Cytogenetic Effect of Cicer Arietinum Ethanolic Extract on the Male Rats Treated with Sorafenib

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Abstract
The present study was conducted to estimate the ameliorative effect of *Cicer Arietinum* ethanolic extract against cytogenetic effect of Sorafenib in male rats. Twenty-four adult male rats were divided into four groups each group contain seven rats; G1(control negative group received distilled water along of period of study), G2(control positive group received 200mg of Sorafenib only), G3(received *Cicer arietinum* extract at dose (314.9 mg/kg) and G4 group treated with Sorafenib at dose (200mg/kg/B.W.) and *Cicer arietinum* extract (314.9mg/kg). The results of chromosomal aberration showed significant P≤0.05 increase in Chromosomal Aberration of G2 group as compared with all groups. While Acentric%, Polyploidy%, Ring% of G3 treated group showed no significant difference (P≤ 0.05), as compared with G1 group. Furthermore, the Acentric% of G4 group showed a significant decrease (P≤0.05) as compared with all other treated groups. Whereas Mitotic Index revealed significant P≤ 0.05 increases in animals exposed to 200mg/kg.bw when compared with groups of plants and control negative group Also, the DNA fragmentation revealed significant P≤ 0.05 increases in fragmented DNA of animals G2 compared with G1 and G3. In conclusion, *Cicer arietinum* seeds ethanolic extract contain potential source of polyphenolis demonstrated biochemical and functional properties against cytogenetic effect of chemotherapy drug Sorfenib. Moreover, it showed DNA damage protection.
Keywords: **Sorafenib, *Cicer Arietinum*, cytogenetic and rat.**

1. Introduction

There are a number of problems associated with the use of drugs in chemotherapy. These included cytotoxicity, drug resistance and induction of new tumors [1]. Their very nature many anticancer drugs were targeted and killed normal cells as well as tumor cells, thus contributing to the common toxic manifestations of chemotherapy such as vomiting, alopecia and diarrhea [2]. Although, these are generally transient side-effects and symptoms can be alleviated by the use of concomitant drug therapies such as anti-emetics, some more serious, irreversible adverse effects such as myelosuppression (i.e., suppression of bone marrow leading to reduced synthesis of blood cells) and cardiac, bladder and pulmonary toxicities can occur [3]. Sorafenib (Nexavar®) is an oral multi-kinase inhibitor that inhibits Raf serine/threonine kinases and receptor tyrosine kinases involved in tumor growth and angiogenesis [4]. Sorafenib has been demonstrated preclinical and clinical activity against several tumor types, as a monotherapy and in combination with other anti-cancer agents [5]. Sorafenib is a vascular endothelial growth factor receptor tyrosine-kinase inhibitor currently used in several malignancies [6]. Several side effects were reported by other studies such as bleeding disorders, myelosuppression and stomatitis, as well as, the major complication for Sorafenib was anemia [7]. So, several studies gave their attention on using herbal medical plant to minimize the dose of chemotherapy drug and then reduce the side effect [8]. The phytochemical analysis of Cicer arietinum seeds revealed the presence of carbohydrates, proteins, amino acids, fixed oils, phytosterols, alkaloids, Polyphenolic compounds and tannins, glycosides, saponins, amino acids, iron, phosphate, sulfate, and chloride. Is *Cicer arietinum* an aphrodisiac, estrogenic, antioxidant, anti-inflammatory, hepatoprotective, anticancer, and many other pharmacological effects [9]. Cicer arietinum seeds ferritin appropriately provides an effective means of controlling the iron deficiency due to a high iron content with better absorption, so it is supposed to be explored a safe and efficient functional source for iron supplement [10]. The present study designed to evaluate the cytogenetic ameliorative effect of *Cicer arietinum* extract on the male rats treated with Sorafenib.

2. Materials and Methods

Animals: This experiment has been done at the Department of Physiology, Biochemistry and Pharmacology, College of the Veterinary Medicine/University of Baghdad. Twenty-four adult male rats 3 months age, weight 150-160g have been kept under the suitable environmental situation,

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temperature range (22-25) C°. The rats were housed in plastic cages dimension 35x25x15 cm in an animal house about 2 weeks before the experiment for acclimatization. They were feed on standard pellets and tap water.

