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Oxidative damage prevention of DNA using passion fruit extract in earthworm as model organism

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ABSTRACT

To study the oxidative damage prevention of DNA using passion fruit extract in earthworm as model organism. In the present study, healthy and young earthworms were collected from Tamil Nadu of Thiruvannamali district. The earthworms was packed in a sterilized bag along with soil and quickly taken to the laboratory. Based on the morphological observation, collected animals were identified as Perionyx ceylanensis with the help of zoological standard manual. The animals were then randomly divided into 3 groups of 6 animals each, placed into cages. The check the potential of passion fruit, extract was subjected to antioxidant activity. The obtained results exhibited the significant effect. The first group was given regular soil (control).The second group A was treated parotta flour with soil in 60:40 ratios (Soil: Parotta flour). Then after one week of incubation the animals were dissected and DNA was isolated to check the DNA damage in earthworm by DNA fragmentation assay. After confirmation of DNA damage, junk food treated three animals were shifted to 3rd group B from group A. Then it was fed passion fruit extract with soil in 60:40 ratios (Soil: Passion fruit extract). Then after one week of incubation the animal were dissected and DNA was isolated to check the DNA damage in earthworm by DNA fragmentation assay. This assay conformed that the passion fruit extract having the ability to repair the DNA damage which induced by junk food

Keywords: Passion fruit, DNA fragmentation, Earthworms, oxidative damage, DNA

INTRODUCTION

Concepts, relationships, lifestyles are metamorphosed to accommodate the new jet age and eating habits too is no exception [1]. Healthy nutritious foods have been replaced by the new food mantra - JUNK FOOD. In the context of world economy, junk food is a global phenomenon. The availability of junk food and snacks at low prices and marketing strategies adapted by manufacturers of such foods has triggered an evolution wherein, consumption of foods that require neither the structure nor the preparation of a formal meal. It seems to have engulfed every age; every race and the newest entrants on stage are children, school going in particular. Hence, a systematic presentation has been made in this review from the articles from various sources highlighting eating habits, nutritional aspects and quality of unhealthy food, their health impact on consumption and preventive measures to be undertaken. Through health education, a change towards good eating practices and adaption of healthy living is possible.

Junk food simply means an empty calorie food. An empty calorie food is a high calorie or calorie rich food which lacks in micronutrients such as vitamins, minerals, or amino acids, and fiber but has high energy (calories). These foods do not contain the nutrients that your body needs to stay healthy. Hence, these foods that has poor nutritional value is considered unhealthy and may be called as junk food Junk food. is an informal term applied to some foods which are perceived to have little or no nutritional value, but which also have ingredients considered unhealthy when eaten regularly, or to those considered unhealthy to consume at all.

The Comet assay applied to earthworms is a valuable tool for monitoring and detection of genotoxic compounds in terrestrial ecosystems. As the worms feed on the soil they live in, they are a good indicator of the genotoxic potential of the contaminants present in the soil and thus used as a sentinel species. Verschaeve demonstrated a dose–response with the extent of DNA damage in coelomic leucocytes (coelomocytes) of earthworms *(Eisenia foetida)* from soil treated with different chemicals as an indication of soil pollution. Coelomocytes from *Eisenia foetida* demonstrated increased DNA damage when worms were exposed to soil samples from polluted coke oven sites or industrialized contaminated areas [2] and even sediment samples from polluted river system.

DNA is like instruction manual for everything that goes on in our body, and genes are the individual instruction. Genes make we look the way we do, but they do lots of other thing too due to bad diets. Half of all cancer caused by DNA damage are due to insufficient foliate. Fast food can induce the oxidative DNA damage. Oxidative DNA damage and may also contribute to the development of chronic disease, including type 2 diabetes, neurodegenerative diseases, cardiovascular diseases and cancer. Oxidative stress is a result of an imbalance between the production and accumulation of reactive species and the organism´s capacity to manage those using endogenous and exogenous antioxidants. The literature data indicates that the *P. edulis* leaf extracts possess *in vitro* and *ex vivo* antioxidant activity against protein oxidative damage, being considered as new sources of natural antioxidants. That's why the present study aimed to ensure that *Passion fruit* can product the DNA damage, which is caused by *parotta* (A common junk food in south India) in earthworms. Earthworm is a wonderful animal model organism to study the oxidative DNA damage, regeneration, stem cells, wound healing, soil toxicology, etc. Earth worm will be useful to develop the application of earthworm into further level. In the present study to ensure the oxidative DNA damage in earthworm by feeding the Junk food *parotta* and passion fruit (*Passiflora flavicarpa*). Antioxidant activity of passion fruit extract was investigated.

