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Cardioprotective effect of wheatgrass (*Triticum aestivum*) on isoproterenol induced H9C2 cardiomyoblast cells

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Abstract
Traditionally, wheatgrass has been used since ages for treating a number of diseases. There is no systematic experiment has been carried out on the effect of Wheat grass juice on ISO-induced myocardial ischemia. Therefore, the present study is to examine whether Wheat grass juice will attenuate the progression of myocardial ischemia induced by iso proterenol. The *in vitro* study shows, increased intracellular ROS levels were observed in ISO-induced H9C2 cells as evidenced by increased DCF florescence. Whereas WG (10 µM) treatment prior to ISO-induction significantly reduced intracellular ROS levels, this is due to the possible scavenging of ROS during ISO-induced cells. Based on our results and along with previous reports we state that the WG could scavenge ISO-induced free radicals. We also examined the involvement of mitochondrial damage in ISO-induced apoptosis. To investigate the effect on mitochondria, we performed Rh-123 staining in H9C2 cells. The present findings showed that ISO-induced loss of Δψm have been restored by WG pretreatment. It suggests that WG protects cells from apoptosis by inhibition of the mitochondrial alteration.

Keywords: *Triticum aestivum*, H9C2, wheat grass and cardioprotective

Introduction

Wheatgrass juice is also called as Green Juice. Wheat Grass means the green leaf of 'baby' red berry wheat plant. In tropical climate Wheatgrass grows to a height of around 6 inches in just 7 days. Cutting the green leaf Wheat Grass at this „jointing stage‟ (before the stem begins to form) and drinking its green chlorophyll rich Wheat Grass juice is known to have many therapeutic benefits. Wheatgrass juice is nature's finest pharmaceutical. It is a capable concentrated fluid supplement. Two ounces of wheatgrass juice has nutritional equivalent called five pounds of the best crude natural vegetables. For instance, wheatgrass has double the measure of Vitamin A as carrots and is higher in Vitamin C than oranges. It contains the all B complex vitamins, and also calcium, phosphorus, magnesium, sodium and potassium in an adjusted proportion (Akram and Aftab, 2015) [1]. Wheatgrass is a finished wellspring of protein, supplying the majority of the key amino acids, and more. It has around 20% of aggregate calories originating from protein. This protein is as poly peptides, simpler and shorter chains of amino acids that the body utilizes all the more productively as a part of the circulation system and tissues (Ghani *et al*., 2015) [11].

Wheatgrass supplies the body with helpful measurements of vitamins, minerals, cancer prevention agents, chemicals, and phytonutrients, wheatgrass is likewise a capable detoxifier, particularly of the liver and blood. It kills poisons and ecological contaminations in the body. This is on account of Wheatgrass contains useful compounds that shield us from cancer-causing agents, including Superoxide Dismutase (SOD), that decreases the impacts of radiation and overview poisons in the body. It scrubs the body from head to toe of any overwhelming metals, poisons and different poisons that might be put away in the body's tissues and organs. It is good to have two ounces of wheatgrass juice every day (Sofi *et al*., 2016) [31].

Wheatgrass is having fundamental vitamins and supplements that keep mind and body solid and energetic. Taking a "shot" of squeezed wheatgrass in morning breakfast every day is considered as a solid approach to begin the day, however it can get exceptionally costly. So, to make wheatgrass a standard piece of your eating routine, developing it yourself at is best way (Bharali *et al.*, 2015) [5].

Wheatgrass juice is especially high in chlorophyll along with some other constituents. Wheatgrass washes down and manufactures the blood because of its high substance of chlorophyll. Chlorophyll is the main result of light and in this manner contains more mending properties than some other component. From the sun, several life's are originated. Only green plants by the process of photosynthesis will utilize the vitality of sun. Chlorophyll is known as the 'life-blood' of the plants. Drinking wheatgrass juice resembles drinking fluid daylight. Chlorophyll conveys elevated amounts of oxygen (in addition to other things) which is particularly intense in helping the body to reestablish irregularities. The chlorophyll which has more oxygen conveys more oxygen to the blood. Red Blood cells numbers will increase and blood oxygen levels raises rapidly with the drinking of wheatgrass squeezed juice and utilizing wheatgrass juice. This is a key indicator of recovery for various abnormalities, ailments and diseases. The Oxygen acts as a crucial element to numerous body parts, especially cerebrum utilizes 25% of the oxygen supply. This high oxygen in body results a sound body

(Rana *et al*., 2011) [29].

