Glyphosate Induced Detrimental Changes In Enzymatic Antioxidants In Experimental Rats

Running title: Glyphosate induced changes in antioxidant levels

ABSTRACT:

BACKGROUND:
Glyphosate is the most enormously used broad spectrum herbicide in the world. Current assessment of carcinogenic capability of glyphosate based herbicides by various regional, national and international agencies have endangered the controversy .Antioxidant enzymes are often used as biomarkers of oxidative stress.Among the biomarkers SOD,GPx,CAT were essential in conservation of homeostasis of cell to function as normal being.

AIM:
To investigate glyphosate induced detrimental changes in the enzymatic antioxidants in experimental rats.

MATERIALS AND METHODS:
Adult male wistar albino rats were divided into 4 groups, each consisting of 6 animals.Group 1 consists of Normal control rats, Group 2 consists of Glyphosate treated at a dose of 50mg/kg body weight/day. Group 3 consists of Glyphosate treated at a dose of 100 mg/kg body weight/day. Group 4 consists of Glyphosate treated at a dose of 250 mg/kg body weight/day..The experimental period was 16 weeks. All chemicals and reagents used in this study were purchased from sigma chemical company,USA.Adult male albino rats weighing 180-200g were used for the study. Parameters analysed were assay of Superoxide Dismutase(SOD),Catalase(CAT),Glutathione Peroxidase(GPx). The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test and it was used to see the statistical significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.

RESULTS:
There was a decrease in the levels of enzymatic antioxidants in experimental rats, when exposed to glyphosate and each bar represents mean$\mp$ SEM (n=6) which is significance at $P<0.05$

CONCLUSION:
Glyphosate has induced oxidative stress in experimental animals by decreasing the expression of Enzymatic Antioxidants.

KEY WORDS:

Glyphosate; Detrimental changes; Enzymatic antioxidants; Experimental rat, innovative technology; novel method.

INTRODUCTION:

Glyphosate is the most enormously used broad spectrum herbicide in the world. Current assessment of carcinogenic capability of glyphosate based herbicides by various regional, national and international agencies have endangered the controversy(1).In glyphosate based herbicides the revealed principle G is mixed with many adjuvants which help to pierce the cell membrane of plants(Gress et al., 2015)(2).Glyphosate,an N-(Phosphonomethyl)glycine is the main constituent in Monsanto ROUNDUP Herbicide.(Owagboriaye et al., 2017). Recent findings indicate glyphAntioxidant enzymes are often used as bio markers of oxidative stress.Among the biomarkers Superoxide dismutase(SOD),Glutathione peroxidase(GPx),Catalase(CAT) were essential in conservation of homeostasis for cell to function as normal(El-Shenawy, 2009).The mRNA expression of Catalase,Glutathione Peroxidase,Cu/Zn Superoxide Dismutase after the 2 weeks the introduction of diabetes was not different from that in the control rats (3). Antioxidant system (TAS,TOS, OSI,PON, Arytelase, Catalase and MDA) and the level of GST plays an important role in mechanism of detoxification in various organs such as liver,lung,kidney and mitochondrial fractions in rats with hyperglycemia where non chronically developed with STZ(Streptozotocin) which is a diabetogenic chemical agent(3,4)osate and its metabolites can also diffuse by wind and soil erosion.(5).Glyphosate which is used as weed control for both domestic horticulture and large crops..(6)

In reality,excessive development of ROS (Reactive Oxygen Species)brings about issues like Lipid Peroxidation and Injury of DNA . Extreme evaluation of ROS give on to construction of cytotoxic metabolites which can induce irreversible disorders like damage of DNA, Peroxidation of lipid.These changes were in agreement with the worsening of kidney function, signifying that renal I/R(Ischaemia\Reperfusion)induced kidney ROS formation, reduced the capacity of cells to get rid of ROS, augmented endogenous antioxidant depletion, and deteriorated renal injury(7) Glyphosate (N-phosphonomethyl [glycine], is an organophosphorus compound with herbicide properties discovered in 1970. It's A competitive inhibitor of the 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme involved in aromatic amino acid biosynthesis in plants and microorganisms(8). In 1974, Monsanto started its commercialization as a broad-spectrum herbicide. This first glyphosate-based herbicide (GBH), RoundUpVR , and the others that followed such as GlyphoganVR , TouchdownVR , or Golflogix VR , are mixtures of glyphosate and various adjuvants used to boost its penetration in plants and enhance its activity(9) .