Ethanolic extraction: The extraction of *Cicer arietinum* seeds performed by the Soxhlet apparatus using 70% ethanol and a temperature of 50c°. The dried powder of *Cicer arietinum* seeds put into a thimble part and placed in the extraction unit till the clear and colorless solvent appeared in the extraction unit. Then, the extract was filtered and evaporated to dryness by using a rotary evaporator with 40 °C and 200 rotations per minute for 4 hours. Later on, a thick, semi-solid mass of deep-yellow color of crude extract appeared. All the dried extract was collected and kept in a freeze at - 20c° until use [11].

Experimental design: Twenty-eight adult male rats were divided into four groups equally and treated orally by stomach tube for a period thirty days as following:

The first group(G1): Seven adult male rats were intubated with distilled water only, as a negative control group.

The second group(G2): Seven adult male rats were treated with Sorafenib at dose (200mg/kg/B.W.) as positive control group.

The third group(G3): the animals were treated with *Cicer arietinum* extract at dose (314.9 mg/kg).

The fourth group (G4): Seven adult male rats were treated with Sorafenib at dose (200mg/kg/B.W.) and *Cicer arietinum* extract (314.9mg/kg).

Preparation of Stock Solutions, Concentrations, and Doses: Stock solutions of *Cicer arietinum* extract at doses 150, 200, 250, 300, and 350mg/kg were prepared to get the final concentrations of *Cicer arietinum* extract included 150, 200, 250, 300 and 350 mg/ml respectively. Each 0.3ml was given to 100gm body weight. Then, the doses were adjusted according to the body weight of each male rat. The recommended human dose of Sorafenib 2.85 mg/kg B.W. according to [12] B.W. was converted to animal dose by multiplying the human dose by factor 6.2 [13], to get the final concentration of stock solution 8.855mg/ ml, in addition, every 0.3 ml of the stock solution of Sorafenib was given orally to each 100g of rat body weight by stomach.

Chromosomal aberration: The Metaphase cells were prepared according to the standard technique [14].

Mitotic index: The MI %was determined as a ratio of the mitotic cells to the cells in interphase in 1000 calculated cells. M.I. %=No. of dividing cells in metaphase / (Total No. of dividing cells +No. of non-dividing cells (1000) cells) X 100 [15].

DNA fragmentation: The assay procedure was adapted from the reported method of [16].

Statistical analysis: Statistical analysis of data was performed on the basis of One way and Two-way analysis of Variance (ANOVA) using a significant level of ($p<0.05$). Specific groups differences were determined using Least Significant Differences (LSD) as described by [17].

3. Results and Discussions

Extraction of Cicer arietinum

The *Cicer arietinum* seeds were extracted using alcoholic solvent 70% and the percentage of extract yield was 14 %, the crude alcoholic extract was obtained and taken to full dryness to form deep yellow in color and semisolid texture of extract. In this study, the *Cicer arietinum* seeds crude extract was converted to thick, semisolid mass of dark yellow color, this result agreed with finding reported by [11].

Chromosomal aberration

The results of chromosomal aberration in table 1 showed significant P≤0.05 increase in Chromosomal Aberration % (Acentric%, Polyploidy%, Ring%) of positive control group as compared with all other treated groups. While Acentric%, Polyploidy%, Ring% of positive *Cicer arietinum* treated group showed no significant difference (P≤ 0.05), as compared with negative control group. Furthermore, the Acentric% of *Cicer arietinum* and sorafenib treated group showed a significant decrease (P≤0.05) as compared with all other treated groups. Whereas, the Polyploidy% and Ring% of *Cicer arietinum* and sorafenib treated group showed no significant difference (P≤0.05) as compared with negative control group. As well as, the chromosomal aberrations were shown in figure (1), in which the acentric, break, Aneuploidy and ring were appeared.

