Journal of Pharmaceutical Research International

34(27B): 18-24, 2022; Article no.JPRI.78830
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Induction of Bax and Activation of Caspases Hydro Ethanoic Leaf Extract of *Citrullus colocynthis*(L)-Mediated Apoptosis in Human Liver Cancer Cells Hep G2

K. Swetha [a], A. Jothi Priya [b≡*], Selvaraj Jayaraman [c#] and R. Gayatri Devi [b≡]

[a] Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai, India.
[b] Department of Physiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
[c] Department of Biochemistry, Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2022/v34i27B36001

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/78830

Original Research Article

Received 18 January 2022
Accepted 24 March 2022
Published 25 March 2022

ABSTRACT

Introduction: *Citrullus colocynthis* is a desert plant of the cucurbitaceae family. *C. colocynthis* grows in many countries with semi dry lands and that seeds are rich in oil which can encourage their sustainable agriculture to produce an economic biodiesel with a competitive price. Apoptosis, also termed as programmed cell death this is a key regulator of tissue homeostasis.
Aim of the Study: To study the induction of bax and activation of caspases hydro ethanolic leaf extract of *Citrullus colocynthis* (L)- mediated apoptosis in human liver cancer cells Hep G2.
Materials and Methods: In the present study, a human liver cancer cell line was procured from the National Centre for Cell science (NCCS), Pune, India. The cytotoxic potential of plant extract was analysed by MTG assay. mRNA expression of BaxmRNA and caspase-mRNA were analysed by

[≡] *Assistant Professor;*
[#] *Associate professor*
**Corresponding author: E-mail: a.jothipriya88@gmail.com, jothipriya.sdc@saveetha.com;*

Real-Time PCR using gene specific primers and a comparative CT method was used to analyse expression of genes. The data were analysed statistically using Graph pad prism software Version 5.

Results: MTT analysis showed that *Citrullus colocynthis* a significant increase in proliferation of human liver cancer cells (P<0.05) compared to untreated cells. However, 400 and 500 /g of the extract showed maximum effect in controlling cell growth. Results of the <mRNA expression analysis showed that *Citrullus colocynthis* reduced the expression of BaxmRNA/ caspase-mRNA in human liver cancer cells at 400 and 500 /g (P<0.05).

Discussion: The anticancer effect of *Citrullus colocynthis* has been via a variety of pathways which includes apoptotic pathway, anti-inflammatory traits, and antioxidant pathway, etc [1]. Effect of HEAM on the mRNA level of tumor related genes (p53, Bcl-2), inflammatory (IL-1β, IL-6 and c-jun) and anti-inflammatory (IL-4) cytokines. Representative photographs of ethidium-bromide-stained 2% agarose gels for each gene after 7 months of treatment.

Conclusion: The hydroethanolic leaf extract obtained from the *Citrullus colocynthis* exhibited anti-proliferative effect on human liver cancer cells HepG2 cell line by affecting the expression of the Bax mRNA and Bax mRNA and hindering the cell viability. Hence, it can be a great scope for using the extract into medicinal properties with further studies to improve herbal medicine for cancer over expensive drugs.

Keywords: Citrullus colocynthis; human liver cancer cells.

1. INTRODUCTION

Cordyceps militaris is fungus which parasites lepidoptera larvae that is extensively used as folk tonic food and crude drug [2]. Apoptosis, also termed as programmed cell death this is a key regulator of tissue homeostasis [3]. A549 and HepG2 are the perfect cell models for lung and liver cancer [4], it aids in precisely cellular mechanisms and gets modulation [5]. Activation of caspases is normally related to induction of apoptosis in many types [6]. Stimulation of normal human T-Lymphocytes with allogeneic antigen or mitogen which leads to cell proliferation and caspases-3 activation [7], Citrullus colocynthis plant was traditionally for the remedy of diabetes in sabzevar city, Iran [8]. Citrullus colocynthis plant having a strong laxative property and application of high doses of this herbal drug can cause diarrhea [9], painful cramps and blood diarrhea in humans [10]. It is currently advised as a dried fruit for the patients who are suffering from diabetes in several cities in different dosages from 300 to 800 mg/day [11]. *Citrullus colocynthis* oil for biodiesel production [12]. *Citrullus colocynthis* belongs to the cucurbitaceae family [13]. *C. colocynthis* grows in many countries with semi dry lands and that seeds are rich in oil which can encourage their sustainable agriculture to produce an economic biodiesel with a competitive price [14].

