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Research Article

Moringa oleifera LEAF EXTRACT PREVENTS *IN VITRO* OXIDATIVE DNA DAMAGE

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ABSTRACT

Moringa oleifera (Sajna in India) is considered to be a very popular vegetable among the Indian and African continent. Among its different parts, the leaf holds the best nutritional and medicinal properties. The present study was aimed to evaluate the protective action of *Moringa oleifera* leaf extract (*MoLE*) against oxidative stress induced DNA damage. To that end, the hydroxyl radical mediated DNA damage by Fenton reaction and hydrogen peroxide (H_2O_2) mediated DNA damage by comet assay were employed. Further, lipid per oxidation (LPO) was assayed to find whether *MoLE* can prevent the subsequent membrane damage. The extract prevented hydroxyl radical induced DNA damage as well as H_2O_2 mediated comet formation as determined by fluorescence microscopy. In addition, *MoLE* inhibits the LPO level by about 30% at 100 µg/ml concentrations. *MoLE* also inhibits the Topoisomerase I activity which is one of enzymes responsible for DNA metabolism. *MoLE* shows high polyphenol content (50 mg polyphenols/g dry leaf), strong reducing power, high metal chelating capacity and DPPH (2, 2-diphenyl-2-picryl hydrazyl) radical scavenging activity. All these parameters can immediately be correlated to its DNA protection efficacy. The present study, first time to our knowledge indicates that *MoLE* possesses significant DNA protective activity and hence suggested for the potential human consumption against oxidative DNA damage and cell damage.

Keywords: Antioxidants. DNA damage. Free radicals. *Moringa oleifera*. Polyphenols.

INTRODUCTION

Reactive oxygen or nitrogen species (ROS or RNS), such as $O_2^{\cdot-}$, $NO^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$, $OH^{\cdot}$ which are generated either endogenously or exogenously by various environmental and metabolic factors[1]. These reactive species induce DNA damage and thus play instrumental role in apoptosis or some immune mechanism which ultimately lead to the etiology of many pathological conditions including lethality, mutagenesis, carcinogenesis and aging. They are capable of chemical reactions with bio*MoLE*cules like proteins, lipids and nucleic acids. As a consequence the cell membrane gets damaged and DNA becomes vulnerable. DNA damage if not protected and repaired it may become fatal for the organism[2]. Apart from the DNA damage free radicals interact with polyunsaturated fatty acids (PUFA) of the biological membranes and leads to a chain reaction called lipid per oxidation (LPO). Aldehydes are formed from lipid peroxidation that produce adducts and perturb Watson Crick base pairing of DNA[3].

Thus antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. They mainly function as free radical scavengers, chain breaking antioxidants, metal chelators, reducing agents, oxidative enzyme inhibitors and quenchers of singlet oxygen[4]. Recently special attention has been paid towards edible plants, especially those that are rich in phytochemicals. The phytochemicals are capable to combat with the free radicals efficiently. The phenolics are major chemical identity among the phytochemicals. This group of compounds is principally correlated with their antioxidant property[5-7]. Phenolic compounds are widely distributed in fruits and vegetables. Polyphenols can donate hydrogen to free radicals and generate relatively non reactive reduced forms, thus, acting as chain-breaking antioxidants[8]. The *Moringa oliefera* is referred to as the "Miracle tree" in tropics and sub-tropics. Its leaf, seed and flowers have been shown to have potent antioxidant activity[9-11]. People consume chiefly the whole leaf or seed or the oils from these parts[12]. The leaf extract possess anti-inflammatory, antihypertensive, hypolipidemic, hepatoprotective and antimicrobial activities. It is employed for the treatment of different ailments in indigenous system of medicine, particularly in South Asia[12]. Charak, Shusrut and other pioneers of Ayurvedic practices implicated the importance of *Moringa oleifera*[12,13]. The Moringa leaf is a rich source of flavonoids like quercetin, kaempferol, catechin, epicatechin, rutin etc, all are potent antioxidants[13,14]. Recently we have demonstrated the *MoLE* ameliorated hepatic lipid peroxidation[15] and hepatoprotection[16] induced by radiation in mice model where the potency of the *MoLE* was substantiated. We also demonstrated the hepatoprotective activity of the *MoLE*[17] by high fat diet induced oxidative stress in mice. Thus, *MoLE* is capable of handling a wide range of ROS executing a beneficial role to the organism.