Wheatgrass found to break down scars formed in the lungs also serves to remove various drug deposits from the body, filters the blood and organs and counteracts acids and poisons in the body. It builds the compound level in our cells, supporting in the revival of the body and the digestion system of supplements. These proteins help with dissolving tumors. Numerous individuals help with fighting tumors naturally and neutralizes poisons. Wheatgrass is promoted to treat various conditions including the common cold, coughs, bronchitis, fevers, infections, and inflammation of the mouth and throat (Amare *et al*., 2016) [2].

Wheatgrass juice is having efficient healing properties. As it contains all minerals and vitamins like A, C, E, K and B complex too. It has many proteins and also contains 17 amino acids. The chlorophyll in the wheatgrass has enzymes, interestingly the super oxide dismutase in wheatgrass helps to slow down the ageing process. The chlorophyll also prevents the growth of bacteria. The wheatgrass is found to be having more than 100 elements needed for a healthy individual (Dey *et al*., 2016) [9].

The wheatgrass ointments helps to decrease various skin problems like eczema, itchy skins, etc. Latest Researches found that insertion of wheatgrass chlorophyll packs into the sinuses clears the congestion. It also helps to cure chronic sinusitis. Chlorophyll also has beneficial effects on heart, vascular system, intestine, uterus and lungs. Wheatgrass juice has superior detoxifying agent. The small amount of wheatgrass in diet regularly prevents tooth decay and reduces tooth aches also. Wheatgrass juice helps to prevent diabetes, hypertension, graying of hair, pyorrhea of mouth, arthritis. It improves digestion and can be used as effective skin cleanser. Wheatgrass juice removes heavy metals and is useful to treat various blood disorders (Kulkarni *et al*., 2006) [21].

The most amazing part of wheatgrass consumption is it is not toxic in any amount. Minor side effects of wheatgrass are it may cause nausea, appetite loss, and constipation. These side effects will not cause any harm to body. The appropriate dose of wheatgrass relies on upon a few elements, for example, the user's age, health, and a few different conditions. As of now there is insufficient logical data to decide a suitable scope of measurements for wheatgrass. Make sure to follow relevant directions on labels of products and consult any drug specialist or doctor or other health care professional before utilizing (Kothari *et al*., 2008) [20].

Materials and Methods

Chemicals

All the chemicals used for this work, were purchased from precision scientific supplies, Trichy. Chemicals used in this study were of analytical grade obtained from E. Merck or HIMEDIA, Mumbai, India.

Collection of plant sample

Wheatgrass (*Triticum aestivum*) free from infection was collected from local market of Ulundurpet, Villupuram District in Tamil Nadu. Then it was transported to the PG and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science, Ulundurpet, where the study was carried out.

Preparation of plant extracts

The 7-10 days old (500g) wheat seedlings were washed thoroughly with running tap water, followed by washing with deionised autoclaved water to remove the dust particles, possible parasites and then used for extraction of juice with the help of a manual extractor/juicer (Preethi Mixer Grinders, India). 310mL juice was collected from 500g seedlings and stored in the deep freezer for pre-freezing at -40º C for 1h. The stored juice was then freeze dried (lyophilized) to form powder (18.3g). The freeze dried powdered sample was then stored in closed air tight bottles for further experimentation. 10g of freeze dried juice powder was extracted simultaneously with water.

