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Research Article

BERYLLIUM DRIVEN HEMATOLOGICAL AND SEROLOGICAL ALTERATION; THAT CURED BY CHELATION VIA NARINGENIN IN ALBINO RATS

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ABSTRACT

Objective: Environment faces range of pollutions threats due to extensive industrialization and deforestation. In exists scenario metal contamination deadly one among them. Beryllium is 44th most abundant element in earth crust which executes their discharge in society through fossils fuel combustion besides daily routines things as in chips of electric gadgets jewelry, dental crown. The objective of the present investigation was the reduction of beryllium harmful effect through naringenin in female albino rats.

Methods: Female rats were administered by 1mg/kg dose intraperitoneally daily for 28 days alone as well as before/along with three doses (12.5, 25, and 50 mg/kg) of naringenin orally (7 days once in day). Hematological and serological analysis was performed for this clinical research.

Results: Beryllium exposure caused severe alteration in hematological markers evident by a significant diminishment of hemoglobin, red blood corpuscles, mean corpuscular hemoglobin concentration and hematocrit and promoted the level of white blood corpuscles and platelets. Similarly, serological parameters; triglycerides cholesterol and glucose were altered significantly as the response of lipid bilayer peroxidation. All these variables were retrieval near to control ardently by three doses of naringenin.

Conclusion: In short naringenin might be useful as in natural chelation for beryllium body burden.

Keywords: Beryllium, Naringenin, Hemoglobin, Triglyceride, Glucose.

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INTRODUCTION

Metal contamination and its health hazards have pose great threat to the general population at costs of deforestation, heavy industrialization, and reconstruction of the world [1]. India has also step-up in these circumstances since past decay by reason of industrial competition over other countries. Numerous metal pollution and their toxic manifestations cases have been well studied previously. Beryllium is one of the alkaline earth metals among 23 elements are comprised in pollutant's list as per the ATSDR (Agency for Toxic Substances and Disease Registry) toxicological report 2018 [2]. In this queue beryllium is one of the light metal that possess unique features makes it reliable to various applications (aerospace, electronic gadgets, heat insulators, non-sparkling tool etc.) [3,4]. Due to their wide-range of regular uses; its exposure and emission become more usual. Fossils fuel combustions a chief source of its emission [5,6]. Several coal mines in India have the main root for electricity and energy generation. However, their ashes have metal particles polluted the near area of a power plant in industries.

During past decay plant containing natural products like polyphenol flavonoids got more attention by scientific communities [7]. Several ailments including diabetes, hypertension, and carcinoma were cured by natural products and their derivative compounds [8,9]. Diet based biomolecules such as flavonoids have therapeutic potential and are thought to be a suitable agent in the prevention and improvement of various diseases [10]. Naringenin (4′,5,7-thrihydroxyflavanone) is a predominant bitter and colorless fl. Narin that occurs naturally in vegetables, fruits, herbs, and nuts widely consumed by humans including *Lycopersicum esculentum* (tomato) [11], *Citrus paradise* (grapefruit) [12], and *Citrus sinensis* (orange) [8,13,14].

An increasing number of pre-clinical studies suggest that naringenin offerings wide range of pharmacological effects, including antioxidant [15], anti-inflammatory [16], antidiabetic [17], antimutagenic effects [18,19], and modulation of hepatic apolipoprotein and lipid synthesis. Previous literature was revealed that naringenin inhibits heavy metal-induced toxicity [20]; however, there is no evidence on the mechanism of preventive action of naringenin against beryllium (a light metal) induced toxic manifestations in albino rats. This preclinical trial, we demonstrate that beryllium caused an impediment in serum and blood cell's functions, through the therapeutic potential of naringenin.

METHODS

Animals

Female albino rat sensitive toward beryllium intoxication (Wistar strain) so on that purpose taken female albino rats for this investigation. About 160±10 g weighted albino rats were purchased from Defense Research and Development Establishment, Gwalior, Madhya Pradesh, India. They were kept standard husbandry situations of light (14 h) and dark (10 h) at 25±2°C temperature and relative humidity of 65–70%. The rats were fed on a standard pelleted diet (Pranav Agro Limited, Pune) and water ad *libitum*. Animals were cared in accordance with the guidelines recommended by the committee for the purpose of control and supervision of experiments on animals (994/Ere/GO/06/CPCSEA).

Chemicals and reagents

Naringenin was procured from Sigma-Aldrich Co. PVT. Limited. Diagnostic kits were purchased from EBRA, Mannheim GmbH Mallaustr, Germany and Meril, India.

Toxicant

Beryllium was obtained from Dr. S. K. Nirala as a gift which utilized for induction of hepatorenal intoxication. Beryllium nitrate was administrated 1 mg/kg dose intraperitoneally daily and once for 4 weeks as per [21,22].

Therapeutic agent

Naringenin was used as a therapeutic agent at three successive doses 12.5 mg/kg, 25 mg/kg, and 50 mg/kg, p.o. for past 7 days after and along with beryllium administration.

Study design

In this experiment, animals were divided into six groups of six animals in each as follows. Beryllium was administered intraperitoneally for 4 weeks once/day. Naringenin served as, at 12.5 25 and 50 mg/kg dose were given last week after and along with beryllium exposure.