The present study thus further explores the unique potential of *MoLE* which is polyphenol rich extract of the leaf which can encounter the DNA damage. We employed a series of technique like microscopy using the fluorescence of ethidium bromide and comet assay. Moreover, we have shown the inhibitory action of this polyphenolic extract from leaf against topoisomerase I activity. The later is known to be an essential enzyme in DNA replication machinery thus it is highly valuable enzyme in cancer pathology and cell cycle regulated disorders. The study bears a unique note that the *MoLE* can efficiently control the DNA damage or inhibits its regulatory enzyme, topoisomerase I.

In conclusion, this report is first to our knowledge that the bioactive polyphenolic components of *MoLE* are efficient agents which restore the DNA structural integrity and functional state, combating the reactive oxidative species. Moreover, it is also a footmark for standardizing or assaying to evaluate the preventive role of any agent including phytocomponents against DNA breakage.

MATERIALS AND METHODS

Chemicals

2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), butylated hydroxyl anisole (BHA), ethidium bromide, agarose, dimethyl sulfoxide were purchased from Sigma, St Louis, MO, USA). 2, 4, 6-tripyridyl-s-triazine (TPTZ), ferrous ammonium sulphate, Trichloro acetic acid (TCA), ferrous chloride were procured from Merck (Darmstadt, Germany). All the chemicals and reagents were of highest analytical grade.

Preparation of *Moringa oleifera* leaf extract

The leaves of *Moringa oleifera* were collected from a specific tree, authenticated at Botanical Survey of India (BSI), Howrah, India

(Voucher no. CNH/I-I/ (310)/2009/Tech.II/352). The leaves were thoroughly washed in distilled water and dried in vacuum oven at 50˚C for 10h. The clean, dry leaves were then crushed and 5g of them was poured in 50 ml of 70% ethyl alcohol separately. Then the mixture was stirred by magnetic stirrer in air-tight container for about 1 h and then filtered. The filtrate was then evaporated in a Rotary Evaporator (adjustment bath: 40-45˚C, rotation: 50 rpm, pressure: ~15psi, condenser: 4˚C) to remove alcohol. The alcohol free residue was then weighed and dissolved in distilled water according to the required concentrations[18].

Estimation of total polyphenol content

Total polyphenol content of the extracts were quantified according to the method of Taga, Miller and Pratt[19] with modifications. The test samples were mixed with 2% Na_2CO_3 and allowed to stand at room temperature for 2 min. At this time 50% Folin-Ciocalteu's phenol reagent was added and the reaction tube was allowed to stand for another 30 minute at room temperature prior to reading the absorbance at 720 nm. Gallic acid was used as the standard for a calibration curve. Polyphenols content of the extracts were expressed in terms of gallic acid equivalent.

Free radical ($DPPH^{\cdot}$) scavenging activity

The free radical scavenging activity of the extract was measured by DPPH using the method described by Oktay et al[20]. About 1ml 0.2 mM of ethanolic DPPH solution was added to 3 ml leaf extract and mixed well. After 30 minutes, absorbance was measured at 517 nm. Radical scavenging activity was expressed as percentage of inhibition and radical scavenging activity was calculated using the formula:

$$\%\ \text{Radical scavenging activity} = [(\text{control O.D} - \text{sample O.D})/\text{control O. D}] \times 100$$