Antioxidants are the constituents manufactured by the body to neutralize the cause of free radicals, but the outcome will be restricted to particular antioxidants. In the human body oxidants and antioxidant ratio will be continued, any changes in these oxidants and antioxidants will bring about collection of ROS within the body, this process is called as oxidative stress.(10)Oxidative stress has a fundamental part in harm of tissue and watches out for neurotic conditions like malignancy.(11)). (12)Glyphosate based herbicides mainly represented by roundup are the most widely used commercial formulations of pesticides worldwide.(12,13) Glyphosate has low harmfulness in warm blooded creatures like mammals. Our team has extensive knowledge and research experience that has translate into high quality publications (14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29),(30),(31),(32),(33) . Hence the present study has been designed to fulfill the deficiency about Glyphosate induced detrimental changes in enzymatic antioxidants in experimental rats.

MATERIALS AND METHODS:

CHEMICALS:

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. glyphosate was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. Dopamine Receptor, Serotonin receptor (The serotonin 1A receptor) and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India and. Animals The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/02-2019/015).

EXPERIMENTAL ANIMALS:

Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha Dental College and Hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. ,

EXPERIMENTAL GROUPS:

Rats were divided into 3 groups, each consisting of 6 animals. Experimental Design Group I Normal control rats fed with normal diet and drinking water Group II Glyphosate treated (dissolved in water at a dose of 50 mg/kg body weight/day at 8 to AM) orally for 16 weeks Group III Glyphosate treated (dissolved in water at a dose of 100 mg/kg body weight/day at 8 to AM) orally for 16 weeks Group IV Glyphosate treated (dissolved in water at a dose of 250 mg/kg body weight/day at 8 to AM) orally for 16 weeks At the end of the treatment, animals were anesthetized with sodium thiopentone (40 mg/kg b.wt), blood was collected through cardiac puncture, sera were separated and stored at −80°C, and 20 ml of isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Gastronemous muscle from control and experimental animals was immediately dissected out and used for assessing the various parameters

Assessment of fasting blood glucose (FBG) :

After the overnight fasting, the blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA). From the rat tail tip the blood was collected and results were expressed as mg/dl

Oral glucose tolerance test (OGTT) :

For the oral glucose tolerance test, animals were fasted overnight. After giving the oral glucose load (10 ml/kg; 50% w/v). blood glucose level was estimated at various time periods (60, 120, and 180 min) by using On-Call Plus blood glucose test strips. Before giving glucose load, the value of blood glucose is considered as 0 min value. Results were marked as mg/dl.

Procedure

Assay of superoxide dismutase (SOD)

Superoxide dismutase was assayed by the method of Marklund and Marklund (1974). Procedure To 1 ml of tissue homogenates 0.25 ml of ethanol and 1.25 ml of chloroform were added, kept in a mechanical shaker for 15 min and centrifuged at 20000xg for 15min. To 0.5 ml of the supernatant, 2.0 ml of 0.1 M Tris-HCl buffer pH 8.2; 1.5 ml of distilled water and 0.5 ml of pyrogallol were added. Change in optical density at 0, 1 and 3 min was read at 420 nm in a spectrophotometer. Control tubes containing 0.5 ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Units/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation.

Assay of Catalase (CAT)

Catalase activity was assayed by the method of Sinha (1972). To 0.1 ml of tissue homogenates 1.0 ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted, and then 2.0ml of Dichromate acetic acid was added. The green colour developed was read at 570 nm using a spectrophotometer. Catalase activity is expressed as μmoles of H2O2 consumed/min/mg protein.