The present work was designed to study the potential cell toxic of some novel lip amino acid coated super paramagnetic iron oxide nanoparticle compared with glycine coated and naked SPION on HepG2 cells and it goes [15], before that toxicity of HepG2 cells and their changes in cell cultures are due to SPION exposure were identified using the MTT assay with comparison of result with previous study [14,16]. BH3 mimetic molecules such are HA14-1 or ABT-737 have been shown selectively kill cancer cells by its activating the intrinsic signaling pathway of apoptosis, [17] and leaving the normal cells unharmed [18].

At low doses cell death occurs through two different mechanisms apoptosis [19] characterised by the activation of initiator caspases which baggersee effectors -cas (cas -3, cas -6, cas -7) to cleave cellular substrate [20]. Human liver cancer cells Hep3b and HepG2 were purchased from a cell bank of the Chinese Academy of science [21]. RAB9 is one of the members of the Rab GTPase family [22]. RAB9 has isoforms, members of the RAB9A and RAB9B [23]. It has been remembered the existence, in the liver as of a strict connections linking development, regeneration and carcinogenesis [24] mild injury is mainly repaired through compensatory hyperplasia of hepatocytes, severe damage implies the activation of the liver progenitor cell compartment [25].

The aim of the study is induction of bax and activation of caspases hydro ethanolic leaf extract of *Citrullus colocynthis* (L)- mediated apoptosis in human liver cancer cells HepG2.

2. MATERIALS AND METHODS

Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.1 Cell Lines and Cell Culture

Human liver cancer cell line(Hep G2) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO_2.

2.2 Cell Viability by MTT Assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability of live cells to convert MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, the cells (1 ×10^4 /well) were exposed to different concentrations of Citrullus colocynthis leaf extract (100-500µg/ml) with HepG2 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37°C for an hour. The formed crystals were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

2.3 Gene Expression Analysis by Real Time-PCR

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were acquired for all samples. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

2.4 Statistical Analysis

The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at p<0.05 level in Duncan's test.

3. RESULTS AND DISCUSSION

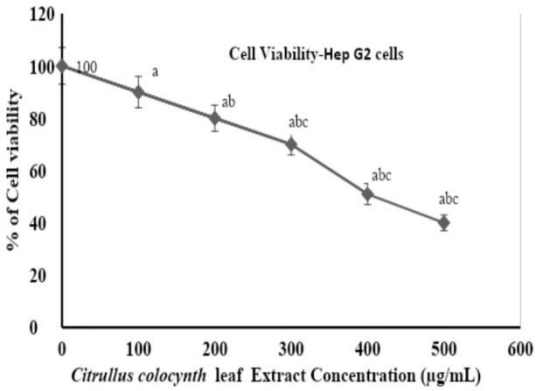

Fig. 1. Effect *Citrullus colocynthis* leaf extract on cell viability in HepG2 cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells, b-compared with 1nM treated HepG2 Cells

3.1 Gene Expression Analysis

Bax mRNA expression (fold change over control) Fig. 2.

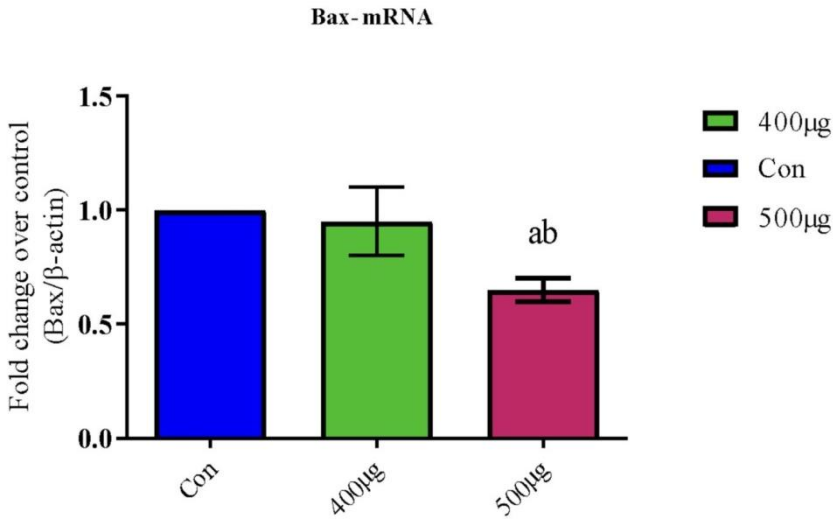

Fig. 2. Effect of *Citrullus colocynthis* leaf extract on Bax mRNA expression in Hepg2 Cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells, b-compared with 400µg treated cells

Caspase-3- mRNA expression (Fold change over control) Fig. 3.