Serological assessment

Animal was euthanized and sacrificed after 24 h of the last dose. Blood is drawn by puncturing retro-orbital sinus and collected to ethylenediaminetetraacetic acid (EDTA) vials as well as centrifuged tubes for separation of serum.

Hematological assessment

For hematological studies, blood sample was collected in EDTA tube to maintain their liquidity then assisted through "HEMA2062+" blood analyzer. The serological biochemical assessment was done through serum separation and their analysis by the kit method. For that, blood samples were allowed to stand at room temperature for 1 h then centrifuged at 3000 rpm for 10 min to obtain serum that was stored at −20°C. Serum was used for the analysis of triglycerides, cholesterol, and glucose using diagnostic kits (Erba Diagnostics, Germany) according to the manufacturer's instructions.

Statistical analysis

Statistical data were expressed as mean±standard error of six animals used in each group. Significance of differences between mean values was determined by one-way analysis of variance (ANOVA) at $^{¥}p\leq0.05$ followed by Student's t-test at $^{ΨΨ}p\leq0.01$ and $^{Ψ}p\leq0.05$ for comparison between experimental control and control group whereas a significant difference among therapeutic group versus experimental control group was marked as $^{ΦΦ}p\leq0.01$ and $^{Φ}p\leq0.05$ [23].

RESULTS AND DISCUSSION

In the present scenario, naturally occurring bioactive compounds utilized to generate novel antidote or synthetic metal chelators [24]. Natural compounds have rich antioxidant potential with low cost, large quantity available in nature and without involving any toxic sign. Natural products are used as a therapeutic approach for the treatment of numerous disease and abnormalities. Naringenin is a ubiquitous flavanone commonly present in a grapefruit juice at a concentration around 0.05 g/L [25] that contributes to several therapeutic actions and chelating effect by due to three hydroxyl groups [26]. Present investigation outcome revealed that administration of beryllium driven severe fluctuations in the hematological parameters that may be caused by mutated hemoglobin molecule. Countless naturally occurring phenolic substances are present in rich amount in the plant kingdom, and their therapeutic potential in reducing toxic metal ion-induced free radical assault is partly attributed to their capability to owe specific chelators which bind to unwanted toxic ions and hence diminish their bioavailability [27,28]. In short, these natural products have hydroxyl moieties may assist as substitute remedies or nutritional supplements to mitigate beryllium intoxication. As per chemical name of naringenin indicates 4′, 5, 7-trihydroxy flavanone to have "trihydroxy" group in their molecular structure which made it more proficient toward beryllium chelation.

Effect of naringenin on hematological assessments

Table 1 depicted significantly ($p\leq0.01$) elevated level of platelet and white blood corpuscles after beryllium administration. Their high level might be delivering upgradation of the immune system to compensate for beryllium incited alterations. Although prior research corroboration and support the present outcome [29,30]. Naringenin successive doses also are drawn back altered range of these variables near to control significantly ($p\leq0.01$) by their chelation action on beryllium-exposed rats. In addition hemoglobin, hematocrit (HCT), red blood corpuscles (RBCs), and mean corpuscular hemoglobin concentration (MCHC) were fallen down in beryllium triggered rats as compared to control group significantly ($p\leq0.01$). It may be due to the bone is one of the target sites for beryllium deposition and it also sites of blood synthesis in adults [31]. Beryllium might be intruding the hemopoietin system which can generate blood-related anomalies. Free beryllium can induce alteration of enzyme involved in hemoglobin synthesis which may result immature erythrocytes with reduced oxygen carrying capacity. Reason of deficient hemoglobin might be due to its higher breakdown in comparison with process of its synthesis may become slower-down. Diminished packed cell volume or HCT can occur through following exposure that causes damage to mature erythrocytes or that damages or inhibits replication of erythrocyte progenitors within the bone marrow [32] mean cell hemoglobin concentration (MCHC), HCT, and MCHC that have also been reported in prior literature [21].

In contranst to that hematological markers retrieval toward control by three doses of naringenin significantly ($p\leq0.01$) except hemoglobin. 12.5 mg/kg dose of naringenin shown significant at $p\leq0.05$ (Table 1) in the case of hemoglobin. Among all doses of naringenin higher doses, 25 and 50 mg/kg were showed remarkable recovery as compared to the lowest dose that is also established in previous studies of natural products [33,34].

Effect of naringenin on serological assessment

Hepatic dysfunction and cell injury tracing a link to beryllium intoxication by an elevated level of serum hepatic biomolecules that leaked out due to damage in the functional integrity of hepatic membrane architecture. Elevation of serum triglycerides and cholesterol on the other hand, blood glucose level were down-regulated by exposure of beryllium which seen in preceding studies [21]. Similarly, outcome observed in the present study where cholesterol and triglyceride levels were upregulated while glucose level was fallen significantly at $p\leq0.01$. All doses of naringenin regulated as procured their optimum levels significantly at $p\leq0.01$, as shown in Table 2, that proven by previously published data which is relevant to the present finding [35,36].

