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Determining the Apoptotic-Inducing Property of Isothiocyanates Extracted from Three Cultivars of *Raphanus sativus* Linn. Using the Comet Assay

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ABSTRACT

The apoptotic inducing property of three cultivars of *Raphanus sativus* L.: white or daikon, red or cherry belle, and large red, were tested on three cancer cell lines: MCF-7, K562, and HT-29. The wild type PAE cells were also incubated with radish extracts for genotoxicity. Each cell line was cultured and treated with either one kind of juice extract or enzymatically hydrolyzed radish sample for 24 hours before harvesting and processing for single cell gel electrophoresis. For each culture set-up, 50 images of cells were captured and analyzed for DNA damage based on three parameters: tail length (*TL*), percent DNA in the tail (*D*), and tail moment (*TM*). Multiple comparisons using Tukey's HSD procedure showed that juice extracts resulted to substantial genotoxicity in MCF-7 cells among the cancer lines based on the superior *TL*, *D*, and *TM* of the comets having 95% CI of [161.16, 231.44], [33.85, 46.63], and [99.97, 157.91], respectively. The juice extracts exhibited no genotoxicity towards normal PAE cells. Extracts from a β-thioglucosidase assisted hydrolysis of white tubers manifested significant disruption of DNA integrity of MCF-7 cells than that of the red tubers ($p < 0.0001$). Furthermore, DNA fragmentation for MCF-7 cells treated with juice and semi-purified preparations of the white tuber were found to be similar with 95% CI for mean differences of [-112.81, 24.21] for *TL*, [-18.03, 5.93] for *D* and [-56.72, 52.15] for *TM*. From the results, it can be construed that preparations of *R. sativus* L. can be a substantial source of biologically active constituents that have the ability to cleave DNA in mutant cell lines while being non-genotoxic to wild type immortalized cells.

INTRODUCTION

In the GLOBOCAN 2008 approximations, roughly 12.7 million cancer cases and 7.6 million cancer deaths were estimated to have occurred in 2008; of these, 56% of the identified cases and 64% of the fatalities transpired in the economically developing world (Jemal *et al*., 2011). The presence of glucosinolates (GLS) in Brassicaceae and other botanical families has been the crux of numerous research due to the possible medical applications of these metabolites, especially in cancer research. Glucosinolates (GLS) are β-thioglucoside N-hydroxy-sulfates [also known as (Z)-(or cis)-N-hydroximinosulfate esters or S-glucopyranosyl thiohydroximates], with a side chain (R) and a sulfur-linked β-D-glucopyranose moiety (Tommaso *et al*., 2007). Five hundred species of non-cruciferous dicotyledonous angiosperms have been reported to contain one or more of the over 120 known GLSs (Prakash *et al*., 2014). The skeleton of GLS consists of a thioglucosidic link to the carbon of a sulphonated oxime. The R group side chain, derived from highly variable amino acids and the sulfate group have an anti stereochemical configuration. It can be aliphatic such as in the case of alkyl, alkenyl, hydroxyalkenyl, w-methylthioalkyl, aromatic or heterocyclic (Li *et al*., 2005). GLSs, however, are only useful when hydrolyzed via the β-thioglucosidase, myrosinase which is stored in specialized vacuoles within the plant structure. It yields a variety of constituents after hydrolysis depending on factors such as plant species and cultivar, site of hydrolysis, the presence of cofactors and the environmental conditions.

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Glucoraphasatin, an alkenyl glucosinolate, has been shown to be present in the tubers of several radish varieties (Bellostas *et al*., 2006). In the absence of myrosinase, as when plants are cooked and the enzyme is subsequently denatured, humans can still efficiently convert GLSs to isothiocyanates (ITC) through the action of the microflora present in the gastrointestinal tract (Hanlon *et al*., 2009).

Glucoraphasatin, a GLS, has been shown to be present in the tubers of several radish varieties (Hanlon *et al*., 2009). The hydrolysis product 4-(methylthio)-3-butenyl isothiocyanate (MTBITC), a compound capable of anti-microbial, anti-mutagenic, and anti-carcinogenic activity, has been isolated from radish roots (Bellostas *et al*., 2006). ITCs have been found to protect normal cells from DNA damage and to induce malignant cancer cells to undergo apoptosis.

In this work, we established the genotoxic capabilities of the juiced and semi-purified preparations of the tubers of three cultivars of radish *R. sativus* L.: white (**W)**, red (**R)** and large red (**L**). Specimens were tested on three aberrant cell lines: breast adenocarcinoma (MCF-7), chronic myelogenous leukemia (K562), and colorectal cancer (HT-29). To the best of our knowledge, this is the first reported study using this methodology of genotoxicity analyses on the extracts of *R. sativus* L. against the aforementioned human cancer cells.