Metal-chelating ability

The metal-chelating activity of *MoLE* was assessed using the method of Decker and Welch[21]. *MoLE* and was first mixed with 3.7ml of distilled water at different concentrations. Then it was reacted with a solution containing 0.1ml 2mM $FeCl_2$ and 0.2ml of 5mM ferrozine. After 10 min, the absorbance of the reaction mixture was measured at 562 nm. The activity was calculated as a percentage applying the following equation:

$$\text{Metal} - \text{chelating ability}\ (\%) = [1 - (\text{O.D.of the sample}/\text{O. D.of the control})] \times 100$$

FRAP (Ferric reducing antioxidant power) assay

The FRAP assay was carried out by using a reagent containing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ) and 20 mM ferric chloride. The *MoLE* at different concentrations were mixed with 1ml of FRAP reagent and absorbance was measured at 593 nm by using a Smartspec spectrophotometer (Biorad, CA). FRAP was subsequently calculated from a standard curve prepared by serial dilution of a 1 mM $FeSO_4$ solution and values were expressed in FRAP Units[22].

Reducing activity

Different concentrations (in µg) of the extracts in 1ml of distilled water were mixed with 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. Then 10% TCA was added to the mixtures which were then centrifuged. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixtures indicated increased reducing power. Ascorbic acid was used as positive control[23].

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined according to the method described by Murthy et al[24].Various concentrations (2.5, 5, 10, 20, 30, 40 µg/ml) of *MoLE* in ethyl alcohol were taken in different test tubes. 1 ml of iron-EDTA solution (0.1% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1 ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90°C for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5% w/v). 3 ml of Nash reagent (75 g ammonium acetate, 3 ml glacial acetic acid, 2 ml acetyl acetone mixed and raised to 1000 ml with distilled water) was added to all of the tubes and left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against reagent blank.

$$\%\ \text{Hydroxyl Radical Scavenging activity} = (1 - \text{Absorbance of sample}/\text{Absorbance of blank}) \times 100$$

Antioxidative Activity in Linoleic Acid Emulsion

The antioxidative activity of the extract against lipid peroxidation in linoleic acid emulsion system was performed using ferric thiocyanate (FTC) method as described by Takao et al[25] with some modifications. A reaction mixture containing 100µg/ml extract, 0.02 M linoleic acid emulsion and 0.2 M phosphate buffer (pH 7) was incubated at 37˚C overnight in dark. An aliquot of 0.1 ml of the reaction solution was then added to 75% ethanol and 30% ammonium thiocyanate. After 3 minutes, 0.02 M $FeCl_2$ in 3.5% (w/v) HCl was added to the reaction mixture. Absorbance was measured at 500 nm using spectrophotometer. Butylated hydroxyanisole (BHA) was used as positive control.

Fluorescence microscopic study of Fenton reaction

Hydroxyl radical was generated following the protocol of Henle et al[26]. with some modifications. The Fe-EDTA reagent was prepared immediately before the reaction by mixing equal volume of 0.6 mM Fe ammonium sulfate and 1.2 mM EDTA.

Whole blood (30 µl) was mixed with modified RPMI 1640 medium (1 ml) supplemented with 10% (v/v) FCS, under layered with Histopaque 1077 (100 µl) and centrifuged at 2000× g for 3 min at 4˚C. One hundred µl of buffy coat were washed in PBS, pH 7.4. This buffy coat contains lymphocytes. Lymphocytes were taken in three microfuge tubes in equal volume of 100 µl and the tubes were marked as tube 1 (control), tube 2 (hydroxyl radical treated) and tube 3 (hydroxyl radical and leaf extract simultaneously treated). In tube 2 and 3 100 µl Fe-EDTA was added. The relevant tubes were treated with 100µl of 0.3% H_2O_2 and 100 µl (10 mg/ml) of leaf extract. Then all the samples were incubated at room temperature for 45 seconds and finally the reactions were stopped by adding 100 µl of 100 mM thiourea in 0.2M EDTA. Then 50 µl of mixture from each tube was taken in three different glass slides and stained with ethidium bromide and finally observed under (magnification: 200x) inverted phase contrast microscope (Leica, Wetzlar, Germany). The digital image was taken using the Camera, Canon Power Shot S70.