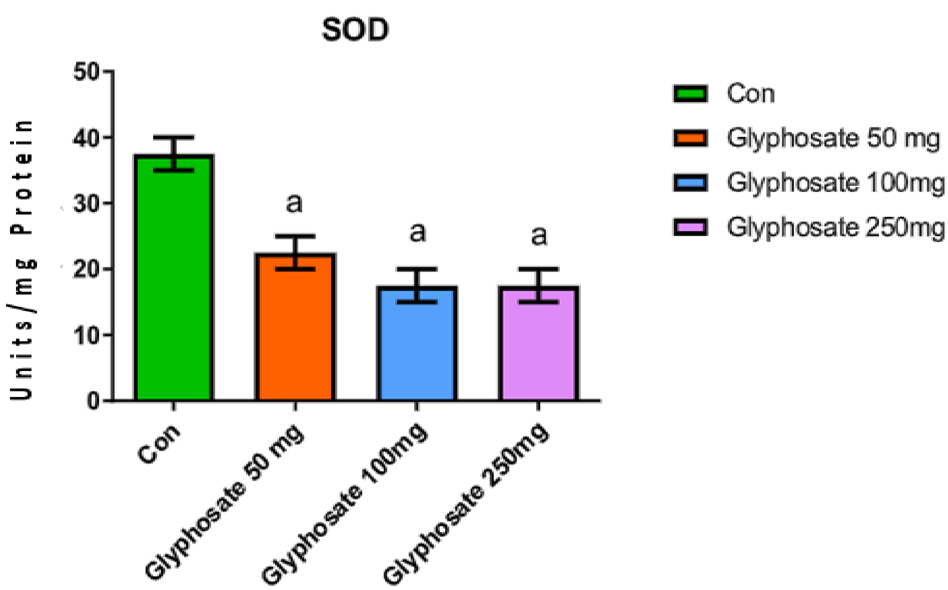
Assay of Glutathione peroxidase (GPX)

Glutathione peroxidase was assayed by the method of Rotruck et al. (1973). 0.2 ml each of EDTA, sodium azide, GSH, H2O2, buffer and tissue homogenates were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.0 ml of phosphate solution and 1.0 ml of DTNB were added and the colour developed was read at 420 nm immediately against blank containing only phosphate solution and DTNB reagent. Graded amounts of standards were also treated similarly. GPX activity is expressed as μg of glutathione utilized/min/mg protein

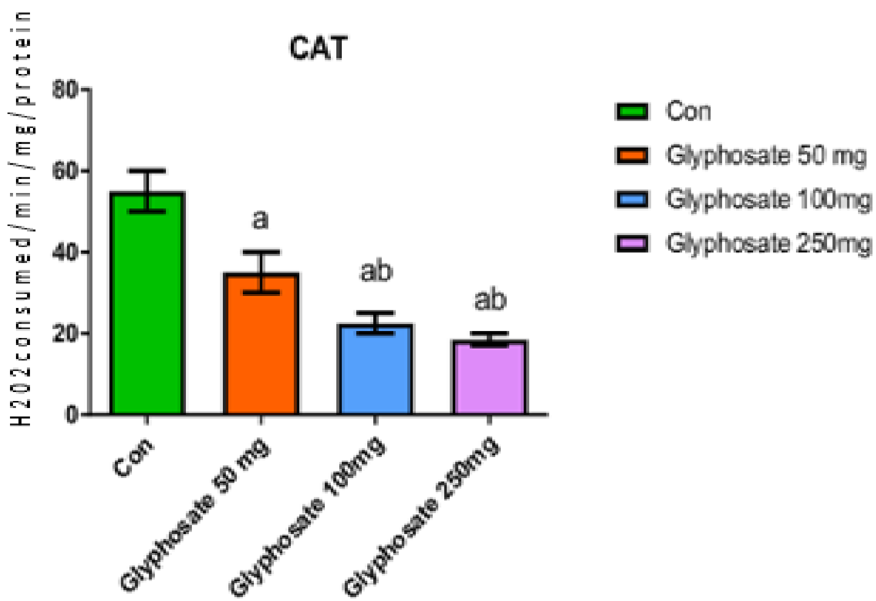
STATISTICAL ANALYSIS:

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation.Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism Version 5. The results with the $p<0.05$ level were considered to be statistically significant.

