estimations supported the above shown activity.

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REFERENCES

1. Cancer, 2018. Available from: https://en.m.wikipedia.org/wiki/Cancer. [Accessed on: 29th November 2018].
2. Richard AH, Pamela CC, Richard F, Luigi XC, Michelle AC. Pharmacology. 4th edition, Lippincott Williams & Wilkins, New Delhi, 2009, 457-88.
3. Ali R, Mirza Z, Ashraf GM, Kamal MA, Ansari SA, Damanhouri GA. New anticancer agents: recent developments in tumor therapy. Anticancer Res, 32(7), 2012, 2999-3005.
4. Kokate CK, Purohit AP, Gokhale SR. Pharmacognosy. 54th edition, Nirali Prakashan, Pune, 2017.
5. OECD, 2013. Available from: http://Iccvam.nieh.nih.gov/suppDocs/OECD/OECD_GL423. [Accessed on: 21st May 2013].
6. Manokaran K, Rajasekaran N, Paramasivam R, Ganesan R, Duraisamy G, Dominic S. *In vivo* and *in vitro* antitumor activity of *Jasminum sambac (Linn)* Oleaceae flower against dalton ascites lymphoma induced swiss albino mice. Int J Pharm Sci, 4(1), 2012, 144-47.
7. Jaslin EJ, Padmaja V. Antitumor activity of *Coleus spicatus* Benth against dalton's ascitic lymphoma. Int J Pharm Tech Res, 5(1), 2013, 189-92.
8. Rajesh R, Chitra K, Padmaa MP, Chidambaranathan N. Anticancer activity of aerial parts of *Aerva lanata Linn* against dalton's ascitic lymphoma. Eur J Integr Med, 3, 2011, 245-50.
9. Lakshmi KS, Shrinivas SS, Rajesh T, Chitra V. Antitumour activity of ethanolic extract of leaves of *Holoptelea integrifolia* on dalton's ascitic lymphoma in swiss albino mice. Int J Green Pharm, 4(1), 2010, 44-47.
10. Chitra V, Shrinivas S, Nandu K. Evaluation of anticancer activity of *Vitex negundo* in experimental Animals: An *in vitro* & *in vivo* study. Int J Pharm Tech Res, 1(4), 2009, 1485-89.
11. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys, 82, 1959, 70-7.
12. Nandi A, Chatterjee IB. Assay of superoxide dismutase activity in animal tissues. J Biosci, 13, 1988, 305-15.
13. Sinha A K. Colorimetric assay of catalase. Anal Biochem, 47, 1972, 389-94.
14. Koiri RK, Mehrotra A, Trigun SK. Dalton's lymphoma as a murine model for understanding the progression and development of T-Cell lymphoma and its role in drug discovery. Int J Immunother Cancer Res, 3(1), 2017, 1-6.
15. Sreenivas SA, Venugopal Y, Ravindranath A, Kalpana G, Rajkapoor B. Antitumor and antioxidant activity of *Capparis sepiaria* against dalton's ascites lymphoma in rodents. Academic J Cancer Res, 5(2), 2012, 46-52.
16. Ahmad O, Mohammad H, Ahmad RH, Adel M. Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in alloxan induced diabetic rats. Asian Pac J Trop Dis, 4(2), 2014, 125-130.
17. Borhade P, Deshmukh T, Patil V and Khandelwal K. Review on *Citrullus colocynthis*. Int J Pharchem, 3(1), 2013, 46-53.

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