Single- cell gel electrophoresis (alkaline comet assay)

Comet assay using lymphocyte cells was performed under alkaline condition following the method of Singh et al[27]. with minor modifications. The lymphocytes were isolated as stated above and were incubated with *MoLE* (100 µl) for 30 min at 37˚C. The cells were subsequently washed with PBS, pH 7.4, centrifuged at 4˚C and incubated in 1 ml PBS, pH 7.4, containing H_2O_2 (200 µM) for 5 min on ice. Next, slides were covered with 400 ml of 0.75% normal melting point agarose in PBS was pre-warmed to 50°C. A cover glass was placed over the agarose solution, and the agarose was allowed to solidify. The cover glass was then removed, and 85 µl of cell-agarose suspension was placed over the first agarose layer and allowed to solidify under a clean cover glass. After removing the cover glass 100 µl of 0.5% low melting point agarose was added and allowed to solidify in a chilled condition. After the cover glass was removed, the slides were gently immersed in a freshly prepared cold lysis solution

(2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO and 1% Triton X-100, pH adjusted to 10 with NaOH) and kept at 4°C in the dark for 1 hour. The slides were placed on the horizontal gel electrophoresis unit filled with fresh, cold electrophoresis buffer for 20 min. Electrophoresis was conducted for the next 20 min at 18V (1.0 V/cm, 250 mA). The slides were then drained, placed on a tray and flooded slowly with three changes of neutralization buffer (0.4 M Tris-HCl, pH 7.5), each for 5 min. The slides were stained with ethidium bromide (10 mg/ml), covered with a cover glass, and analyzed within 1 hour at 200x magnification using a fluorescent microscope (Motic BA 400, Germany) with green filter. The photograph was taken through the attached digital camera (Canon power shot, S70).

Protective action of *MoLE* against DNA unwinding of Topoisomerase I

The assay was done according to the method of Roy et al[28]. with some modifications. The supercoiled pBS (SK^+) [pBluescript (SK^+)] DNA was incubated with type I DNA topoisomerase and various concentrations of plant extracts and run in agarose gel for the study. The assay was carried out by incubating 90 fmol of supercoiled DNA with 30 fmol of topoisomerase enzyme, reaction buffer (containing 1 M tris pH 7.5, 80% glycerol, 4 M KCl, 1 M $MgCl_2$, 1 M dithiothreitol and 10 mg/ml BSA) and graded concentrations of the plant extract for 30minutes at 37ºC. The reaction products were electrophoresed in a 1% agarose gel containing 0.5µg/ml ethidium bromide. The gel was then photographed using Gel Doc 2000 under UV illumination (Bio-Rad Quality One software).

Statistical analysis

The values are given as Mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) with Tukey's post hoc test was done for statistical evaluation of the data and for the determination of level of significance in various groups of animals. $p < 0.05$ was considered as the level of significance.

RESULTS

Total polyphenol content

The *MoLE* shows high polyphenol content in terms of gallic acid equivalence (50 mg of polyphenols/ g of leaf). As a whole, the polyphenols are shown to be potent antioxidants[5-7].

***In vitro* antioxidant activity**

The extract shows considerable amount of in-vitro antioxidant power as found in the Table 1. DPPH· scavenging activity has become routine in establishing the antioxidant activity of phytochemicals[11]. 24 µg/ml of the extract is required to reduce the 50% of the initial DPPH· concentration.

The extract may be effective in neutralizing the reactive oxygen family which is accounted by its high reducing power and ferric reducing activity. The EC_{50} values for the above parameters are 20 and 45 µg/ml respectively (Table 1). *MoLE* specifically can scavenge the hydroxyl radical. The *MoLE* shows a hydroxyl radical scavenging potency with EC_{50} value 16 µg/ml (Table 1). It has been reported that chelating agents are effective as secondary antioxidants, because they reduce redox potential, thereby stabilizing the oxidized form of the metal ion[29]. To support the above reference we found only 23 µg/ml of the extract is good enough to reduce the initial concentration by 50% in ferrozine assay (Table 1).