RESULTS AND DISCUSSION:



Graph 1 represents the impact of glyphosate on antioxidant- SOD(Superoxide Dismutase) activity in adult male albino wistar rats. The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Superoxide Dismutase enzyme (SOD).Green colour represents the controlled rats. Orange colour represents Group 1 rats exposed to about 50 mg of glyphosate. Blue represents Group 2 rats exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats exposed to about 250 mg of glyphosate. Each bar represents mean∓ SEM (n=6). Significance at $P < 0.05$glyphosate.



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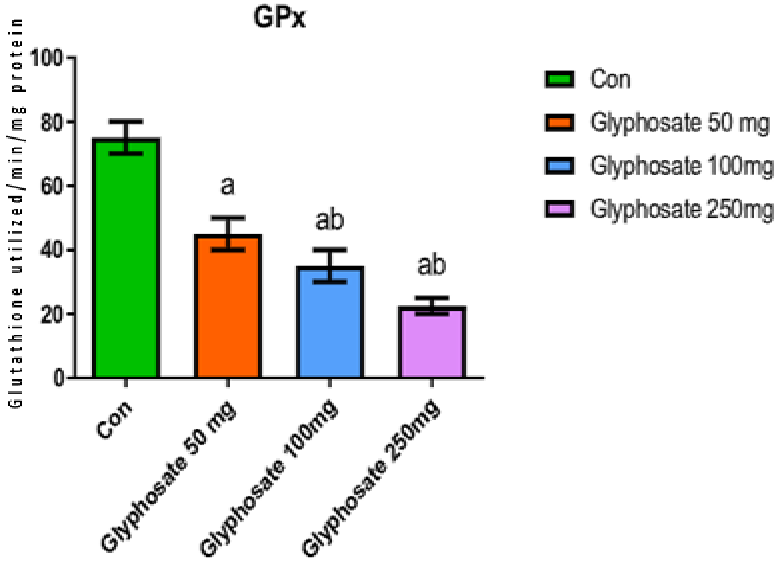
Graph 2 represents the impact of glyphosate on antioxidant- CAT(Catalase) activity in adult male albino wistar rats. The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Catalase enzyme (CAT).Green colour represents the controlled rats. Orange colour represents Group 1 rats exposed to about 50 mg of glyphosate. Blue Group 2 represents rats exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats exposed to about 250 mg of glyphosate. Each bar represents mean∓ SEM (n=6). Significance at $P<0.05$.



Graph 3 represents the impact of glyphosate on antioxidant- GPx (Glutathione peroxidase) activity in adult male albino wistar rats.The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Glutathione peroxidase enzyme (GPx).Green colour represents the controlled rats. Orange colour represents Group 1 rats who were exposed to about 50 mg of glyphosate. Blue colour represents Group 2 rats who were exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats who were exposed to about 250 mg of glyphosate. Each bar represents mean∓ SEM (n=6). Significance at P<0.05

From the Graph 1 we know that,Group 1 rats were exposed to about 50 mg of glyphosate there is a decrease in the activity of SOD from change over value , Group 2 rats were exposed to about 100 mg of glyphosate there is more decrease in the activity of SOD from change over value ,Group 3 rats were exposed to about 250 mg of glyphosate there is also a decrease in the activity of SOD.From the Graph 2 we know that,Group 1 rats were exposed to about 50 mg of glyphosate there is a decrease in the activity of CAT, Group 2 rats were exposed to about 100 mg of glyphosate there is more decrease in the activity of CAT,Group 3 rats were exposed to about 250 mg of glyphosate there is more decrease in the activity of CAT.From the Graph 3 we know that,Group 1 rats were exposed to about 50 mg of glyphosate there is a decrease in the activity of GPx, Group 2 rats were exposed to about 100 mg of glyphosate there is more decrease in the activity of GPx.Group 3 rats were exposed to about 250 mg of glyphosate there is more decrease in the activity of GPx. Antioxidants play a significant role in protecting the cells by inhibiting the oxidative stress while glyphosate is exposed to rats. These enzymatic antioxidants level gets decreased and there is a dose dependent decrease in the activity of these enzymes on glyphosate exposure.