EXPERIMENTAL SECTION

ISOLATION OF GENOMIC DNA FROM EARTHWARM

Isolation of genomic DNA from the earthworm species followed by the method of Adlouni *et al*., [3]. Briefly, Put 60-80mg of tissue in a petri dish and wash it thoroughly with distilled water. Grind the tissue in a chilled pestle mortar. Transfer the homogenate in a sterilized centrifuge tube. Add 500ul TE buffer. Add 100ul proteinase K (10mg/ml) and 240ul 10% SDS, shake gently, and incubate for 2hrs at 65^0C in the water bath. The tissue should be digested completely. If there are still some tissue pieces visible, add proteinase K again, shake gently, and incubate for another 1hr at 65^0C. Add 500ul of phenol, shake by hand for 5 to 10 min, and centrifuge at 10,000 rpm for `5 min at 10^0C. Pipette the supernatant into a new tube, add equal volume of chloroform/isoamyl alcohol (24:1); Shake by hand for 5 to 10 min, and centrifuge at 10,000 rpm for 15 min. Pipette the supernatant into a new tube, repeat extraction with chloroform/isoamyl alcohol (24:1). If necessary, Add equal volume of chloroform/isoamyl alcohol (24:1); shake by hand for 5 to 10 min, and centrifuge at 10,000 rpm for 15 min. Pipette the supernatant into a new tube, 25µl 3 M sodium acetate (pH 5.2) and 500ul isopropanol, shake gently until the DNA precipitates. Pellet the DNA at 10,000rpm for 5 min, discard the supernatant carefully. Wash the DNA in 70%ethanol and air dries it. Dissolve the DNA in 50µl sterile or T.E Buffer and store at 20 ^{0}C

DNA FRAGMENTATION ASSAY BY AGAROSE GEL ELECTROPHORESIS

The Single Cell Gel Electrophoresis assay (also known as comet assay) for the detection of DNA damage at the level of the individual eukaryotic cell described by Singh et al., [4]. Dissolve agarose in 1x TAE buffer. Gradually bring the solution to boil in a water bath mixing occasionally by hands. Boil gently till agarose dissolves. Cool the solution up to 55 and add ethidium bromide to the solution in electrophoretic unit with app well forming comb 4. 250ml TAE buffer was poured in electrophoresis unit. Prepared gel was placed in such a way that the wells are towards cathode loads the sample in the well and run the gel at 50v for 2hrs. Observe the gel at uv trans illuminator for the intactness and sharpness of DNA.

In vitro Antioxidant activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, [5]. The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., [6]. The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu et al., [7].

RESULTS AND DISCUSSION

The passion fruit was taken and made into powder**. Plate 1** represents the passion fruit powder .the fruits were made into dry and grind it by using mixer. Natural antioxidant in plant resources can protect biology systems from oxidative stress [8] such as yellow passion fruit (*Passiflora flavicarpa*). Passion fruit rind, the main by-product of the juice industry, contains pectin, a highly valued functional food ingredient widely used as a gelling agent and

stabiliser. These rinds have also been studied for use in the production of candy and flour for human consumption. Due to its high nutritional value and flavonoid contents, investigations to evaluate the potential of passion fruit as a functional food or a source of active compounds for antioxidant or anti-inflammatory purposes are very important. Moreover, although agroindustrial by-products may be rich sources of bioactive compounds, the use of passion fruit rinds still requires further studies. Recent studies have shown the potential of passion fruit and its rind for several purposes, such as the antihypertensive effect of passion fruit rind attributed partially to the vasodilatory effect of polyphenols, especially the flavonoid luteolin [9].

Plate -1: Passion fruit Powder

Plate 2 represents junk food flour (parota flour) it has been taken from the parota which is consider as junk food made it dry and grinded finely. The emergence of the fast food industry has, transformed urban food culture in India to some extent. In India, fast food culture emerged after independence. Eating at home used to be a significant aspect of Indian culture. However, over a period of time, with a growth in the number of nuclear families, economic growth and increasing per capita income as well as globalization, fast food culture gained prominence. Similarly, children also resorted to fast food due to their exposure to global urban culture and western cuisine which accelerated their desire for cheap and delicious fast food. Moreover, fast food costs less than traditional meals commencing with appetizer and concluding with dessert. With the liberalization of the economy in 1992, new multinational fast food giants targeted India as a huge potential market with their outlets. Burger King, Pizza Hut, Domino's Pizza, McDonald's and KFC outlets are functioning in shopping malls and other public areas.