We compared the reactivity between the same concentration (100 µg/ml) of the *MoLE* and positive control BHA in terms of different chemical assays (Fig. 1a). *MoLE* scavenges almost 60% DPPH· which is comparable to BHA with no significant difference ($p < 0.05$). In case of Ferrozine the chelating activity of both *MoLE* and BHA is close to 20% without any significant difference ($p < 0.05$). *MoLE* scavenges almost 60% hydroxyl radicals in comparison to BHA which scavenges 70% radicals. The difference is also insignificant ($p < 0.05$). Dose dependent curve of FRAP and hydroxyl radical scavenging power of *MoLE* were shown (Fig. 1b and 1c) comparing with BHA. Fig. 1b shows that *MoLE* is less effective at lower concentration in FRAP assay at lower concentrations, however at highest concentration (100 µg/ml) *MoLE* shows similar efficacy as BHA. Hydroxyl radical scavenging activities of *MoLE* and BHA show similar efficacy (Fig. 1c).

Inhibition of Lipid per oxidation assay

LPO is a reliable marker of oxidative stress both *in vivo*and *in vitro* as the membrane structures become vulnerable in presence of the ROS. The DNA becomes accessible by the ROS in a state of the damaged membrane and its components. Polyphenols can inhibit LPO to a considerable amount by acting as a chain breaking peroxyl- radical scavenger[30]. Fig. 2 shows about 30% inhibition of Lipid peroxidation at 100 µg/ml concentration in comparison to about 40% inhibition by BHA with no significant difference ($p < 0.05$). Hence this extract possesses enough power to prevent peroxidation of lipid molecules in the membrane. Therefore, we studied whether *MoLE* can prevent the DNA damage with the help of microscopic techniques.

Fluorescence microscopic study of Fenton reaction

In Fig. 3 we demonstrated DNA damage and its protection from the hydroxyl radical generated by Fenton's reaction. The hydroxyl radical is generated by the Fe^{2+} and H_2O_2 in presence of EDTA. This radical can damage almost every biological *MoLE*cule found in living cells[31]. The cell nuclei become ripped apart since the cells were exposed to hydroxyl radicals generated (Fig. 3b). On the contrary when the cells were *MoLE* pretreated (Fig. 3c) the slide enumerated substantial protection as very little damage is found.

Alkaline comet assay

MoLE prevented the hydrogen peroxide-induced DNA damage of lymphocyte as demonstrated by the alkaline 'comet assay' (Fig. 4). The cells were incubated with H_2O_2 which reacted with iron or copper to form hydroxyl radical that induces DNA damage[32]. DNA damage was visualized using fluorescence microscope as 'comets' with a bright fluorescent head and a tail streaming away from it. Such damage was clearly demonstrated by the appearance of comets (Fig. 4b) where the cells were exposed with H_2O_2. When *MoLE* was applied prior to H_2O_2 no such damage found (Fig. 4c). We further analyzed the gel photograph with the Comet Score Software (Comet score, version 1.5). The result (Fig. 4d) shows significant increase of comet score ($p < 0.05$) in the comet group where lymphocytes were incubated with H_2O_2 in contrast to control. By *MoLE* treatment the comet score is significantly reduced ($p < 0.05$).

DNA Topoisomerase inhibition assay

To explore whether this polyphenol rich *MoLE* is effective to inhibit DNA metabolic enzyme activity, we employed the DNA Topoisomerase I inhibition assay using supercoiled plasmid DNA. These ubiquitous enzymes carry out vital cellular processes, e.g. replication, repair, recombination, transcription, integration and chromosomal segregation[33-35]. In general cancer or tumour cells show high levels of topoisomerase activity. Therefore the compound which inhibits this enzyme activity will also prevent the occurrence of cancer or tumour[33]. From Fig. 5 it has been found that *MoLE* at different concentration inhibits the Topoisomerase activity even at its lowest concentration (10 µg/µl). Thus, *MoLE* can prevent the enzymatic activity efficiently either by acting directly on the catalytic site or altering the protein structure.