MATERIALS AND METHODS

Sample collection

Mature tubers of several *R. sativus* L. varieties were processed immediately after procurement. Certification that the specimens were of the same family and species was obtained from the National Museum, Botany Division in Manila, Philippines.

Preparation of extracts

An electric juicer (table – top model) was used to prepare radish juice from white tubers (**WJ**), red tubers (**RJ**), and large red tubers (**LJ**). The raw, peeled tubers were diced and placed in the juicer which then separated the juice from the pulp. The fresh juice samples were stored at -80 ^{0}C and thawed for subsequent use in the bioassay.

Semi-purified hydrolyzates, using a modified method developed by Al–Gendy and Lockwood (2005), were prepared for white tubers (**W**) and cherry belle, red radishes (**R**). Measured quantities of lyophilized hydrolyzates were extracted with dichloromethane (DCM) and the resultant mixture was then centrifuged. The residues were re-extracted twice using the same conditions, after which the supernatants was combined and passed through an anhydrous sodium sulfate column and concentrated over nitrogen (N_2) gas.

Maintenance and preparation of cells

The radish extracts used for the comet assays were juiced preparations and DCM extractions of hydrolyzed tubers. Incubation of the immortalized cells was done in T-flasks at a controlled temperature of 37^{0}C, 5% CO_2, and 98% humidity and the concentrations of the radish juices and DCM extracts were 500 µL and 50 µg/mL, respectively. The immortalized cell lines were also exposed to 0.01% hydrogen peroxide for twenty minutes and the comets produced served as a positive control with which all the respective trials using extracts from *R. sativus* L. extracts were compared.

Comet assay

Monitoring of DNA damage in PAE, MCF-7, K562, and HT-29 immortalized cell lines after incubation with juice and semi-purified radish extracts were examined using an alkaline single cell electrophoresis technique. PAE cells served as the control for this study to test for possible genotoxicity and for optimization of the concentrations of the radish extracts. Cells with minimal DNA damage were observed to have distinct nuclei or heads with no tail or relatively short or less dispersed cellular nuclei.

Trevigen's COMET assay kit was used throughout the research and strict adherence to the stipulated protocol was followed. Each of the dried slides were stained with 50 µl of SYBR Green and dried prior to image capture using fluorescence microscopy. An Olympus BX61 microscope at a magnification of 20X was used to view the stained slides. A total of 50 images per sample were procured for analysis and the free version of a manual scoring program CometScore was used to calculate three parameters: tail length (*TL*), percent DNA (*D*), and tail moment (*TM*).

Multivariate analysis of variance (MANOVA) was performed using Pillai's Trace statistic to determine if significant differences in the means of *TL*, *D* and *TM* exist among the radish extracts being compared. Multiple comparisons were carried out to identify the source of any significant difference while controlling experiment-wise error rate using the Tukey's HSD procedure. Tukey's HSD results give simultaneous confidence intervals or are shown as letters beside means. Means found to be significantly different are not sharing the same letter. All statistical analyses were performed using SAS 9.3 software at 5% level of significance. Graphical representations were done using GraphPad Prism 7.01 (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

The chemopreventive activity of dietary ITCs has been attributed to the decreased activation and excretion of carcinogens. ITCs and their cysteine conjugates inhibited cytochrome P450 isozymes 1A1, 1A2, 2B1 and 2E1 (phase I enzymes). They also induced increased activities of phase II enzymes (glutathione S-transferases, quinone reductase, epoxide hydrolase and UDP-glucuronosyl-transferases) involved in the detoxification and conjugation of carcinogens for elimination (Hayes *et al*., 1995). Another feature of the pharmacological activity of dietary ITCs and related S-(N-thiocarbamoyl) cysteine derivatives was their anticancer activity. Transformed cells and cancer cells are much

more susceptible to ITCs than their normal untransformed counterparts (Sahu *et al*., 2009). Inhibition of tumor growth in pre-clinical development by ITCs may contribute to the association of decreased cancer prevalence with dietary GLS consumption (Hanlon *et al*., 2009).

The effects of ITCs extracted from three cultivars of *R. sativus* L. on immortalized cell lines were investigated in this study. In particular, the genotoxicity of the different preparations **WJ**, **RJ**, **LJ**, **W**, and **R** on MCF-7, K562, HT-29, and PAE was explored. Results provided evidence on the anticancer activity of ITCs from *R. sativus* L.

Comparison of juice from three cultivars of R. sativus L.

