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Research Article

Effect of Aqueous Extracts of Green Tea in Arsenic induced Toxicity in Mice

Abstract

Ground water arsenic contamination is a global problem affecting thousands of people worldwide, thus present study was aimed to evaluate the hypothesis that green tea extracts, having high polyphenolic content, might be able to combat the oxidative cellular damage caused by arsenic toxicity and to prevent further development of free radicals.

In the present study treatment groups received sodium arsenite orally at the dose of 3 mg kg-1 body weight daily for 4 weeks followed by administration of Camellia sinensis (green tea) 300 mg kg-1 body weight daily by gavage method for 6 weeks. Their biochemical levels like liver and kidney function tests were assayed and were found with elevated levels. Furthermore, their free radical assessment like lipid peroxidation levels were assayed which was found to be many folds higher. But, after administration of aqueous extract of green tea, there was significant amelioration in the biochemical and lipid peroxidation levels. The protective effect of these antioxidants was shown in the form of normalization of enzymatic and non-enzymatic activities represented by normalization of liver and kidney functions.

Introduction

Production of free radicals inside the cell are main cause of toxicity, aging and diseases (El-Zayat et al, 2003). The main source of arsenic toxicity is the drinking of ground water contaminated with an inorganic form [1]. Most of the arsenic compounds are readily absorbed from the gastrointestinal tract [2]. Generally, oxidative stress of arsenic is due to the production of free radicals like super oxide and hydrogen peroxide which were supposed to initiate lipid peroxidation [3].

Liver is considered as the first target organ in arsenic metabolism where the element is subjected to methylation [4]. Cytotoxic and physiological dysfunctions in the liver, caused by arsenic toxicity, are associated with oxidative DNA damage, enhanced cell proliferation, altered DNA methylation, genomic instability and general heptotoxicity [5], Gaim et al. 2015 [6]. Kidney also has been considered as the second target organ for arsenic toxicity. Pentavalent arsenic and organic arsenic are rapidly and completely eliminated via kidney (Singh et al, 2015).

Since there is no specific treatment for chronic arsenicosis yet, therefore stopping further intake of arsenic contaminated water and drinking arsenic-free water helps to combat the arsenic issues. However, recent studies confirmed that the poorest fraction of the society are facing worst arsenic problems. So, it has become a necessity to find a safe, effective and affordable treatment of arsenic toxicity.

Thus, in the present scenario preference is being given to natural antioxidants which can be utilized in diets for controlling and treating diseases. Keeping in view present study has been conducted on natural antioxidants like green tea on liver and kidney of mice against arsenic toxicity.

Green tea, commonly consumed beverage in the world and it is a rich source of polyphenolic compounds [7,8], which is known as the tea flavonoids. Polyphenolic compounds are effective against oxidative damage in various pathological conditions. Tea polyphenols possess strong antioxidative properties [9,10], (Mandel *et al.*, 2004), antilipid peroxidation capacity [11], and are excellent chemopreventors against reactive oxygen and nitrogen species (Sarkar and Bhaduri, 2001).

Thus present study was aimed to evaluate the hypothesis that green tea extracts, having high polyphenolic content, might be able to combat the oxidative cellular damage caused by arsenic toxicity and to prevent further development of free radicals

Materials and Methods

Animals

Swiss albino mice (*Mus musculus*), weighing 30g to 35g of 8 weeks old, were obtained from animal house of Mahavir Cancer

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Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. 2015/3D-16/12/15. Food and water to mice were provided *ad libitum* (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The mice were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±2^{0}C with 12 h light/dark cycle.

Chemicals

Sodium Arsenite (98.5%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5), was obtained from the Scientific store of Patna of Bihar India.

Preparation of camellia sinensis aqueous extract

In the present study, extracts of *green tree* were procured locally from Patna, Bihar, India The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected extract of *green tea* were shade dried and were grinded to fine powder. The aqueous extract dose was calculated after LD_{50} estimation which was found to be 3000 mg kg-1 body weight and the final dose was fixed to 300mg kg^{-1} body weight.

Study groups & sampling

The control group of 6 mice received distilled water as drinking water. The treatment groups (n= 18) received Sodium arsenite daily at the dose of 3 mg kg-1 body weight for 4 weeks orally (after estimation of LD_{50} value which was found to be 8 mg kg-1 body weight) followed by administration of green tea 300mg kg-1 body weight daily by gavage method for 6 weeks. Mice were sacrificed after completion of their treatment and there blood were collected and serum were extracted for biochemical assays and lipid peroxidation estimation.