Analysis of Fourier transform infrared Spectrophotometer (FT-IR) Spectroscopy

Fourier transform infrared spectrophotometer analyse is used to analyse the functional groups present in a molecule based on their frequencies of vibration between bonds of the atoms. Aqueous extract of WG were characterized using Fourier transform infrared spectrophotometer (FT-IR; IR Affinity-1, Shimadzu, Tokyo, Japan) for FT-IR spectra measurement in the frequency range of 400 to 4,000 cm^{-1}.

GC-MS Analysis

The GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused Silica Capillary Column (30 x 0.25 mm ID x 1μMdf, composed of 100% dimethyl poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999 %) was used as carrier-gas at a constant flow of 1ml/min and an injection volume of 2μl was employed (split ratio of 10.1); Injector temperature 250°C; Ion-source temperature 280°C. the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 260°C, ending with a 9min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 45 to 450Da. The Total GC running time was 36 min

The Interpretation of the mass spectrum GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components. The name, molecular weight and structure of the components of the tested materials were ascertained.

In vitro free radical scavenging assays

hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of *WG* was determined by the method of (Halliwell *et al*., 1987) [14, 15]. In this assay, $OH^{\bullet}$ has been generated by reduction of H_2O_2 the transition metal (iron) in the presence of ascorbic acid. Hydroxyl radical can detect by its ability to degrade deoxyribose to form products, which on heating with thiobarbituric acid (TBA) form a pink colour chromogen. Addition of *WG* competes with deoxyribose for $OH^{\bullet}$ and diminishes the colour formation. The incubation mixture in a total volume of 1 mL contained 0.1 mL of buffer, varying volumes of *WG* sample (10, 20, 30, 40 and 50 μM), 0.2 mL of 500 μM ferric chloride, 0.1mL of 1mM ascorbic acid, 0.1 L of 1M EDTA, 0.1mL of 10mM H_2O_2 and 0.2 mL of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then 1 mL of 1% TBA (1 g in 100 mL of 0.05N NaOH) and 1 mL of 28% TCA were added. All the tubes were kept in a boiling water bath for 30 min. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of *WG*. The efficiency of *WG* was compared with various concentrations (10, 20, 30, 40 and 50 µM) of ascorbic acid as standard. Decreased absorbance of the reaction mixture indicates increased hydroxyl radical scavenging activity. The percentage scavenging was calculated as given below:

$$\% \text{ of scavenging } [OH] = \frac{A0\text{-}A1}{A0} \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of *WG*.

Superoxide anion scavenging assay

Superoxide anion radical scavenging activity of *WG* was determined by the method of (Nishimiki *et al*., 1972) with modifications. The assay was based on the oxidation of NADH by phenazine methosulphate (PMS) to liberate PMSred. PMSred convert oxidized nitroblue tetrazolium (NBToxi) to the reduced form NBTred, which formed a violet coloured complex. The colour formation indicated the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of colour after the addition of the antioxidant was a measure of its superoxide scavenging activity. 1 mL of NBT (100 µm of NBT in 100 mM phosphate buffer, (pH 7.4), 1 mL of NADH (468 µmol in 100 mM phosphate buffer, pH 7.4) solution and varying concentrations of *WG* (10, 20, 30, 40 and 50 µM) were mixed well. The reaction was started by the addition of 100 µl of PMS (60 µm/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30 ˚C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the ethanol extract was used as blank. Ascorbic acid was used as a standard for comparison. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage scavenging was calculated as given below:

$$\% \text{ scavenging } = \frac{A0\text{-}A1}{A0} \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the sample of *WG* or ascorbic acid as standard.