Cancer cell lines exhibit different morphology due to abnormal cell division and an irregular regulatory system. Hence, it is expected that the degree of damage in these cells would not be uniform (**Figure 1**).

Significant differences in the mean scores measuring DNA damage was observed in PAE cells ($F(9, 588) = 22.65$, $p < 0.0001$) (Table 1). Results showed that juices from all the cultivars exhibited significantly lower DNA damage in PAE cells as compared to the control with 95% CI for *TL* [13.11, 15.70], for *D* [20.38, 23.32] and for *TM* [3.12, 3.73]. This nuclear cell disintegration in PAE cells could be attributed to apoptotic induction since hydrogen peroxide is a known apoptotic agent. The minimal DNA damage in PAE cells only reached as high as the mean *TL* of 2.96 (SE 0.36) in **WJ**, mean *D* of 3.60 (SE 0.37) in **RJ**, and mean *TM* of 0.12 (SE 0.02) in **RJ**. Only six of the 50 PAE cells were observed to have minimal comet morphology while the majority of the cells exhibited a compact head with very little DNA migration. **Figure 2** exemplifies this result by visualization of the compact spherical nuclei of the normal cells. Since these cells are assumed to have an intact regulatory system, induction of pathways such as the cyclin-dependent kinase pathway could stimulate the cytoprotection of normal cells. However, increased concentrations of the extracts caused the lysis of cells given that ITCs are electrophilic species capable of producing reactive oxygen species (ROS)**.**

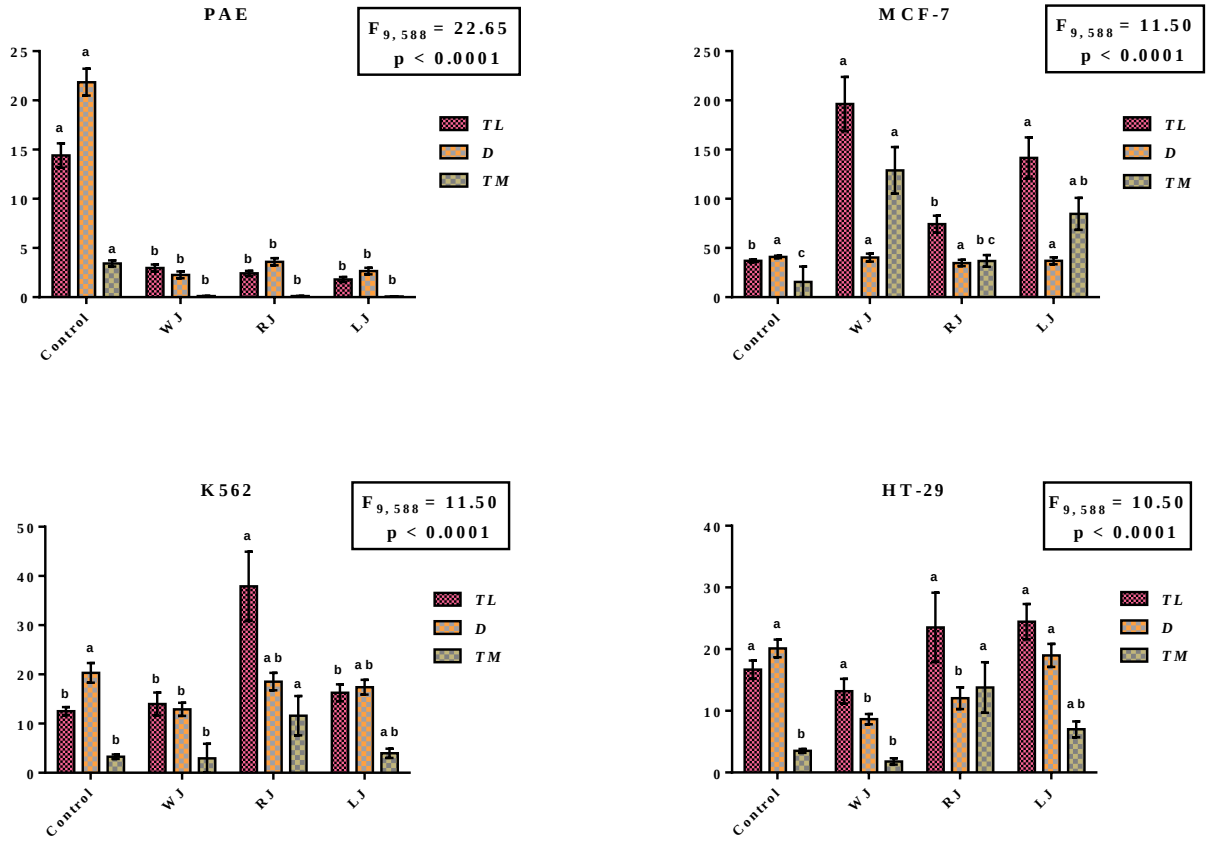
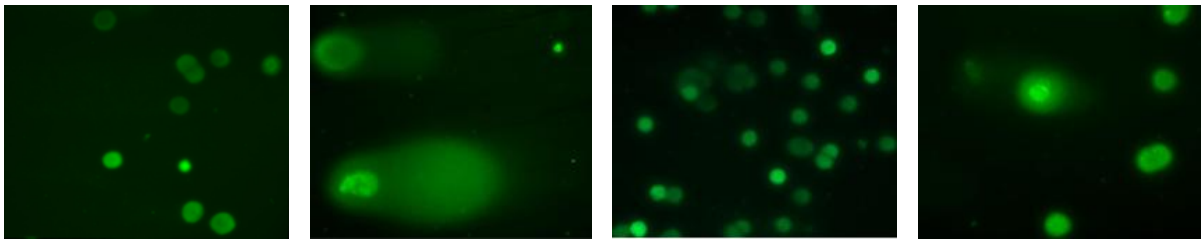