Table (1): Effect of sorafenib, *Cicer arietinum* and *Cicer arietinum* with sorafenib on Chromosomal Aberration % of male rats for 30 days.

Groups	Chromosomal Aberration %				
	Acentric%	Deletion%	Polyploidy%	Ring%	Total
G1	0.13± 0.07 b	0.11± 0.005 b	0.10± 0.02 c	0.14 ± 0.02 c	0.48± 0.07 b
G2	0.20± 0.02 a	0.27± 0.02 b	2.09± 0.25 a	1.52 ± 0.18 a	4.08± 0.25 a
G3	0.12± 0.008 b	0.09± 0.008 c	0.08± 0.03 c	0.11± 0.004 c	0.40 ± 0.01 b
G4	0.16±0.009c	0.11±0.006 b	0.14±0.03 b	0.16 ±0.01 b	0.57 ± 0.03 b
LSD	0.102	0.032	0.313	0.196	0.391

* Mean ± Standard Deviation. a, b, c means in the same raw with different superscripts differ significantly at probability value (P≤0.05).

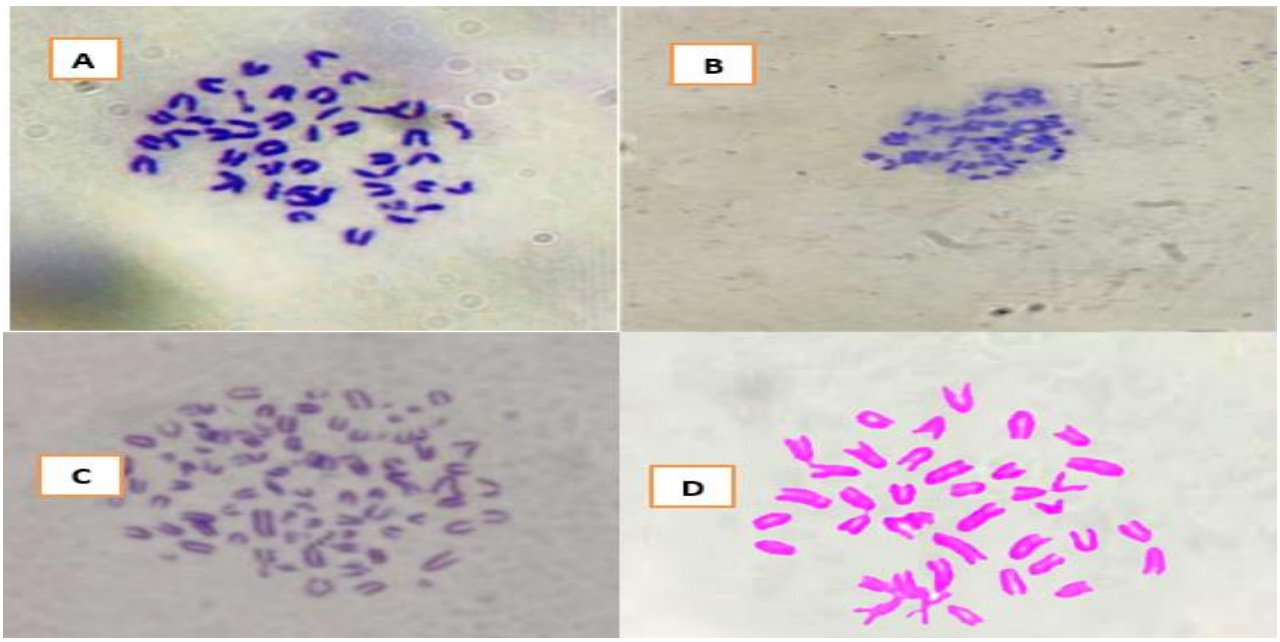


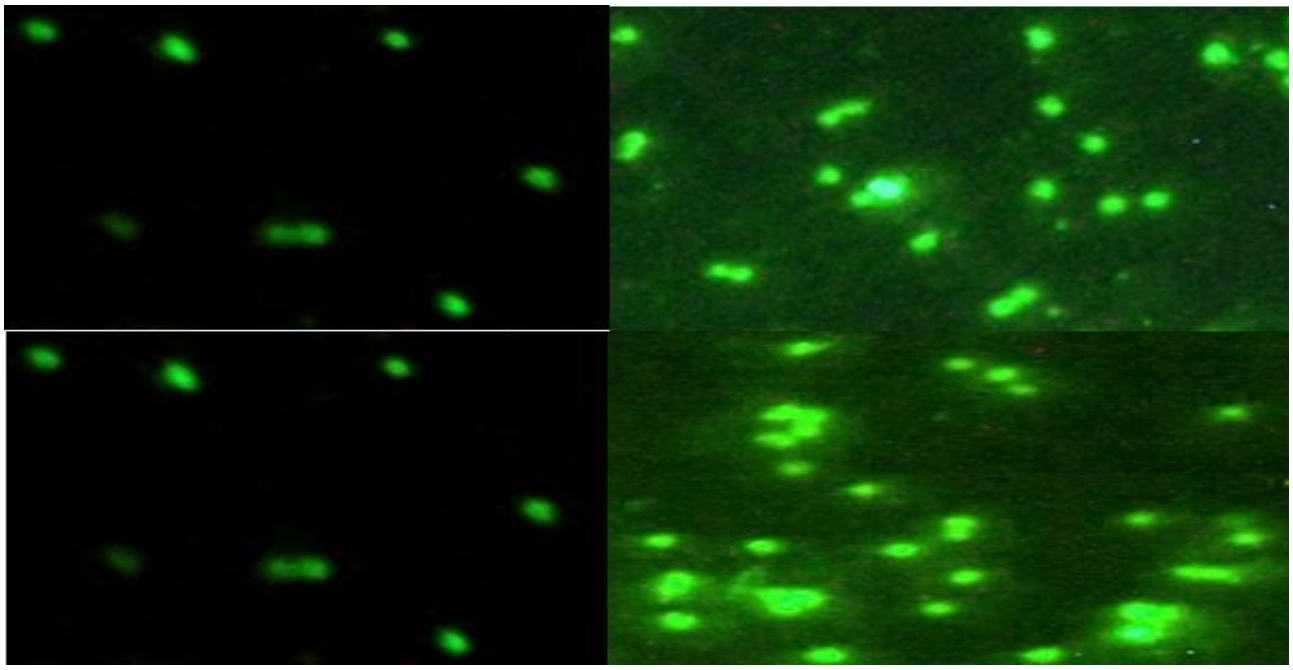
Figure (1): Chromosomal aberration in different experimental groups. A: Acentric, B: Break, C: Aneuploidy, D: Ring.

Mitotic Index and DNA Fragmentation: The results of Mitotic Index revealed significant P≤ 0.05 increases in mitotic index of animals exposed to 200mg/kg.bw when compared with groups of plants and control negative group Table (2).For the DNA fragmentation the results revealed significant P≤ 0.05 increases in fragmented DNA of animals exposed to 200mg/kg.bw when compared with groups of plants and control negative group table (2).

Table (2): Effect of sorafenib, *Cicer arietinum* and *Cicer arietinum* with sorafenib on Mitotic index and DNA fragmentation of male rats for 30 days.

Groups	Mitotic index	DNA Fragmentation
G1	6.4± 0.12 b	11.26±0.58 c
G2	10.3± 0.28 a	38.19±0.48 a
G3	5.48 ± 0.09 c	10.61±0.92 c
G4	6.98 ±0.14 b	16.38±0.69 b
LSD	0.594	3.016

* Mean ± Standard Deviation a, b, c: means in the same raw with different superscripts differ significantly at probability value (P≤0.05)



Recently, plant-derived natural compounds or phytochemicals are gaining increasing importance (due to their wide availability, low toxicity, and safety) to analyze their protective effects against drug-induced toxicity. In the present study, experiments were performed to screen whether *C. arietinum* has any potential preventive effects against the Sorafenib-induced toxicity. The genotoxicity of