Junk food allows people to eat without planning. Eat not only when it is pre-set meal time, but also when they have spare time. Ingredients of junk foods give great taste and make them addictive [10]. Fat and sugar in combination are capable of producing a dopamine-driven surge of intense pleasure in people with a propensity for addictive behaviour. On the other side, it must be noted that they are hazardous to health too. High fat content, particularly cholesterol, sugar and salts have their adverse effects on health. Soaring calorie content with sugar can lead to obesity [11].

Plate - 2: Junk food Powder

Plate 3 indicates the soxhlett apparatus extraction of passion fruit 10g of passion fruit powder taken and covered the powder by using muslin cloth kept inside the apparatus. Here methanol has been used for the separation of the compound .each cycle required half an hour six cycles are essential. Kept in water bath at 60˚c.However, the pulp biological activity that has been the most extensively studied is its antioxidant activity, using various methods, such as DPPH, FRAP, ABTS and DMPD. These methods explore mainly the stoichiometric activity of extracts by measuring the ability of polyphenolic molecules to trap or neutralise radical species generated by in vitro molecular models.

Some in vivo studies have detected anti-inflammatory activity of P. edulis and P. alata leaves by using a carrageenan-induced pleurisy model in mice. These studies showed a decrease of MPO activity, which was associated with a decrease of neutrophil influx. However, the effect of these extracts on ROS produced by stimulated neutrophils and on the true enzymatic activity of MPO, considered as a target for new drug development [12] has not been studied.

Plate -3: Extraction of passion fruit

Table 4: DPPH radical scavenging activity of *Passion fruit extract*

S.No	Concentration (µg/ ml)	DPPH radicals scavenging activity (%)			
		Standard ascorbic acid	% of inhibition	Fruit	% of inhibition
1.	20	0.30	52	0.30	9.0
2.	40	0.25	60	0.23	30
3.	60	0.20	62	0.17	48
4.	80	0.18	66	0.12	63
5.	100	0.15	73	0.07	78

Table 4 represents DPPH radical scavenging of *Passion fruit* extract respectively. 1,1- Diphenyl- 2- picrylhydrazyl (DPPH), is a kind of stable organic radical. The capacity of biological reagents to scavenge DPPH radicals can be expressed as its magnitude of antioxidant ability. The DPPH oxidative assay is used worldwide in the quantification of radical scavenging capacity. The antioxidant activities of plant extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable DPPH free radical activity [6]. The results are expressed as the IC50 value (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%). The results of the DPPH free radical scavenging assay suggest that leaves of all Cleome species have potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy.

Plate -4: Treatment of junk food in earthworm

Plate 4 represents the junk food flour and the first group was given regular soil (control). The second group A was treated parotta flour with soil in 60:40 ratios (Soil: Parotta flour). Then after one week of incubation the animals were dissected and DNA was isolated to check the DNA damage in earthworm by DNA fragmentation assay. Junk food comprises of anything that is quick, tasty, convenient and fashionable. Clever junk food advertising and the lure of convenience in addition to taste drag people to junk food addiction. Following factors generally makes it appealing: Time factor: Junk food ad ction is so high because of its simplicity. They are easy to prepare and ready to consume within no time. Taste factor: Great taste also, is another important reason to an extent that influences to opt for junk food. This taste is achieved owing to lavish usage of oils, salts and/or sugar. Attractiveness: Packing of such foods has very attractive appearance by adding food additives and colours in addition to enhancement in flavour. Ad factor: Advertising has a major role in attracting the public, particularly children and adolescent to the junk food selling joints.

Plate 5 indicates the effect of junk food after treated with the junk food flour. Junk food allows people to eat without planning. Eat not only when it is pre-set meal time, but also when they have spare time. Ingredients of junk foods give great taste and make them addictive [10]. Fat and sugar in combination are capable of producing a dopamine-driven surge of intense pleasure in people with a propensity for addictive behaviour. On the other side, it must be noted that they are hazardous to health too. High fat content, particularly cholesterol, sugar and salts have their adverse effects on health. Soaring calorie content with sugar can lead to obesity [11].