SOD,CAT,GPx are three important antioxidants which play a major role in protecting the cell. In this study we observed the effect of glyphosate on SOD,CAT,GPx.When glyphosate was given to rats, these enzymatic antioxidant levels got decreased because of glyphosate induced oxidative stress.Antioxidants are natural defence mechanism which fights against free radicals which result in oxidative stress. In most of the cases, free radical accumulation may lead to various non pathological disorders like Diabetes mellitus, Cancer, Ageing related disorders etc. The imbalance between the antioxidant and free radicals levels is the root cause for the generation of free radicals and its associated disorders. In the current study, after the administration of non specific herbicide- Glyphosate has resulted in the decrease in the expression of the natural defence mechanism- SOD,GPx,CAT. The study has focussed on the alarming decrease in the levels of the natural antioxidants and other related consequences.From the previous study, it has been proved that the administration of Rutin (polyphenolic flavonoid) in streptozotocin-induced diabetes wistar rats has resulted in the increase of non- enzymatic anti- oxidants significantly($P<0.05$). Treatment of normal rats with Rutin did not significantly alter any of the parameters studied. These results show that Rutin exhibits anti-hyperglycemic and anti- oxidant activity in streptozotocin-induced diabetic rats(34). The study by Magdalena Gorny et al has proved the alternations in the antioxidant enzyme activities in the neurodevelopmental rats model. Glutathione deficiency during early postnatal life has resulted in the decrease in SOD activity. Comparison of CAT activity in kidney examined groups did not show significant differences between them((4,35) . Previous study byCindy Peillex etal has proved the role of antioxidants in diabetes -induced oxidative stress in glomeruli of daibetic rats.Decreased antioxidant levels has resulted in excessive oxidative stress.(9)). From the study it was evident that prolonged use of Glyphosate can lead to detrimental decrease in the enzymatic antioxidants, which needs to be taken seriously.The examination was finished with only three fluctuated portions of glyphosate .Studying more shifted dosages will help in acquiring more precise consequences for articulation with additional time and test.In future, other associated parameters also need to to be checked in order to prove the ill effects of prolonged exposure to this non specific herbicide.

CONCLUSION:

Antioxidants play a significant chemical role in protecting the cells by inhibiting oxidative stress while glyphosate is given to male albino wistar rats these enzymatic antioxidants levels decrease and there is dose dependent decrease in the activity of enzymes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

REFERENCES:

1. Zhang L, Rana I, Shaffer RM, Taioli E, Sheppard L. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence [Internet]. Vol. 781, Mutation Research/Reviews in Mutation Research. 2019. p. 186–206. Available from: http://dx.doi.org/10.1016/j.mrrev.2019.02.001

2. Gress S, Lemoine S, Séralini G-E, Puddu PE. Glyphosate-based herbicides potently affect cardiovascular system in mammals: review of the literature. Cardiovasc Toxicol. 2015 Apr;15(2):117–26.

3. Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. J Am Soc Nephrol. 2003 Aug;14(8 Suppl 3):S250–3.

4. Çelık VK, Şahın ZD, Sari İ, Bakir S. Comparison of oxidant/antioxidant, detoxification systems in various tissue homogenates and mitochondria of rats with diabetes induced by streptozocin. Exp Diabetes Res. 2012 Mar 28;2012:386831.

5. Owagboriaye FO, Dedeke GA, Ademolu KO, Olujimi OO, Ashidi JS, Adeyinka AA. Reproductive toxicity of Roundup herbicide exposure in male albino rat. Exp Toxicol Pathol. 2017 Sep 5;69(7):461–8.

6. Milesi MM, Lorenz V, Beldomenico PM, Vaira S, Varayoud J, Luque EH. Correction to: Response to comments on: Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats. Arch Toxicol. 2020 Aug;94(8):2897–8.