Sorafenib was studied with respect to gene mutations in bacteria, chromosomal aberrations in vitro in Chinese hamster V79 cells, and in vivo in the mouse micronucleus test in bone marrow. Sorafenib was investigated using the Salmonella/microsome plate incorporation test for point mutagenic effects in doses of up to 5000 µg per plate on five Salmonella typhimurium LT2 mutants [18]. In the present study, the results showed the combination treatment with *C. arietinum* and Sorafenib in *Cicer arietinum* **and sorafenib group** showed a preventive and protective effect of *C. arietinum* as indicated by a decrease in the mean number of chromosomal aberrations when compared with **Positive control group** that received just Sorafenib, thus confirming the anti-genotoxic/protective effects of *C. arietinum*. The genotoxicity assays revealed that Sorafenib had a statistically significant mutagenic effect on the chromosomes, indicating an increase in the mean number of chromosomal aberrations, these findings were in the same line of a studies of [19] and [20]. In the current study the results of chromosomal aberration in *Cicer arietinum* **treated group** and *Cicer arietinum* **and sorafenib group** that dosing *C. arietinum* ethanolic extract showed protective effect against cytotoxicity induced by Sorafenib. These findings could be linked to the bioactive components such as flavonoids, saponins, alkaloids in *Cicer arietinum* seeds which reported by many studies [21; 22]. The ability of *Cicer arietinum* to preserve chromosomes confirmed its safety on the molecular level. Thus, the present study explained the supportive role of *C. arietinum* in cellular function by the molecular mechanisms. The oxidative damage is one of the mechanisms that can elicit DNA fragmentation and oxidation [21, 23]. Results in the present study showed that mitotic index decreases as abnormality index increased. The altered mitotic rate of the plant subjected to Sorafenib is mostly attributed to interference in normal steps of mitosis and spindle formation [24], failure of DNA replication and proteins synthesis [25], enzyme production, function and regulation and low level of ATP generation due to decreased oxidative photophosphorylation induced by Sorafenib over ROS production [26]. In addition, the diet rich in anti-oxidants scavenges different kinds of ROS, thereby preventing chromosomal aberration development [27]. In our study, the results showed that Sorafenib treated group **Positive control group** revealed maximum ROS leading to induced cytotoxicity, as discussed and reported by [28] when they mentioned that formation of ROS by Sorafenib.

4. Conclusion

Cicer arietinum seeds extract had protective cytogenetic effect against chemotherapy drugs such as Sorafenib by several mechanisms regarded to anti-oxidants scavenges different kinds of ROS, thereby preventing chromosomal aberration development.

References

1- Luqmani YA. Mechanisms of drug resistance in cancer chemotherapy. Medical principles and practice. 2005;14(Suppl. 1):35-48. https://www.karger.com/Article/PDF/86183

2- Chu E, Sartorelli AC. Cancer chemotherapy. Lange's Basic and Clinical Pharmacology,. 2018:948-76. https://d1wqtxts1xzle7.cloudfront.net/62835124/Katzung2.

3- Miltenburg NC, Boogerd W. Chemotherapy-induced neuropathy: a comprehensive survey. Cancer treatment reviews. 2014 Aug 1;40(7):872-82. https://www.sciencedirect.com/science/article/abs/pii/S0305737

4- Takimoto CH, Awada A. Safety and anti-tumor activity of sorafenib (Nexavar®) in combination with other anti-cancer agents: a review of clinical trials. Cancer chemotherapy and pharmacology. 2008 Apr;61(4):535-48. https://link.springer.com/article/10.1007/s00280-007-0639-9

5- Blanchet B, Billemont B, Cramard J, Benichou AS, Chhun S, Harcouet L, Ropert S, Dauphin A, Goldwasser F, Tod M. Validation of an HPLC-UV method for sorafenib determination in human plasma and application to cancer patients in routine clinical practice. Journal of pharmaceutical and biomedical analysis. 2009 May 1;49(4):1109-14. https://www.sciencedirect.com/science/article/abs/pii/S0731708509001125