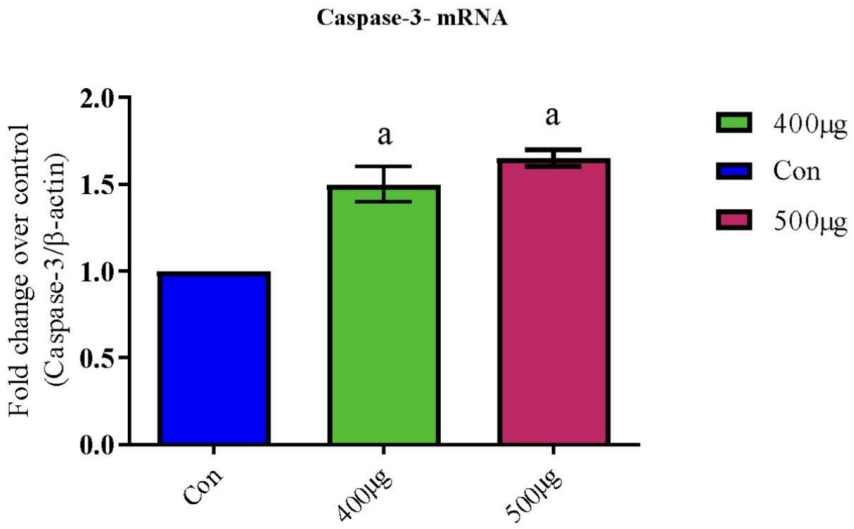

Fig. 3. Effect of *Citrullus colocynthis* leaf extract on caspase-9mRNA expression HepG2 cells. Each bar represents mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells

4. DISCUSSION

The anticancer effect of *Citrullus colocynthis* has been via a variety of pathways which includes apoptotic pathway, anti-inflammatory traits, and antioxidant pathway, etc [1]. Effect of HEAM on the mRNA level of tumor related genes (p53, Bcl-2), inflammatory (IL-1β, IL-6 and c-jun) and anti-inflammatory (IL-4) cytokines [26]. Representative photographs of ethidium-

bromide-stained 2% agarose gels for each gene after 7 months of treatment [27]. Adult stem cells are the only cells that persist in the tissue for a sufficient length of time to acquire the requisite number of genetic changes for neoplastic development [28]. In contrast to intestinal mucosa and epidermis where a steady flux of cells occurs from the stem cell zone to the terminally differentiated cells that are imminently to be lost, [29] liver normally exhibits a very low level of cell turnover [30]. Levels of messenger RNA in COL1A1 & COL3A1 and production of type I procollagen were drastically amplified by AMF and several compounds [31] (especially HDNC) as shown by RT-PCR and ELISA [32] analysisTransition of Fibroblast to Myofibroblast plays a critical role in cutaneous wound healing [33], Fibroblast-to-myofibroblast transition requires a combination of several types of factors, the most important of which are divided into humoural and mechanical factors, as well as certain extracellular matrix proteins [34].

5. CONCLUSION

The hydroethanolic leaf extract obtained from the *Citrullus colocynthis* exhibited anti-proliferative effect on human liver cancer cells hepG2 cell line by affecting the expression of the Bax mRNA and Bax mRNA and hindering the cell viability. Hence, it can be a great scope for using the extract into medicinal properties with further studies to improve herbal medicine for cancer over expensive drugs.