Biochemical evaluation

The Liver Function Test (LFT) were assayed by methods as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxalate Transaminase (SGOT) (Reitman & Frankel, 1957), Alkaline Phosphatase ALP (Kind & King, 1954), Bilirubin (Jendrassik & Grof, 1938). The Kidney Function Test (KFT) were assayed by methods as Urea by (Berthelot 1859 and Fawcett & Scott 1960) Uric acid by (Bones 1945) and Creatinine by (Toro & Ackermann 1975).

Lipid Peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method (Draper and Hadley 1990). The principle of the method was a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 min at 90^{0}C. After cooling in tapwater, the mixture was centrifuged at 3000g for 10min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and again incubated for 15 min at 90^{0}C. The solution was then cooled in tap water and its absorbance was measured using Thermo Scientific UV-10 (UV –Vis) spectrophotometer (USA) at 532nm.

Statistical analysis

Results are presented as mean ± SD and total variation present in a set of data was analysed through one way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnet's t-test. Calculations were performed with the Graph Pad Prism Program (Graph Pad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at $P< 0.05$.

Results

In the present study green tea extract treatment after 6 weeks showed a significant change in the biochemical parameter and LPO level. Following the green tea extract treatment, after 6 weeks the SGPT, SGOT, ALP and Bilirubin concentrations in the arsenic-treated mice decreased from 150.3 ± 2.33 U/ml to 37.00 ± 2.18 U/ml in SGPT level, from 128.7 ± 2.60 U/ml to 26.33 ± 1.32 U/ml in SGOT level, from 29.67 ± 1.76 K.A. Unit to 9.067 ± 0.49 K.A. Unit in ALP level, from 1.677 ± 0.06 mg/dl to 0.7100 ± 0.04 mg/dl in bilirubin levels respectively, compared with the post-arsenic values.

Following the administration of green tea extract after 6 weeks the urea, uric acid and creatinine concentrations in the arsenic-treated mice decreased from 86.67 ± 2.96 mg/dl to 21.07 ± 1.02 mg/dl in urea levels, from 9.733 ± 0.23 mg/dl to 6.130 ± 0.09 mg/dl in uric acid levels, from 29.67 ± 1.76 K.A. unit to 9.067 ± 0.49 K.A. units in ALP level, from 3.733 ± 0.23 mg% to 1.281 ± 0.14 mg% in creatinine levels respectively, compared with the post-arsenic values. Administration of green tea leaf extract resulted in a significant reduction of the elevated Lipid peroxidation concentrations. Lipid peroxidation concentrations decreased from 58.73 ± 1.36 nmol/ml to 8.060 ± 0.21 nmol/ml after 6 weeks.

Changes in Liver Function Test of mice exposed to sodium arsenite at the dose of 3 mg/Kg body weight daily for 6 weeks and its amelioration by green tea extract for 6 weeks (Table 1).

Changes in the Liver Function Test, Kidney Function Test and Lipid peroxidation levels of mice exposed to sodium arsenite at the dose of 3 mg/kg body weight daily for 4 weeks and its amelioration by green tea extract for 6 weeks (Tables 1-3).

Discussion

The present study was aimed to evaluate the hypothesis that green tea extract, having high polyphenolic content, might be able to combat the oxidative cellular damage caused by arsenic toxicity and to prevent further development of free radicals.

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Table 1: Showing the Liver Function Test parameters values as comparative results of treatment (control, arsenic treated and green tea extract administered). The data are presented as mean ± S.D, n = 6, significance at p< 0.0001.

Biochemical Parameters	Control (n= 6)	Arsenic 4 weeks (n= 6)	Camellia sinensis 6 weeks (n= 6)
SGPT (U/ml)	25.67 ± 1.20	209.7 ± 6.74	37.00 ± 2.18
SGOT (U/ml)	22.00 ± 2.64	128.7 ± 2.60	26.33 ± 1.32
ALP (K.A Units)	6.567 ± 0.35	29.67 ± 1.76	9.067 ± 0.49
Bilirubin (mg/dl)	0.5633 ± 0.029	1.677 ± 0.06	0.7100 ± 0.04

Table 2: Showing the Kidney Function Test parameters values as comparative results of treatment (control, arsenic treated and green tea extract administered). The data are presented as mean ± S.D, n = 6, significance at p< 0.0001.