6- Je Y, Schutz FA, Choueiri TK. Risk of bleeding with vascular endothelial growth factor receptor tyrosine-kinase inhibitors sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. The lancet oncology. 2009 Oct 1;10(10):967-74. https://www.sciencedirect.com/science/article/abs/pii/S1470204509702220

7- Aslam S, Jahan N, Rehman KU, Asi MR. Development of sodium lauryl sulphate stabilized nanosuspension of Coriandrum sativum to enhance its oral bioavailability. Journal of Drug Delivery Science and Technology. 2020 Dec 1;60:101957.https://www.sciencedirect.com/science/article/abs/pii/S1773224720312466.

8- Wasan, H.S., Gibbs, P., Sharma, N.K., Taieb, J., Heinemann, V., Ricke, J., Peeters, M., Findlay, M., Weaver, A., Mills, J. and Wilson, C., 2017. First-line selective internal radiotherapy plus chemotherapy versus chemotherapy alone in patients with liver metastases from colorectal cancer (FOXFIRE, SIRFLOX, and FOXFIRE-Global): a combined analysis of three multicentre, randomised, phase 3 trials. *The Lancet Oncology*, *18*(9), pp.1159-1171. https://www.sciencedirect.com/science/article/pii/S1470204517304576

9- Al-Snafi AE. Medicinal plants with anticancer effects (part 2)-plant based review. Sch Acad J Pharm. 2016;5(5):175-93.

https://d1wqtxts1xzle7.cloudfront.net/51039937/149-with-cover-page-

10- Huang Y, Xin M, Li Q, Luo X, Wang X, Zou X, Liu X, Chen B, Tang J. Chickpea seeds ferritin as a potential source in the treatment of iron deficiency anemia. Journal of Food and Nutrition Research. 2014 Nov 5;2(12):876-9. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.962.7583&rep=rep1&type=pdf

11- Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30. https://books.google.iq/books?hl

12- Shailendra B, Shailendra M, Manish J, Singh TY, Priti T. Design and optimization of domperidone fast dissolving tablet using central composite design. Current Drug Delivery. 2015 Dec 1;12(6):736-44. https://www.ingentaconnect.com/content/ben/cdd/2015/00000012/00000006/art00013

13- Kumar S, Ravulapalli SY, Tiwari SK, Gupta S, Nair AB, Jacob S. Effect of sex and food on the pharmacokinetics of different classes of BCS drugs in rats after cassette administration. International Journal of Pharmaceutics. 2021 Dec 15;610:121221. https://www.sciencedirect.com/science/article/abs/pii/S0378517321010279

14- Agarwal K, Sharma A, Talukder G. Clastogenic effects of copper sulphate on the bone marrow chromosomes of mice in vivo. Mutation Research Letters. 1990 Jan 1;243(1):1-6. https://www.sciencedirect.com/science/article/abs/pii/016579929090115Z

15- Collan YU, Kuopio T, Baak JP, Becker R, Bogomoletz WV, Deverell M, Van Diest P, Van Galen C, Gilchrist K, Javed A, Kosma VM. Standardized mitotic counts in breast cancer evaluation of the method. Pathology-Research and Practice. 1996 Jan 1;192(9):931-41. https://www.sciencedirect.com/science/article/abs/pii/S0344033896800756

16- Abid-Essefi S, Baudrimont I, Hassen W, Ouanes Z, Mobio TA, Anane R, Creppy EE, Bacha H. DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: prevention by Vitamin E. Toxicology. 2003 Nov 5;192(2-3):237-48. https://www.sciencedirect.com/science/article/abs/pii/S0300483X0300329.

17- Al-Ukaelii SA, Al-Shaeb SM. Statically Analysis by used SPSS program. Al-Shoroq house for Publishers and advertisement Amaan, Jordan. 1998. https://www.researchgate.net/profile/Majid

18- Kieboom J, Abee T. Arginine-dependent acid resistance in Salmonella enterica serovar Typhimurium. Journal of Bacteriology. 2006 Aug 1;188(15):5650-3. https://journals.asm.org/doi/full/10.1128/JB.00323-06.