NOTE

The study highlights the efficacy of "herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable

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 AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Marzoqi AH, Kareem SM, Alhuchaimi S, Kadhim Hindi NK, Ghasemian A. Decreased vancomycin susceptibility among Staphylococcus aureus clinical isolates and postulated platforms to explore rational drugs [Internet]. Vol. 31, Reviews in Medical Microbiology. 2020; 111–6. Available:http://dx.doi.org/10.1097/mrm.0000000000000204
2. Solai Prakash AK, Devaraj E. Cytotoxic potentials of *S. cumini* methanolic seed kernel extract in human hepatoma HepG2 cells. Environ Toxicol. 2019;34(12):1313–9.
3. Shao LW, Huang LH, Yan S, Jin JD, Ren SY. Cordycepin induces apoptosis in human liver cancer HepG2 cells through extrinsic and intrinsic signaling pathways. Oncol Lett. 2016;12(2):995–1000.
4. Wadhwa R, Paudel KR, Chin LH, Hon CM, Madheswaran T, Gupta G, et al. Anti-inflammatory and anticancer activities of Naringenin-loaded liquid crystalline nanoparticles in vitro. J Food Biochem. 2021;45(1):e13572.
5. Jacob K, Peter KJP, Nair S, Chandramohanakumar N. Cell cycle arrest and apoptosis in human liver cancer cell line and A549 cell lines by Turbiconol– A novel sterol isolated from Turbinaria conoides [Internet]. Vol. 15, Pharmacognosy Magazine. 2019;189. Available:http://dx.doi.org/10.4103/pm.pm_643_18
6. Nambi G, Kamal W, Es S, Joshi S, Trivedi P. Spinal manipulation plus laser therapy versus laser therapy alone in the treatment of chronic non-specific low back pain: A randomized controlled study. Eur J Phys Rehabil Med. 2018;54(6):880–9.
7. Jayaraman S, Barrett JN. Coordinated activation of caspases and apoptosis in allogeneic antigen stimulated human t cells [Internet]. Vol. 1, The Scientific World Journal. 2001;37–37.

Available:http://dx.doi.org/10.1100/tsw.2001.23.163

8. Rajakumari R, Volova T, Oluwafemi OS, Rajesh Kumar S, Thomas S, Kalarikkal N. Grape seed extract-soluplus dispersion and its antioxidant activity. Drug Dev Ind Pharm. 2020;46(8):1219–29.
9. Tahmasebi S, Qasim MT, Krivenkova MV, Zekiy AO, Thangavelu L, Aravindhan S, et al. The effects of oxygen-ozone therapy on regulatory T-cell responses in multiple sclerosis patients. Cell Biol Int. 2021;45(7): 1498–509.
10. Shukla AK, Iravani S. Green synthesis, characterization and applications of nanoparticles. Elsevier. 2018;548.
11. Barghamdi B, Ghorat F, Asadollahi K, Sayehmiri K, Peyghambari R, Abangah G. Therapeutic effects of Citrullus colocynthis fruit in patients with type II diabetes: A clinical trial study. J Pharm Bioallied Sci. 2016;8(2):130–4.
12. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2020;306–12.
Available:http://dx.doi.org/10.1016/j.oooo.2020.06.021
13. Kumar S, Krishnan S, Samal SK. Recent Developments of Epoxy Nanocomposites Used for Aerospace and Automotive Application [Internet]. Diverse Applications of Organic-Inorganic Nanocomposites. 2020;162–90.
Available:http://dx.doi.org/10.4018/978-1-7998-1530-3.ch007
14. Ali Elsheikh Y, Hassan Akhtar F. Biodiesel from *Citrullus colocynthis* oil: Sulfonic-ionic liquid-catalyzed esterification of a two-step process. ScientificWorldJournal. 2014;29: 540765.
15. Mudigonda SK, Murugan S, Velavan K, Thulasiraman S, Krishna Kumar Raja VB. Non-suturing microvascular anastomosis in maxillofacial reconstruction- a comparative study. J Craniomaxillofac Surg. 2020; 48(6):599–606.
16. Gholami A, Rasoul-amini S, Ebrahiminezhad A, Seradj SH, Ghasemi Y. Lipoamino acid coated superparamagnetic iron oxide nanoparticles concentration and time dependently enhanced growth of human hepatocarcinoma cell line (Hep-G2) [Internet]. Vol. 2015, Journal of Nanomaterials. 2015;1–9.
Available:http://dx.doi.org/10.1155/2015/451405
17. Santhakumar P, Roy A, Mohanraj KG, Jayaraman S, Durairaj R. Ethanolic extract of capparis decidua fruit ameliorates methotrexate-induced hepatotoxicity by activating Nrf2/HO-1 and PPARγ mediated pathways [Internet]. Vol. 55, Indian Journal of Pharmaceutical Education and Research. 2021;s265–74.
Available:http://dx.doi.org/10.5530/ijper.55.1s.59
18. Raychaudhuri S, Das SC. Low probability activation of Bax/Bak can induce selective killing of cancer cells by generating heterogeneity in apoptosis. J Healthc Eng. 2013;4(1):47–66.
19. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma [Internet]. Vol. 48, Journal of Oral Pathology & Medicine. 2019;299–306.
Available:http://dx.doi.org/10.1111/jop.12835
20. Meng J, Candas M, Keeton TP, Bulla LA. Expression in Spodoptera frugiperda (Sf21) Insect Cells of BT-R1, a Cadherin-Related Receptor from Manduca sexta for Bacillus thuringiensis Cry1Ab Toxin [Internet]. Vol. 22, Protein Expression and Purification. 2001;141–7.
Available:http://dx.doi.org/10.1006/prep.2001.1421
21. Vivekanandhan K, Shanmugam P, Barabadi H, Arumugam V, Daniel Raj Daniel Paul Raj D, Sivasubramanian M, et al. Emerging therapeutic approaches to combat COVID-19: Present Status and future perspectives. Front Mol Biosci. 2021;8:604447.
22. Bharath B, Perinbam K, Devanesan S, AlSalhi MS, Saravanan M. Evaluation of the anticancer potential of Hexadecanoic acid from brown algae Turbinaria ornata on HT–29 colon cancer cells [Internet]. Vol. 1235, Journal of Molecular Structure. 2021;130229.
Available:http://dx.doi.org/10.1016/j.molstruc.2021.130229
23. Sun P, Li L, Li Z. RAB9A Plays an Oncogenic Role in Human Liver Cancer Cells. Biomed Res Int. 2020;2020: 5691671.