TABLE 1: TABLE SHOWS EC_{50} VALUES OF *MOLE* FOR DIFFERENT *IN VITRO* ANTIOXIDANT PARAMETERS.

PARAMETERS	EC_{50} (µg/ ml)
DPPH	24
Ferrozine	23
FRAP	45
Reducing power	20
Hydroxyl radical scavenging activity	16

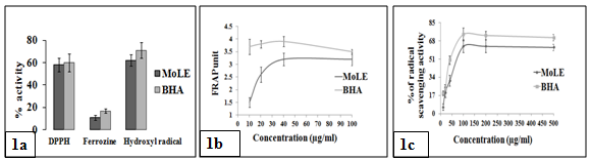

FIGURE1: IT SHOWS COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITY OF *MOLE* WITH BHA.

Figure 1a. It shows comparative DPPH, Ferrozine and hydroxyl scavenging activity of *MoLE* with BHA at 100 µg/ml concentration ($p < 0.05$).Figure 1b. It shows line diagram of FRAP activities at different concentrations of *MoLE* and BHA. Figure 1c. It shows line diagram of hydroxyl radical scavenging activities at different concentrations of *MoLE* and BHA.

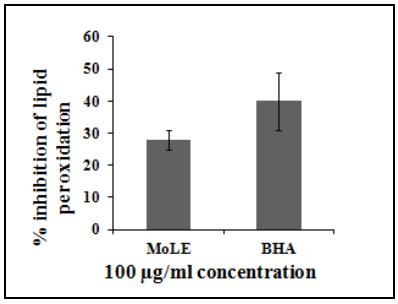

FIGURE 2: IT SHOWS % INHIBITION OF LIPID PEROXIDATION OF *MOLE* IN COMPARISON TO BHA ($P < 0.05$).

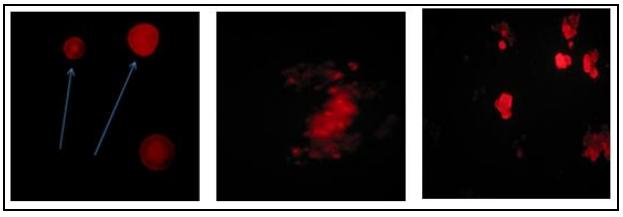

FIGURE 3: IT SHOWS PHOTOMICROGRAPH OF LYMPHOCYTES STAINED WITH EtBr OBSERVED UNDER FLUORESCENCE

Figure 3a. It shows lymphocyte nuclei stained with EtBr. The blue arrows indicate the stained nuclei. Figure 3b. It shows lymphocytes incubated with 100µl of 0.3% H_2O_2 that generates Hydroxyl radicals and stained with EtBr. Figure 3c. It shows lymphocytes incubated with 100 µl (10mg/ml) *MoLE* prior to generation of Hydroxyl radicals and stained with EtBr.

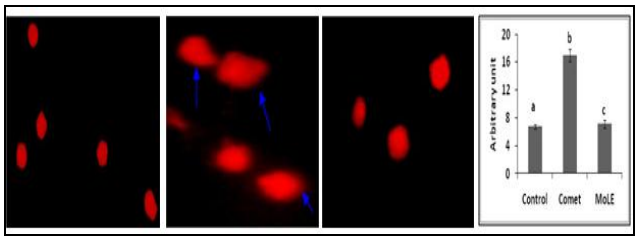

FIGURE 4: IT SHOWS PHOTOMICROGRAPH OF SINGLE CELL GEL ELECTROPHORESIS OF LYMPHOCYTES STAINED WITH EtBr OBSERVED UNDER FLUORESCENCE MICROSCOPE (200X MAGNIFICATION).