19- Das S, Das J, Paul A, Samadder A, Khuda-Bukhsh AR. Apigenin, a bioactive flavonoid from Lycopodium clavatum, stimulates nucleotide excision repair genes to protect skin keratinocytes from ultraviolet B-induced reactive oxygen species and DNA damage. J Acupunct Meridian Stud. 2013 Oct;6(5):252-62. https://www.sciencedirect.com/science/article/pii/S2005290113001532

20- Singh D, Khan MA, Akhtar K, Arjmand F, Siddique HR. Protective Effects of Dietary Flavonoid, Apigenin Against Sorafenib-Induced Oxidative, Genetic, and Hepato-Renal Toxicity in Swiss Albino Mice.32(5):2022. https://www.sciencedirect.com/science/article/abs/pii/S0041008X22002174

21- Sayed AA, Ali AA, Mohamed HR. Fertility enhancing efficacy of Cicer arietinum in male albino mice. Cellular and Molecular Biology. 2018 Mar 31;64(4):29-38. http://www.cellmolbiol.org/index.php/CMB/article/view/1733

22- Gaur PM, Samineni S, Thudi M, Tripathi S, Sajja SB, Jayalakshmi V, Mannur DM, Vijayakumar AG, Ganga Rao NV, Ojiewo C, Fikre A. Integrated breeding approaches for improving drought and heat adaptation in chickpea (Cicer arietinum L.). Plant Breeding. 2019 Aug;138(4):389-400. https://onlinelibrary.wiley.com/doi/full/10.1111/pbr.12641.

23- Mittal G, Vadhera S, Brar AP, Soni G. Protective role of chickpea seed coat fibre on N-nitrosodiethylamine-induced toxicity in hypercholesterolemic rats. Experimental and Toxicologic Pathology. 2009 Jul 1;61(4):363-70. https://www.sciencedirect.com/science/article/abs/pii/S0940299308001474

24- Qureshi ST, Memon SA, Abassi AR, Sial MA, Bughio FA. Radiofrequency radiations induced genotoxic and carcinogenic effects on chickpea (Cicer arietinum L.) root tip cells. Saudi journal of biological sciences. 2017 May 1;24(4):883-91. https://www.sciencedirect.com/science/article/pii/S1319562X16000589

25- Shen B, Singh P, Liu R, Qiu J, Zheng L, Finger LD, Alas S. Multiple but dissectible functions of FEN-1 nucleases in nucleic acid processing, genome stability and diseases. Bioessays. 2005 Jul;27(7):717-29. https://onlinelibrary.wiley.com/doi/abs/10.1002/bies.20255

26- Hao H, Zhang D, Shi J, Wang Y, Chen L, Guo Y, Ma J, Jiang X, Jiang H. Sorafenib induces autophagic cell death and apoptosis in hepatic stellate cell through the JNK and Akt signaling pathways. Anti-cancer drugs. 2016 Mar 1;27(3):192-203. https://www.ingentaconnect.com/content/wk/ancad/2016/00000027/00000003/art00006

27- Cho E, Allemang A, Audebert M, Chauhan V, Dertinger S, Hendriks G, Luijten M, Marchetti F, Minocherhomji S, Pfuhler S, Roberts DJ. AOP report: Development of an adverse outcome pathway for oxidative DNA damage leading to mutations and

chromosomal aberrations. Environmental and Molecular Mutagenesis. 2022 Mar 21. https://onlinelibrary.wiley.com/doi/full/10.1002/em.22479

28- Islam BU, Habib S, Ahmad P, Allarakha S, Moinuddin, Ali A. Pathophysiological Role of Peroxynitrite Induced DNA Damage in Human Diseases: A Special Focus on Poly(ADP-ribose) Polymerase (PARP). Indian J Clin Biochem. 2015 Oct;30(4):368-85. https://link.springer.com/article/10.1007/s12291-014-0475-8