24. Saraswathi I. et al. Impact of COVID-19 outbreak on the mental health status of undergraduate medical students in a COVID-19 treating medical college: A prospective longitudinal study. Peer J. 2020;8:e10164.
Available:http://dx.doi.org/10.7287/peerj.10164v0.2/reviews/2
25. Cesselli D, Beltrami AP, Poz A, Marzinotto S, Comisso E, Bergamin N, et al. Role of tumor associated fibroblasts in human liver regeneration, cirrhosis, and cancer. Int J Hepatol. 2011;2011:120925.
26. Kamath SM, Manjunath Kamath S, Jaison D, Rao SK, Sridhar K, Kasthuri N, et al. *In vitro* augmentation of chondrogenesis by Epigallocatechin gallate in primary human chondrocytes - sustained release model for cartilage regeneration [Internet]. Vol. 60, Journal of Drug Delivery Science and Technology. 2020;101992.
Available:http://dx.doi.org/10.1016/j.jddst.2020.101992
27. Clarizia G, Bernardo P. Diverse applications of organic-inorganic nanocomposites: Emerging research and opportunities: Emerging Research and Opportunities. IGI Global; 2019.
28. Egbuna C, Mishra AP, Goyal MR. Preparation of phytopharmaceuticals for the management of disorders: The development of nutraceuticals and traditional medicine. Academic Press. 2020;570.
29. Barabadi H, et al. Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles. Inorganic Chemistry Communications. 2021;108647.
DOI: 10.1016/j.inoche.2021.108647..
30. Kishi Y, Hasegawa K, Sugawara Y, Kokudo N. Hepatocellular carcinoma: Current management and future development—improved outcomes with surgical resection [Internet]. Vol. 2011, International Journal of Hepatology. 2011; 1–10.
Available:http://dx.doi.org/10.4061/2011/728103
31. PC J, Marimuthu T, CK, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res. 2018;20(4):531–4.
32. Wahab PUA, Madhulaxmi M, Senthilnathan P, Muthusekhar MR, Vohra Y, Abhinav RP. Scalpel versus diathermy in wound healing after mucosal incisions: A split-mouth study. J Oral Maxillofac Surg. 2018;76(6):1160–4.
33. Gowhari Shabgah A, Ezzatifar F, Aravindhan S, Olegovna Zekiy A, Ahmadi M, Gheibihayat SM, et al. Shedding more light on the role of Midkine in hepato-cellular carcinoma: New perspectives on diagnosis and therapy. IUBMB Life. 2021; 73(4):659–69.
34. Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases', Hepatobiliary & pancreatic diseases international: HBPD INT 2020;19(5):429–434.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/78830