Dense sugar content can cause dental cavities and type 2 diabetes mellitus [13]. A short-term adverse effect as a result of eating junk foods .lack of energy. Which occurs because junk foods don.t provide essential nutrients, even though they can be very much sufficing, due to which one feels weakened. Unfortunately, meals consisting of junk food don.t fill up for long. Because they are lacking in fibre, and are made of processed foods, they are rated high on the glycaemic index, which means they provide a quick rise in blood sugar, but this also falls quickly, and giving rise to hunger

Plate - 5: Effect of junk food on earthworm

Plate 6 Represents the control group as C and damaged DNA as A and repaired the DNA with passion fruit as B. This assay conformed that the passion fruit extract having the ability to repair the DNA damage which induced by junk food. Gel electrophoresis is a method that separates macromolecules either nucleic acid or protein on the basis of size, electric charge and other physical properties. The term electrophoresis means the migration of charged particles under the influence of an electric field. Junk food has been linked to hypertension, cardiovascular disease, diabetes, and certain cancers so that the rise in obesity has become serious public concern Junk foods have certainly carved up the Third World. Due to globalization. It is an integral part of life in the developed and also the developing world, and coming with it is a massive increase in obesity and associated problems. The key to eating these junk foods is moderation, occasional consumption and preferably in small portions. It is not impossible to win war with junk foods against healthy foods. However, one must beware; entice is so strong that you will be addicted. It must be remembered that the addiction to junk is great for business. It is all in our hands to choose junk food or health. Fast food can induce the oxidative DNA damage. Oxidative DNA damage and may also contribute to the development of chronic disease, including type 2 diabetes, neurodegenerative diseases, cardiovascular diseases and cancer. Oxidative stress is a result of an imbalance between the production and accumulation of reactive species and the organism´s capacity to manage those using endogenous and exogenous antioxidants. The literature data indicates that the *P.edulis* leaf extracts possess *in vitro* and *ex vivo* antioxidant activity against protein oxidative damage, being considered as new sources of natural antioxidants. That's why the present study aimed to ensure that

Passion fruit can product the DNA damage, which is caused by parottain earthworms, anyhow further study need to investigate the effect of junk food in genomic and proteomics level.

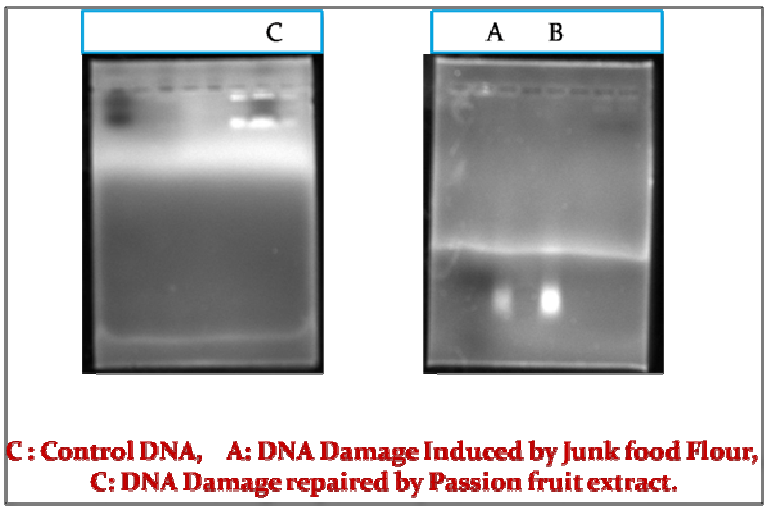

Plate - 6: Agarose gel electrophoresis of DNA damaged & repaired of earth worm

CONCLUSION

In the present study, healthy and young earthworms were collected during the time of january-2016 from Tamilnadu state of Thiruvannamali district. Photographs were taken before and after the collection from their habitat. Then the earthworms was packed in a sterilized bag along with soil and quickly taken to the laboratory. Based on the morphological observation, collected animals were identified as *Perionyx ceylanensis* with the help of zoological standard manual. The animals were then randomly divided into 3 groups of 6 animals each, placed into cages. The check the potential of passion fruit, extract was subjected to antioxidant activity. The obtained results exhibited the significant effect. The first group was given regular soil (control).The second group A was treated parotta flour with soil in 60:40 ratios (Soil: Parotta flour). Then after one week of incubation the animals were dissected and DNA was isolated to check the DNA damage in earthworm by DNA fragmentation assay. After confirmation of DNA damage, junk food treated three animals were shifted to 3rd group B from group A. Then it was fed passion fruit extract with soil in 60:40 ratios (Soil: Passion fruit extract). Then after one week of incubation the animal were dissected and DNA was isolated to check the DNA damage in earthworm by DNA fragmentation assay. This assay conformed that the passion fruit extract having the ability to repair the DNA damage which induced by junk food

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