Biochemical parameters	Control (n= 6)	Arsenic 4 weeks (n= 6)	*Camellia sinensis* 6 weeks (n= 6)
Urea (mg/dl)	17.33 ± 2.02	86.67 ± 2.96	21.07 ± 1.02
Uric acid (mg/dl)	4.800 ± 0.45	9.733 ± 0.23	6.130 ± 0.09
Creatinine (mg%)	0.5167 ± 0.03	3.733 ± 0.23	1.281 ± 0.14

Table 3: Showing the Lipid Peroxidation levels as comparative results of treatment (control, arsenic treated and green tea extract administered). The data are presented as mean ± S.D, n = 6, significance at p< 0.0001.

Biochemical parameters	Control (n= 6)	Arsenic 4 weeks (n= 6)	Camellia sinensis 6 weeks (n= 6)
MDA levels in serum (nmol/ml)	1.987 ± 0.06	58.73 ± 1.36	8.060 ± 0.21

Long-term exposure to arsenic in drinking water results in increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. Arsenic causes cellular toxicity by damaging the body's oxidative defense mechanisms [12].Chronic arsenic exposure causes excessive free radical generation in the body [13-16], Arsenic induced oxidative stress generates the superoxide radical and the hydroxyl radical primarily associated with mitochondria, microsomes and peroxisomes [17]. As a result, cells under oxidative stress display various dysfunctions due to lesions caused by reactive oxygen species (ROS) to lipids, proteins and DNA [18].

Polyphenolic compounds are effective against oxidative damage in various pathological conditions. Tea extract increases antioxidant parameters [19,20], and its aqueous extract has been shown to quench reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals [21,22].

In the present study green tea extract treatment after 6th weeks showed a significant change in the Biochemical parameter and LPO level. Following the tea treatments, after 6 weeks the SGPT, SGOT, ALP and Bilirubin concentrations in the arsenic-treated mice decreased compared with the post-arsenic values whereas, after 6 weeks the Urea, Uric acid, and creatinine concentrations in the arsenic-treated mice decreased compared with the post-arsenic values. Administration of green tea extract resulted in a significant reduction of the elevated Lipid peroxidation concentrations.

Tea contains minerals act as co-factors in antioxidant enzymes: zinc, selenium and manganese. Additional mechanisms for Polyphenols in which they minimize oxidation level besides direct role as antioxidants [23]. Binding of metal ions such as iron and copper and prevents its participation in oxidation reactions (leading to the formation of hydroxyl radical) [24]. Prevention of redox sensitive transcription factors activation that amongst others things serve as mediators of inflammatory reactions [25]. Suppression of oxidation stimulants such as induced nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), lipoxygenase 2 (LOX-2) and xanthine oxidase. Induction of antioxidant enzymes such as glutathione S-transferase and super oxide dismutase [26]. The aqueous extract of green tea has an efficient protection against arsenic which induces biochemical toxicity in experimental mice which is in support of my work.

Conclusion

In conclusion, the present study evaluated the protective role of green tea extract against a toxic dose of 3 mg/Kg body weight sodium arsenite. The protective effect of these antioxidants was shown in the form of normalization of enzymatic and non-enzymatic activities represented by normalization of liver and kidney functions. There was also a reasonable consistency between the biochemical and Lipid peroxidation (LPO) findings. It was obvious that arsenic-induced toxicity was clearly seen in both kidney and liver tissues and the natural antioxidants showed hepato-renal protective capacities.

References

1. Islam MZ, Awal MA, Mostofa M, Ghosh A, Khair A (2009) Effect of Spinach against Arsenic Toxicity in Rats. Bangladesh J Vet Med 7: 358-363. J Biol Sci 11: 1405-1413. Link: https://goo.gl/Qz7Y7U
2. Karmakar R, Mondal T, Saha B, Ban DK, Dey B, et al. (2011) Arsenic induced Biochemical perturbation in Swiss Albino Mice and Cytoprotective activities of Curcumin. Inter J Environ Sci 2: 228-236. Link: https://goo.gl/Zj9joo
3. Hong IS, Lee HY, Kim HP (2014) Anti-oxidative effects of Rooibos tea induces apoptosis in mouse liver is mitochondria dependent and is abrogated by N-acetylcysteine. Toxicol Appl Pharmacol 220: 146-155. Link: https://goo.gl/2ksdkp
4. Jancsó Z, Hermesz E (2015) Impact of acute arsenic and cadmium Jayaprakasha G K, Rao J. Phenolic constituents from lichen Parmon tremastuppeum. Hale and antioxidant activity.ZeitschriftFürNaturforscung 55: 1018-1022. Link: https://goo.gl/ZzhLLj
5. Bouaziz H, Soudania N, Essafia M, Ben Amara I, Hakim A, et al. (2015) brain. PLoS One 9: e87061. by arsine gas. Toxicology 63: 25-34. Link
6. Yasmin S, Das J, Stuti M, Rani MD, Souza D (2011) Sub Chronic Toxicity of Arsenic Trioxide on Swiss Albino Mice Inter J Environ Sci 1: Link: https://goo.gl/1jTg4P
7. Beecher GR, Warden BA, Merken H (1999) Analysis of tea polyphenols. Proc Soc Exp Biol Med 220: 267–270. Link: https://goo.gl/Ts1f2J
8. Blazovics A, Szentmihalyi K, Lugasi A, Lugasi A, Balázs A, et al. (2003) In vitro analysis of the properties of Beiqishen tea. Nutrition 19: 869–875. Link: https://goo.gl/vwF2Up
9. Azam S, Hadi N, Khan NU, Hadi SM (2004) Prooxidant property of green tea polyphenols epicatechin and epigallocatechin- 3-gallate: implications for anticancer properties. Toxicol In Vitro 18: 555–561. Link: https://goo.gl/M4GjCh