1, 1-Diphenyl-2-Picrylhydrazyl radical scavenging assay

The stable free radical 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Brand-Williams *et al*., 1995). *WG*at various concentrations ranging from

10-50 µM, were mixed in 1mL of freshly prepared 0.5 mM DPPH ethanolic solution and 2 mL of 0.1 M acetate buffer at pH 5.5. The resulting solutions were then incubated at 37 ºC for 30 min and measured at 517 nm in a Shimadzu UV-1601 spectrophotometer (Tokyo, Japan). DPPH$^{\bullet}$ radical scavenging activity of the *WG* was calculated from the decrease in absorbance at 517 nm in comparison with the negative control. IC_{50} value is the concentration of compound required to inhibit 50% of DPPH$^{\bullet}$ radical production. The percentage of DPPH radical scavenging was calculated as given below:

$$\% \text{ DPPH}^{\bullet} \text{ scavenging} = \frac{A0\text{-}A1}{A0} \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the sample of *WG* or ascorbic acid.

Experimental protocol

Cultured H9C2 cells were divided into four experimental groups as follows:

Group I : Normal cardiomyoblasts without any treatment
Group II: Normal cardiomyoblasts with 10 µM of WG
Group III: ISO-induced cardiomyoblasts (31.25 µM)
Group IV: ISO-induced cardiomyoblasts pretreated with 10µM of WG

Treatment of apigenin and ISO in H9C2 cells

H9C2 cells pretreated with apigenin (10 µM) for 2 h prior to ISO (31.25 µM) treatment and then cellular and molecular changes were analyzed after 24 h of incubation.

Cardioprotective effect of apigenin on ISO-induced H9C2 cells

MTT assay

The cytotoxic effect of ISO-induced H9C2 was determined by MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells (Mosmann, 1983).

Cultured H9C2 (1x106 cells/mL) cells were taken into a 96 well plate. Then the cells were pretreated with different concentration of apigenin (1, 5, 10, 20, 40, and 80 µM) for 2 h prior to ISO treatment. Then the cells were incubated at 5% CO_2 and 95% O_2 environment at 37 ºC for 24 h. MTT (0.5 mg/mL) was added to the incubated cells and then further incubated for another 4 h. The cells were centrifuged for 10 min and the supernatant was removed, 200 µL of DMSO were added into each tubes. Absorbance was measured in a microplate reader at 540 nm. Images captured under microscope. Percentage viability was calculated as follows:

$$\% \text{ Cell Viability} = \frac{\text{Test optical density}}{\text{Control optical density}} \times 100$$

Evaluation of protective effect of apigenin on ISO-induced ROS generation

ROS levels were measured by using a non-fluorescent probe, 2, 7,-diacetyl dichlorofluorescein diacetate (DCFH-DA). DCFH-DA is transported across the cell membrane in acetylated form and deacetylated by esterase to generate the non-fluorescent 2’, 7’-dichlorofluorescein (DCFH). This compound is drapped inside of the cells; DCFH is converted to highly fluorescent DCF through the action of hydrogen peroxide generated by the presence of peroxidase (Hafer *et al*., 2008) [13]. Fluorescence measurements were made with excitation and emission filters set at 485 ± 10 nm and 530 ± 2.5 nm, respectively. The results were expressed as percentage fluorescence intensities. Further, the cells were subjected to fluorescence microscopic analysis using blue filter (460 nm).

The percentage of ROS levels was estimated in the control, ISO-induced plus apigenin treated H9C2 cells. Briefly, an aliquot of the isolated cells ($8x10^6$ cells/mL) were made up to a final volume of 2 mL in PBS (pH 7.4). Then, 1 mL cell suspension were taken, to which 10 µL DCFH-DA (10 µM) was added and incubated at 37 °C for 30 min. Then, apigenin pretreated and/or ISO-induced H9C2 were incubated for 30 min in 6 well plates with 10 µg/mL of DCFH-DA in PBS. Finally, cells were washed thrice with PBS and the fluorescence intensity was recorded using multimode reader and the images were captured using fluorescence microscope (460 nm).

Evaluation of protective effect of apigenin on ISO-induced changes in mitochondrial transmembrane potential

Alteration in mitochondrial membrane potential (depolarization) is an indication of early stages of apoptosis. Rhodamine 123 (Rh 123) is a lipophilic cationic dye, highly specific for mitochondria. Polarized mitochondria are marked by orange-red fluorescence and depolarized mitochondria are marked by green fluorescence.