Table 1: Influences of naringenin on hematological marker against beryllium-induced toxicity

Parameter Unit	Hemoglobin g/dL	RBCs 10^6/mm^3	HCT %	MCHC g/dL	WBC 10^6/mm^3	Platelets 10^3/mm^3
Group 1	14.1±0.98	7.33±0.57	40.9±2.86	39.5±2.96	9.71±0.82	802±72.9
Group 2	14.2±1.02	7.31±0.60	41.3±3.30	39.2±3.13	9.51±0.86	812±73.1
Group 3	11.8±0.83^{ΨΨ}	3.45±0.30^{ΨΨ}	30.8±2.27^{ΨΨ}	33.1±2.84^{ΨΨ}	19.9±1.71^{ΨΨ}	1226±84.5^{ΨΨ}
Group 4	12.6±0.63^{Φ}	5.65±0.53^{ΦΦ}	34.9±2.89^{ΦΦ}	45.5±3.19^{ΦΦ}	12.0±1.15^{ΦΦ}	1036±96.3^{ΦΦ}
Group 5	12.8±1.02^{ΦΦ}	6.04±0.50^{ΦΦ}	35.4±2.47^{ΦΦ}	36.1±3.42^{ΦΦ}	11.9±0.95^{ΦΦ}	950±81.7^{ΦΦ}
Group 6	13.1±0.92^{ΦΦ}	6.71±0.57^{ΦΦ}	36.3±3.26^{ΦΦ}	38.7±3.52^{ΦΦ}	11.7±0.88^{ΦΦ}	915±84.1^{ΦΦ}
ANOVA	51.57$^{¥}$	21.69$^{¥}$	63.87$^{¥}$	58.9$^{¥}$	16.99$^{¥}$	23.4$^{¥}$

Data were expressed as mean±standard error of n=6; $^{¥}$significant at 0.05% for ANOVA. ^{ΨΨ}Beryllium against Control at $p\leq0.01$ and ^{Φ}Be+Naringenin against Beryllium at ≤0.05; ^{ΦΦ}Be+Naringenin against Beryllium at $p\leq0.01$ for Student's t-test. RBCs: Red blood corpuscles, MCHC: Mean corpuscular hemoglobin concentration, HCT: Hematocrit, WBC: White blood corpuscles. Group 1: Control, Group 2: Naringenin *per se* (Naringenin 50 mg/kg), Group 3: Beryllium (1 mg/kg i.p. Once in a day; 4 weeks), Group 4: Beryllium+Naringenin 12.5 mg/kg, Group 5: Beryllium+Naringenin 25 mg/kg, Group 6: Beryllium+Naringenin 50 mg/kg

Table 2: Efficacy of naringenin on serological marker against beryllium exposure

Parameter	Cholesterol	Triglycerides	Glucose
Unit	mg/dL	mg/dL	mg/dL
Group 1	17.2±0.95	42.1±2.32	108±5.97
Group 2	19.1±1.05	48.4±2.67	102±5.63
Group 3	34.3±1.89$^{\Psi\Psi}$	97.6±5.39$^{\Psi\Psi}$	68.1±3.76$^{\Psi\Psi}$
Group 4	30.1±1.66$^{\Phi\Phi}$	78.8±4.35$^{\Phi\Phi}$	73.2±4.04$^{\Phi\Phi}$
Group 5	26.3±1.45$^{\Phi\Phi}$	70.6±3.90$^{\Phi\Phi}$	74.5±4.67$^{\Phi\Phi}$
Group 6	22.5±1.24$^{\Phi\Phi}$	67.3±3.72$^{\Phi\Phi}$	89.1±4.92$^{\Phi\Phi}$
ANOVA	28.5$^{¥}$	40.6$^{¥}$	11.12$^{¥}$

Data were expressed as mean±standard error of n=6, $^{¥}$significant at 0.05% for ANOVA. $^{\Psi\Psi}$Beryllium against Control at p≤0.01 and $^{\Phi}$Be+Naringenin against Beryllium at ≤0.05, $^{\Phi\Phi}$Be+Naringenin against Beryllium at p≤0.01 for Student's t-test. Group 1: Control, Group 2: Naringenin *per se* (Naringenin 50 mg/kg), Group 3: Beryllium (1 mg/kg i.p. Once in a day; 4 weeks), Group 4: Beryllium+Naringenin 12.5 mg/kg, Group 5: Beryllium+Naringenin 25 mg/kg, Group 6: Beryllium+Naringenin 50 mg/kg

CONCLUSION

The above study suggested that the oral administration of naringenin enable to minimize beryllium incited toxic manifestation in blood and serum markers. 25 and 50 mg/kg doses of naringenin were found to be more significant than the lowest dose, for diminishing beryllium harmful effects. It might be helpful in chelation of reactive free beryllium ions. Their structural affinity toward reactive free beryllium ion seized inside their lattice and kept as inactive form or assists to eliminate from the body of individual more easily. Hence, we could favor naringenin as a chelating agent against beryllium injury in the near future after an extensive investigation in it.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to this investigation.

CONFLICTS OF INTEREST

The authors have declared that there are no conflicts of interest.

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