Fig. 1: Comparison of the comet assay parameters incubated with juice from different radish cultivars by immortalized cell lines
* Means of the comet assay parameter from an immortalized cell line with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

Table 1: Mean (± Standard Error) of the comet assay parameters incubated with juice from different radish cultivars by immortalized cell lines.

EXTRACT	COMET ASSAY PARAMETER		
	TL	*D*	*TM*
PAE ($F(9, 588) = 22.65$, $p < 0.0001$)			
Control	14.40 ± 1.21^{a}	21.85 ± 1.37^{a}	3.43 ± 0.31^{a}
WJ	2.96 ± 0.36^{b}	2.27 ± 0.35^{b}	0.11 ± 0.02^{b}
RJ	2.42 ± 0.26^{b}	3.60 ± 0.37^{b}	0.12 ± 0.02^{b}
LJ	1.80 ± 0.25^{b}	2.65 ± 0.33^{b}	0.08 ± 0.01^{b}
MCF-7 ($F(9, 588) = 11.50$, $p < 0.0001$)			
Control	36.99 ± 1.49^{b}	40.95 ± 1.23^{a}	15.55 ± 0.90^{c}
WJ	196.30 ± 27.46^{a}	40.24 ± 4.10^{a}	128.94 ± 23.71^{a}
RJ	74.18 ± 8.65^{b}	34.77 ± 3.28^{a}	36.81 ± 5.90^{bc}
LJ	141.46 ± 20.97^{a}	36.95 ± 3.60^{a}	84.63 ± 16.28^{ab}
K562 ($F(9, 588) = 8.56$, $p < 0.0001$)			
Control	12.51 ± 0.83^{b}	20.32 ± 1.99^{a}	3.27 ± 0.47^{b}
WJ	13.98 ± 2.35^{b}	12.88 ± 1.33^{b}	2.95 ± 0.82^{b}
RJ	37.90 ± 7.03^{a}	18.54 ± 1.77^{ab}	11.60 ± 4.00^{a}
LJ	16.28 ± 1.71^{b}	17.41 ± 1.50^{ab}	3.96 ± 0.95^{ab}
HT-29 ($F(9, 588) = 10.50$, $p < 0.0001$)			
Control	16.67 ± 1.47^{a}	20.12 ± 1.43^{a}	3.50 ± 0.33^{b}
WJ	13.20 ± 2.00^{a}	8.65 ± 0.83^{b}	1.79 ± 0.52^{b}
RJ	23.52 ± 5.62^{a}	12.04 ± 1.76^{b}	13.79 ± 4.09^{a}
LJ	24.46 ± 2.85^{a}	18.99 ± 1.87^{a}	7.01 ± 1.28^{ab}

*Means of the comet assay parameter from an immortalized cell line with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