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10. Chen JH, Tipoe GL, Liong EC, So HS, Leung KM, et al. (2004) Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by downregulating inducible nitric oxide-derived prooxidants. Am J Clin Nutr 80: 742–751. Link: https://goo.gl/fkTVpC

11. Sur-Altiner D, Yenice B (2000) Effect of black tea on lipid peroxidation in carbon tetrachloride treated male rats. Drug Metab Drug Interact 16: 123–128. Link: https://goo.gl/9JJ6BM

12. Rabbani GH, Saha SK, Akhtar M, Marni F, Mitra AK, et al. (2003) Antioxidants in detoxification of arsenic-induced oxidative injury in rabbits: preliminary results. J Environ Sci Health A Tox Hazard Subst Environ Eng 38: 273–287. Link: https://goo.gl/3QXUTZ

13. Patrick L (2003) Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. Altern Med Rev 8: 106–128. Link: https://goo.gl/GyLod1

14. Rao MV, Avani G (2004) Arsenic induced free radical toxicity in brain of mice. Indian J Exp Biol 42: 495–498. Link: https://goo.gl/BJUZr3

15. Kumagai Y, Pi J (2004) Molecular basis for arsenic-induced alteration in nitric oxide production and oxidative stress: implication of endothelial dysfunction. Toxicol Appl Pharmacol 198: 450–457. Link: https://goo.gl/yNEBr9

16. Shi H, Hudson LG, Ding W, Wang S, Cooper KL, et al. (2004) Arsenite causes DNA damage in keratinocytes via generation of hydroxyl radicals, Chem Res Toxicol 17: 871–878. Link: https://goo.gl/fZ5pCt

17. Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12: 1161–1208. Link: https://goo.gl/edzas7

18. Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress Part I: mechanisms involved in metalinduced oxidative damage. Curr Top Med Chem 1: 529– 539. Link: https://goo.gl/T3xHp1

19. Leenan R, Roodenburg AJ, Tijburg LB, Wiseman SA (2000) A single dose of tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr 54: 87–92. Link: https://goo.gl/VszZwC

20. Luczaj W, Skrzydlewska E (2005) Antioxidative properties of black tea. Prev Med 40: 910–918. Link: https://goo.gl/bGUK2v

21. Thiagarajan G, Chandani S, Sundari CS, Rao SH, Kulkarni AV, et al. (2001) Antioxidant properties of green and black tea, and their potential ability to retard the progression of eye lens cataract Exp Eye Res 73: 393–401. Link: https://goo.gl/uQYDfd

22. Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, et al. (1999) Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radic Biol Med 26: 1427–1435. Link: https://goo.gl/MyUxpu

23. Scalbert A, Johnson I, Saltmarsh M (2005) Polyphenols: Antioxidants and beyond. Am J ClinNutr 81: 215-217 Link: https://goo.gl/JhYFV7

24. Yang C, Wang H, Li G, Yang Z, Guan F, et al. (2011) Cancer prevention by tea: evidence from laboratory studies. Pharmacol Res 64: 113-122. Link: https://goo.gl/uYZBv1

25. Mei Y, Qian F, Wei D, Liu J (2004) Reversal of cancer multidrug resistance by green tea polyphenols. J Pharm Pharmacol 56: 1307-1314. Link: https://goo.gl/ZnAhUP

26. Ling G, Gu J, Genter MB, Zhuo X, Ding X (2004) Regulation of cytochrome P450 gene expression in the olfactory mucosa. Chem Biol Interact 147: 247–58. Link: https://goo.gl/nJsUG8

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