7. Moslemi Z, Gheitasi I, Doustimotlagh AH. Protective Effect of Hydroalcoholic Extract of Stachys pilifera on Oxidant-Antioxidant Status in Renal Ischemia/Reperfusion Injuries in Male Rats [Internet]. Vol. 2021, Journal of Toxicology. 2021. p. 1–8. Available from: http://dx.doi.org/10.1155/2021/6646963

8. Mesnage R, Defarge N, de Vendômois JS, Séralini GE. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits [Internet]. Vol. 84, Food and Chemical Toxicology. 2015. p. 133–53. Available from: http://dx.doi.org/10.1016/j.fct.2015.08.012

9. Peillex C, Pelletier M. The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. J Immunotoxicol. 2020 Dec;17(1):163–74.

10. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health [Internet]. Vol. 4, Pharmacognosy Reviews. 2010. p. 118.

Available from: http://dx.doi.org/10.4103/0973-7847.70902

11. Kattappagari K, Teja CSR, Kommalapati R, Poosarla C, Gontu S, Reddy BR. Role of antioxidants in facilitating the body functions: A review [Internet]. Vol. 7, Journal of Orofacial Sciences. 2015. p. 71. Available from: http://dx.doi.org/10.4103/0975-8844.169745

12. El-Shenawy NS. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate [Internet]. Vol. 28, Environmental Toxicology and Pharmacology. 2009. p. 379–85. Available from: http://dx.doi.org/10.1016/j.etap.2009.06.001

13. Gillezeau C, van Gerwen M, Shaffer RM, Rana I, Zhang L, Sheppard L, et al. The evidence of human exposure to glyphosate: a review. Environ Health. 2019 Jan 7;18(1):2.

14. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.

15. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019 Aug;197:111518.

16. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111773

17. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020 Nov;131:110702.

18. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020 Dec;48(1):1206–13.

19. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3577–84.

20. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018 Nov;107:1514–22.

21. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert

Truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea [Internet]. Food Reviews International. 2021. p. 1–20. Available from: http://dx.doi.org/10.1080/87559129.2021.1889581

22. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environmental Nanotechnology, Monitoring & Management. 2020 Dec 1;14:100333.

23. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitroandin vivostudies. Arabian Journal of Chemistry. 2020 May 1;13(5):5501–16.

24. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020 Oct 27;1–8.

25. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

26. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

27. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.

28. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells [Internet]. Vol. 119, Materials Science and Engineering: C. 2021. p. 111656. Available from: http://dx.doi.org/10.1016/j.msec.2020.111656

29. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021 Jun;36(6):1011–20.

30. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021 Jul;28(7):3633–40.

31. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021 Jul;28(7):3641–9.

32. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15,

Journal of Dental Sciences. 2020. p. 562–3. Available from: http://dx.doi.org/10.1016/j.jds.2020.02.009

33. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. Cell Mol Immunol. 2020 Oct;17(10):1114–6.

34. Kamalakkannan N, Prince PSM. Antihyperglycaemic and Antioxidant Effect of Rutin, a Polyphenolic Flavonoid, in Streptozotocin-Induced Diabetic Wistar Rats [Internet]. Vol. 98, Basic Clinical Pharmacology Toxicology. 2006. p. 97–103. Available from: http://dx.doi.org/10.1111/j.1742-7843.2006.pto_241.x

35. Website [Internet]. [cited 2021 Mar 8]. Available from:)Alterations in the Antioxidant Enzyme Activities in the Neurodevelopmental Rat Model of Schizophrenia Induced by Glutathione Deficiency during Early Postnatal Life by Magdalena Górny 1,Anna Bilska-Wilkosz 1,Małgorzata Iciek 1,Marta Hereta 2,Kinga Kamińska 2,Adrianna Kamińska 3,Grażyna Chwatko 3,Zofia Rogóż 2 andElżbieta Lorenc-Koci 2,*Antioxidants 2020, 9(6), 538; https://doi.org/10.3390/antiox9060538 Received: 22 May 2020 / Revised: 10 June 2020 / Accepted: 17 June 2020 / Published: 19 June 2020