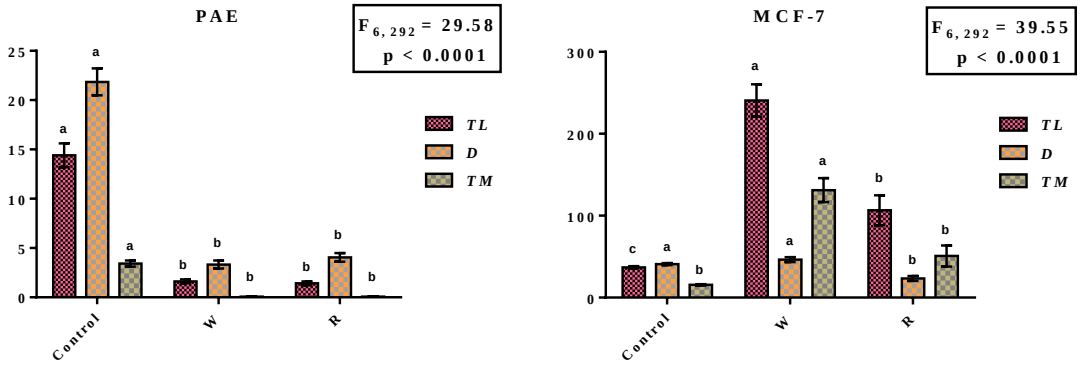


PAE **MCF-7** **K562** **HT-29**

Fig. 2: Sample images of comets for different cell lines incubated with **WJ.**

Similarly, significant effects of the juice from three cultivars of *R. sativus* L. were also evident in the MCF-7 cells ($F(9, 588) = 11.50$, $p < 0.0001$) (**Table 1**). MCF-7 cells incubated with **WJ** and **LJ** displayed a substantial degree of DNA damage as evidenced by the superior *TL* of the comets having 95% CI of [161.16, 231.44] and [106.32, 176.60], respectively (**Figure 1**). Although the data did not provide evidence of a significant difference among the mean *D* based on the multiple comparisons done, the results demonstrated that the mean *TM* of **WJ** was highest (95% CI [99.97, 157.91]) but not significantly different from that of **LJ** (95% CI [55.67, 113.60]). On the other hand, the effect of **RJ** was found to be comparable to that of the control having 95% CI for mean differences of [-102.50, 28.11] for *TL* and [-75.08, 32.57] for *TM*. **Figure 2** shows the DNA damage in MCF-7 cell with well - defined comet tails.

Since migration length is generally related to fragment size and is proportional to the level of strand breaks, the sizeable degree of DNA fragmentation in MCF-7 would indicate single or double strand breaks in the nuclei of the cells after 24 hours incubation with **WJ** or **LJ,** as perceived in all the parameters analyzed. In the K562 cell line, significant differences were likewise observed in damages to DNA ($F(9, 588) = 8.56$, $p < 0.0001$) (**Table 1**). Among the treatments being compared, **RJ** demonstrated the highest activity as exhibited in its *TL* (95% CI [30.35, 45.45]) and *TM* (95% CI [7.44, 15.76]) (**Figure 1**). The effect of **RJ** on *D* was no different from the effect of **LJ** with a 95% CI for mean differences of [-7.24, 4.98]. Genotoxicity of **WJ** was comparable with that of **LJ** as well as that of the control for comet assay parameters *TL* (**WJ**: 95% CI [6.43, 21.53]; **LJ**: 95% CI [8.73, 23.83]; control: 95% CI [4.96, 20.06]) and *TM* (**WJ**: 95% CI [-0.20, 8.12]; **LJ**: 95% CI [-1.21, 7.11]; control: 95% CI [-0.89, 7.43]). For the trials involving HT-29 cell lines, significant differences in DNA damage were also found ($F(9, 588) = 10.50$, $p < 0.0001$) (**Table 1**). Multiple comparisons performed indicated that these differences were for the comet assay parameters *D* and *TM*. **LJ** recorded a significantly higher *D* (95% CI [15.98, 22.00]) than that of **WJ** and **RJ**, but was found to be not significantly different from the control. On the other hand, **RJ** reported the significantly higher *TM* (95% CI [9.52, 18.06]) compared to **WJ** and the control. No significant differences were found between the *TM* of **RJ** and **LJ** (95% CI for mean difference [-14.71, 1.15]). Among all the mutant cell lines, pronounced genotoxicity which was induced by the three cultivars was observed in MCF-7. MCF-7 cells incubated with **WJ** and **LJ** displayed significant genetic damage as evidenced by the superior *TL* and *TM* of the comets; however, large standard deviations can be seen due to irregular cell morphology. The juice from **WJ** and **LJ** were found to be the most effective against MCF-7 cells.



* Means of the comet assay parameter from an immortalized cell line with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

Fig 3. Comparison of the comet assay parameters from DCM extracts of **W** and **R** on PAE and MCF-7 cell lines

Table 2: Mean (± Standard Error) of the comet assay parameters incubated with DCM extracts from red and white radish cultivars by immortalized cell lines.