H9C2 ($1x10^6$ cells/ml) cells were cultured in 6 well plates. Apigenin (30 µM) was added and the cells were kept incubation for 24 h at 37 °C for 24 hrs. After incubation, fluorescent dye Rhodamine-123 (10 µg/ml) was added, then the dishes were kept incubation for 30 minutes in CO_2 incubator. The cells were washed by the addition of warm PBS and observed under fluoresence microscope using bule filter (460 nm).

Evaluation of apoptotic morphological changes by acridine orange/ethidium bromide dual staining

Ethidium bromide (EtBr) is a cyclic planar membrane impermeable molecule that binds between the stacked base-pairs of relaxed DNA. Apoptotic cells uptake EtBr and emits red/orange fluorescence under 550 nm. Acridine orange is a DNA selective and membrane permeable fluorescent cationic dye freely enters normal cell nuclei and emits green fluorescence under 525 nm. (Lakshmi *et al*., 2008). H9C2 cells exhibiting typical changes of apoptosis i.e. nuclear condensation, membrane blebbing etc. were stained with AO/EtBr and % apoptotic cells were calculated as a function of total number of AO stained cells present in the field.

H9C2 cells were seeded in 6-well plate ($1x10^6$/well) and incubated in CO_2 incubator for 24 h. The cells were fixed with methanol: glacial acetic acid (3:1) for 30 min at room temperature. Then, the cells were washed in PBS, and stained with 1:1 ratio of AO/EtBr. Stained cells were immediately washed again with PBS and viewed under a fluorescence microscope with a magnification of 40x. The number of cells showing features of apoptosis was counted as a function of the total number of cells present in the field

Results

FTIR analysis of aqueous extract of WG

The characterization of the plant extract and the functional groups was analyzed in Fourier Transform Infrared Spectroscopy (FT-IR). The absorbance spectra of soluble plant extract was shown in figure 1. The absorbance bands analysis were observed in the region of 4000-400cm-1 are 8 compounds derived. The compounds represented the ranges from 3448.83 to 853.05 peaks were shown Table 1 and Fig 1. The Stretching of Alcohol and Phenol group, Alkyl C-H Stretch, Aldehyde, Alkanes, aromatic amines, aliphatic amines and Aromatics are present in the peaks.

Fig 1: FT-IR analysis of aqueous extract of WG

Table 1: Functional group of FT-IR spectrum analysis in aqueous extract of WG

S. No	Peak Area	Bond	Functional Group
1.	3244.12	O-H	Stretching of Alcohol and Phenol group
2.	2942.61	C-H bonds	Alkyl C-H Stretch
3.	2855.37	H–C=O: C–H stretch	Aldehyde
4.	1635.21	–C=C– stretch	Alkanes
5.	1489.94	C–H bond	Alkanes
6.	1384.07	C–N stretch	aromatic amines
7.	1027.71	C–N stretch	aliphatic amines
8.	853.05	C–H “oop”	Aromatics

GC MS analysis of aqueous extract of WG

Crataeva nurvala bark extract has been analyzed by GC-MS technique. The results are given in Table 2. The Crataeva nurvala bark extract was shown to contain a mixture of components. 8 components were identified. The analysis of Crataeva nurvala bark extract showed 2-Hydroxy-4-Methoxybenzaldehyde, 1'-Acetonaphthone, 1,2-Bis (Trimethylsilyl) Benzene, Cyclotrisiloxane, 4-Hexadecen-6-Yne, (E), Limonen-6-ol, Pivalate, Lup-20(29)-En-3-ol and Methoprene.