Figure 4a. It shows control lymphocyte nuclei stained with EtBr. Figure 4b. It shows lymphocytes incubated with H_2O_2 (200 µM), stained with EtBr. The arrows indicate formation of comets. Figure 4c. Lymphocytes incubated with 100 µl *MoLE* (10mg/ml) prior to H_2O_2 (200µM), stained with EtBr. Figure 4d. It shows statistical comparison of comet scores by bar diagram among the 3 groups ($p < 0.05$).

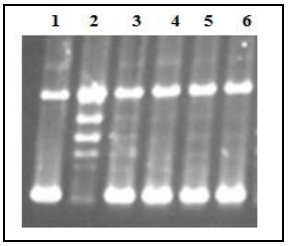

FIGURE 5: IT SHOWS *MOLE* INHIBITS DNA TOPOISOMERASE I ACTIVITY.

Figure 5. *MoLE* inhibits DNA Topoisomerase I activity: Increasing doses of *MoLE* prevented Topo I activity. Lanes 1= 90 fmol of pBS (SK^+) DNA
2= same as before+ 30 fmol of Topoisomerase I
3-6= increasing concentration of *MoLE* (10, 20, 40 and 60 µg/µl).

DISCUSSION

The bioactive elements of food are responsible for nutritive as well as nonnutritive functions such as reduction of pathophysiological situations. Human beings are exposed to various kinds of radiations and other ways of oxidative stress from natural as well as man-made sources. It includes endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer[36]. All these incidents form free radicals which are being scavenged by body's natural antioxidant system. But when formed in excess they can attack the cell membrane, causing lipid peroxidation and consequently damage the DNA[37]. One of the deleterious consequences of DNA damage from exposure to oxidative stress is cancer. Protecting cellular DNA from radical damage might result in the prevention of the cancers. Phytochemicals can neutralize such oxidative stress either by eliciting the synthesis of *in vivo* antioxidants or themselves acting as radical scavengers[38].

In our present study it is found that *MoLE* directly prevents the hydroxyl radical induced DNA damage in mice lymphocytes found from the fluorescence microscopic study. Further, the extract is also capable of preventing the hydrogen peroxide-induced DNA damage by comet assay. Its specific role as a savior of DNA can further be established from the topoisomerase I inhibition.

The extract has shown considerable free radical scavenging potency. The single strand or double strand breaks may prove fatal if not repaired or protected from oxidizing environment[39]. We further identified Quercetin, Rutin, Epicatechin, Ferulic acid and other important polyphenols using HPLC from the *MoLE* and other analyses (data not shown). These compounds have strong antioxidant activity. In fact we have recently shown the strong reducing power of Quercetin[40] and other polyphenols in biological systems (data not shown). Moreover, we have recently addressed the role of *MoLE* and its major component quercetin alleviates the HFD generated oxidative stress and subsequent inflammatory developments[41]. Thus, the preventive potential is lying in the chemical properties of the multitude of the phytocomponents present in *MoLE*. We have substantiated this statement with a series of logical experiments leading to our establishment of the biochemical basis with the recent publications[15,16,17]. The present article is perhaps the first validation how this popular vegetable provides substantial protection against environmental toxicological stress with its bioactive components. On a different note, the present article is not substantiating any single bioactive component of *MoLE* conferring the protection of the genomic content, nor it is advocating anything in favour of the epigenetic modification. As a whole, the ultimate goal of the nutritional genomics is to seek the role of whole foods of our diet on our genomic functions, therefore the article presented a conclusive elucidation and validation of the physiological and nutritional benefits by the whole bioactive elements of the vegetables or fruits which were written in our traditional literature or established in the last century scientific literatures.

CONCLUSION

We conclude that the above preventive capabilities of *MoLE* against the changes of DNA structure-function which are presumably due to the additive or synergistic contribution of its bioactive compounds. Therefore, this extract has a strong potential as curative and therapeutic agent against oxidative stress originating from any source which warrants further *in vitro* and *in vivo* experiments.

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