EXTRACT	COMET ASSAY PARAMETER		
	TL	*D*	*TM*
	PAE (F(6, 292) = 29.58, p < 0.0001)		
Control	14.40 ± 1.21 [a]	21.85 ± 1.37 [a]	3.43 ± 0.31 [a]
W	1.62 ± 0.20 [b]	3.33 ± 0.41 [b]	0.08 ± 0.02 [b]
R	1.42 ± 0.20 [b]	4.05 ± 0.44 [b]	0.08 ± 0.02 [b]
	MCF-7 (F(6, 292) = 39.55, p < 0.0001)		
Control	36.99 ± 1.49 [c]	40.95 ± 1.23 [a]	15.55 ± 0.90 [b]
W	240.60 ± 19.79 [a]	46.30 ± 2.99 [a]	131.23 ± 14.69 [a]
R	106.68 ± 18.21 [b]	23.34 ± 3.08 [b]	50.75 ± 12.93 [b]

* Means of the comet assay parameter from an immortalized cell line with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

***Comparison of DCM extracts from red and white tubers of R. sativus* L.**

DCM extracts of radish tubers were also tested for their anticancer properties. These extracts should constitute most if not all hydrolysis products of GLS. Exogenous myrosinase was added to the extracts to release all products especially ITCs which have potential anticancer properties. MTBITC has been extracted in large quantities in *R. sativus* L. tubers (You *et al*., 2015).

Since there was a dramatic effect on MCF-7 cells, trials were done on these cells to verify if these results were due to the presence of ITCs. Analyses were also done to verify the appropriate concentration of **W** and **R** which should exhibit no genotoxicity towards normal PAE cells and significant genotoxicity to MCF-7 cells. The concentration found to produce these effects was 50 µg/mL. Statistical analyses showed that these have been achieved since differences in DNA damage to PAE cells were observed (F(6, 292) = 29.58, p < 0.0001) (**Figure 3**). Both **W** and **R** reported a significantly lower mean comet assay parameters compared to the control (**Table 2**). DNA damage in PAE cells only reached as high as the mean *TL* of 1.62 (SE 0.20) in **W**, mean *D* of 4.05 (SE 0.44) in **R**, and mean *TM* of 0.08 (SE 0.02) for both **W** and **R**.

Significant effects of DCM extracts from **W** and **R** were also observed in the MCF-7 cells (F(6, 292) = 39.55, p < 0.0001) (**Figure 3**). Cells incubated with DCM extracts from **W** had the most pronounced comets and therefore suffered the most DNA fragmentation having 95% CI of [209.87, 271.33] for *TL*, [41.21, 51.39] for *D*, and [108.87, 153.58] for *TM*.

Comparison of juice and DCM extracts from R. sativus* L. *on MCF-7 cells

The degree of damage in MCF-7 cells was not the same for different preparations of the white and red cultivars of *R. sativus* L. (**Figure 4**). Significant differences in the mean scores measuring DNA damage were observed (F(12, 735) = 18.11, p < 0.0001). Enzymatically hydrolyzed **W** exhibited the most DNA fragmentation in MCF-7 cells as manifested in all three parameters with 95% CI for *TL* [205.88, 275.32], for *D* [40.27, 52.37] and for *TM* [103.64, 158.81] (Table 3). Results showed that 50 µg/mL of ITCs present in semi-purified **W** induced similar bioactivity on MCF-7 cells as did 500 µL of **WJ** due to the extensive and comparable comet formation that was found as evidenced by the 95% CI for mean differences of [-112.81, 24.21] for *TL*, [-18.03, 5.93] for *D* and [-56.72, 52.15] for *TM*. Similar bioactivity was also observed between **R** (50 µg/mL) and **RJ** (500 µL) having 95% CI for mean differences of [-101.01, 36.01] for *TL*, [-0.55, 23.41] for *D* and [-68.38, 40.49] for *TM*. The effects of ITCs extracted from three cultivars of *R. sativus* L. on immortalized cell lines had been shown to differ in this study. The degree of damage in these human cancer cells was not uniform because of numerous factors including maintenance of a critical balance between phase I and phase II enzymes (Gupta *et al*., 2014). Chemopreventive

effects of ITCs have been found to be exerted by inhibition of the bioactivation of carcinogens by phase I drug metabolizing enzymes. This phenomenon leads to several chemicals or pro-carcinogens which are activated or converted into highly reactive electrophilic metabolites (Yoshigae *et al*., 2013). The generated electrophiles can disturb the genomic stability by causing DNA damage (Gupta *et al*., 2014).

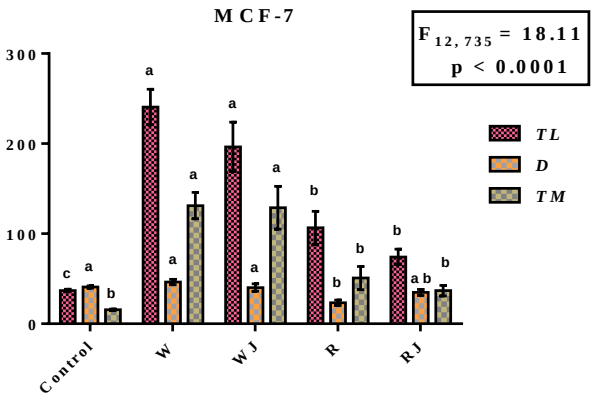


Fig. 4: Comparison of the comet assay parameters from Juice and DCM extracts of **W** and **R** on MCF-7 cell line. *Means of the comet assay parameter with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

Table 3: Mean (± Standard Error) of the comet assay parameters from MCF-7 cell lines incubated with juice and DCM extracts from red and white radish cultivars

EXTRACT	COMET ASSAY PARAMETER		
	TL	*D*	*TM*
Control	36.99 ± 1.49 [c]	40.95 ± 1.23 [a]	15.55 ± 0.90 [b]
W	240.60 ± 19.79 [a]	46.30 ± 2.99 [a]	131.23 ± 14.69 [a]
WJ	196.30 ± 27.46 [a]	40.24 ± 4.10 [a]	128.94 ± 23.71 [a]
R	106.68 ± 18.21 [b]	23.34 ± 3.08 [b]	50.75 ± 12.93 [b]
RJ	74.18 ± 8.65 [b]	34.77 ± 3.28 [ab]	36.81 ± 5.90 [b]

* Means of the comet assay parameter with the same letter are not significantly different at 5% level of significance based on the Tukey's HSD comparison.

Glutathione-S-transferase (GST), a phase II enzyme, catalyzes the conjugation of glutathione with electrophilic compounds making them more hydrophilic and thereby facilitating their removal (Mannervik, 1985). ITCs bind with other vital cellular proteins causing their thiocarbamoylation (Thornalley, 2002). The increase of the intracellular concentration of ITCs was by in part due to the formation of dithiocarbamates, as ITCs rapidly conjugate with thiols, particularly glutathione (GSH). The transferring of ITC-GSH causes rapid attenuation of intracellular GSH, ensuring the perturbation of cellular redox homeostasis. This could be one possible mechanism of ROS produced by ITCs (Gupta *et al*., 2014). As electrophiles, ITCs could potentially trigger apoptosis by binding to macromolecules including DNA and proteins (Zhang *et al*., 2005); although no studies have been reported on the direct binding of ITCs to cellular DNA (Beklemisheva *et al*., 2007). In a previous study, inhibition of human leukemia 60 cell macromolecule syntheses and commitment to apoptosis developed in the initial 24 hours due to the activities of caspase-3 and caspase-8 during ITC-induced apoptosis (Adesida *et al*., 1996). Previous studies have shown that the anti-proliferative activities were limited by hydrolysis of the ITC.

ITC-induced apoptosis may suppress the growth of pre-clinical tumors and contribute to the well-established decreased cancer incidence associated with a *Brassica* vegetable-rich diet. Also, ITCs particularly, phenethyl isothiocyanate (PEITC) has been shown to induce apoptosis in certain cancer cell lines, and, in some cases, is even able to induce apoptosis in cells that are resistant to some currently used chemotherapeutic drugs such as in drug-resistant leukemia cells that produce the powerful apoptosis inhibitor protein BCl-2 (Thomson *et al*., 2006).

The chemotherapeutic potential of raphasatin or MTBITC was due to the potent growth inhibitory action as seen in human liver cancer cells. It was found that MTBITC inhibits the expression of telomerase which invokes cells to pursue apoptosis induction. Mediation of this pathway MAPK activation was also found to be unrelated to the production of ROS. This mode of action was observed within one hour with a transient increase in hTERT mRNA expression which then turned into telomerase suppression. In contrast, activated extracellular signal-regulated kinases (ERK1/2) and P38 but not JNK caused telomerase attenuation and subsequent cell death through apoptosis (Lamy *et al*., 2013).

The mechanism involved has been related to the modulation of enzymes of carcinogen activation and elimination, and countering the malignant phenotype, they may exercise both anti-carcinogenic and pre-clinical anticancer activity in vivo. Recent work suggests they may also be effective as secondary preventive agents against metastatic tumors which are mediated by apoptosis (Adesida *et al*., 1996). It has been suggested that in ITC-induced apoptosis, the caspase pathway has an essential role and the JNK pathway a supporting role (Thornalley *et al*. 2001). Arguments on whether p53 is required for ITC or more specifically PEITC-induced apoptosis or whether the PEITC-induced apoptosis is mediated by ERK1/2 are still being highly debated (Xiao *et al*., 2002).

CONCLUSION

MCF-7, in all the experiments on juiced cultivars, exhibited the greatest extent of genetic fragmentation of all the aberrant cell lines used, with **WJ** being the most genotoxic. Similar moderate DNA damage was detected in both HT-29 and K562 trials. From the dried semi – purified DCM extracts of **W** and **R**, it was observed that constituents of *R. sativus* could be the major anticancer proponent since results showed that there were significant DNA migrations in all three parameters. Since there was no significant difference between the juiced and semi – purified hydrolysates of **W** and **R**, it can be concluded that the bioactivity found in these trials could be caused by ITCs such as the hydrolysis product of glucoraphasatin, MTBITC.

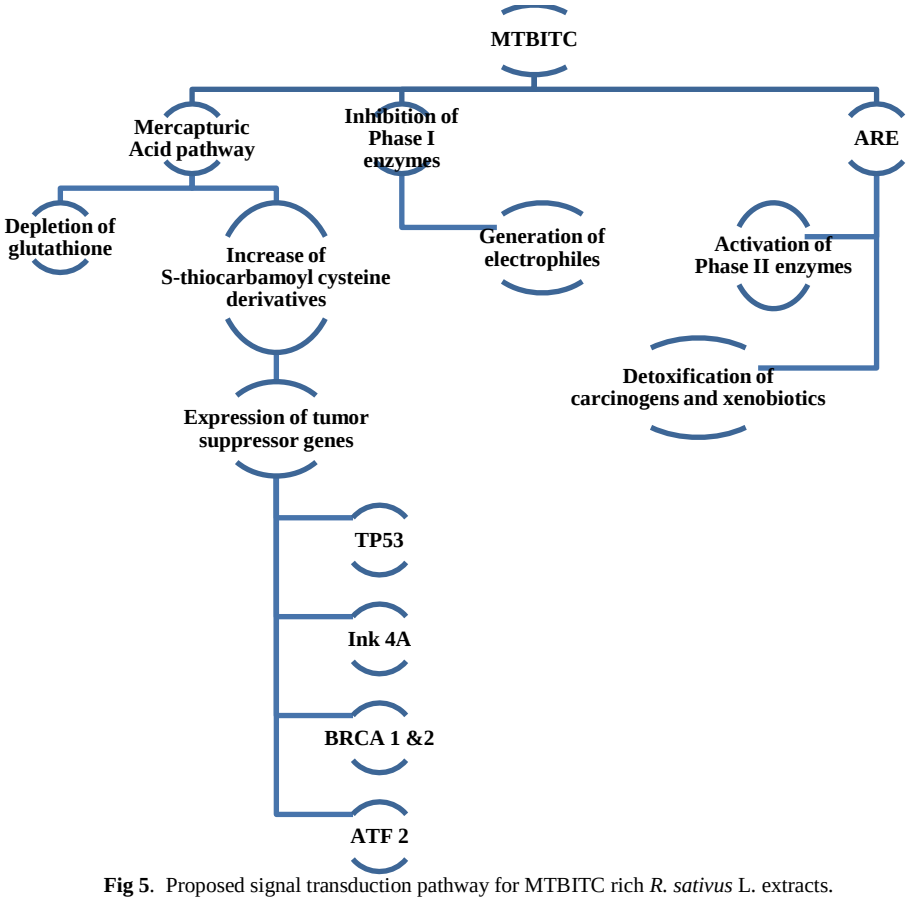


Fig 5. Proposed signal transduction pathway for MTBITC rich *R. sativus* L. extracts.

Apoptosis of MCF-7, HT-29, and K562 was in all probability due to the presence of MTBITC since ITCs have been found to participate in the mercapturic acid pathway. This proposed pathway, as seen in **Figure 5**, causes the depletion of glutathione and increase of S-thiocarbomyl cysteine derivatives in the cytosol and initiates signal transduction(s) to occur stimulating the upregulation of tumor suppressor genes such as TP53, INK4a, BRCA 1&2, and ATF 2. Expression of these genes could also be due in part to the inhibition of Phase I enzymes which are primarily from the cytochrome P450 family and intensification of Phase II enzymes, mediated in part by the antioxidant response element (ARE), such as glutathione S-transferases, quinone reductase, epoxide hydrolase and UDP glucuronosyl transferases**.**

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