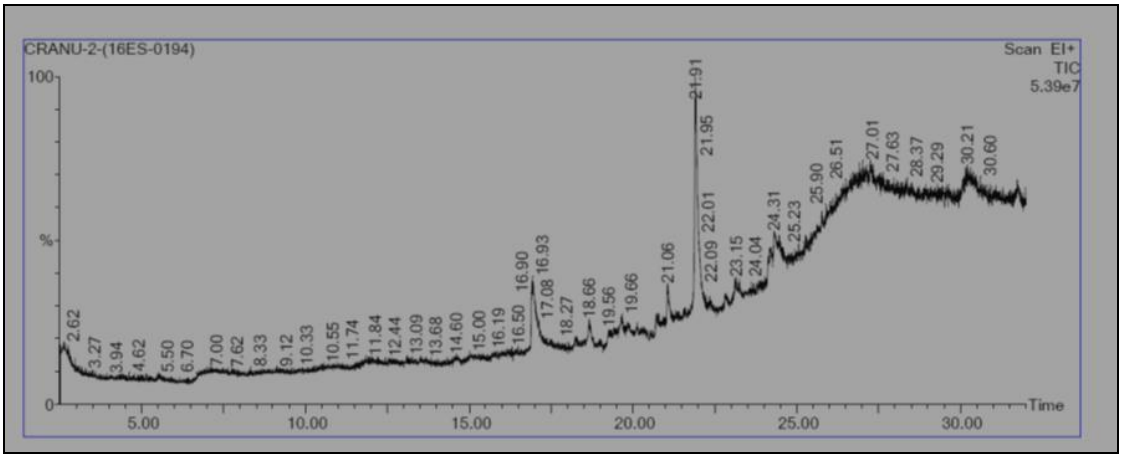

Fig 2: Chromatogram of GC-MS analysis of aqueous extract of WG

Table 2: Functional group of GC-MS analysis in aqueous extract of WG

S. No	RT	Name of the compound	Molecular weight	Molecular formula
1	2.523	1,1-Dichloropentane	140	$C_5H_{10}Cl_2$
2	2.633	ether, 3-Butenyl Propyl	114	$C_7H_{14}O$
3	16.934	Cyclohexanol	184	$C_{12}H_{24}O$
4	18.665	Bisnorallocholanic Acid	332	$C_{22}H_{36}O_2$
5	19.660	4-Hexadecen-6-YNE, (E)-	220	C16H28
6	21.056	Limonen-6-OL, Pivalate	236	$C_{15}H_{24}O_2$
7	21.906	Caryophyllene Oxide	220	$C_{15}H_{24}O$
8	24.212	Methoprene	310	$C_{19}H_{34}O_3$
9	24.307	5,9-Undecadien-1-Yne	176	$C_{13}H_{20}$
10	26.818	Cyclotrisiloxane	222	$C_6H_{18}O_3SI_3$
11	27.253	Carvone OXIDE, CIS-	166	$C_{10}H_{14}O_2$

Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT).

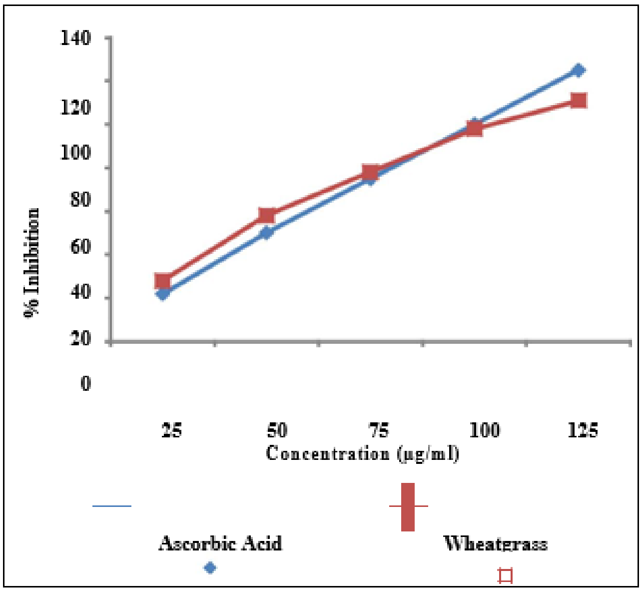

Fig 3: Effect of aqueous extract of WG on hydroxyl radical scavenging ability

Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT).

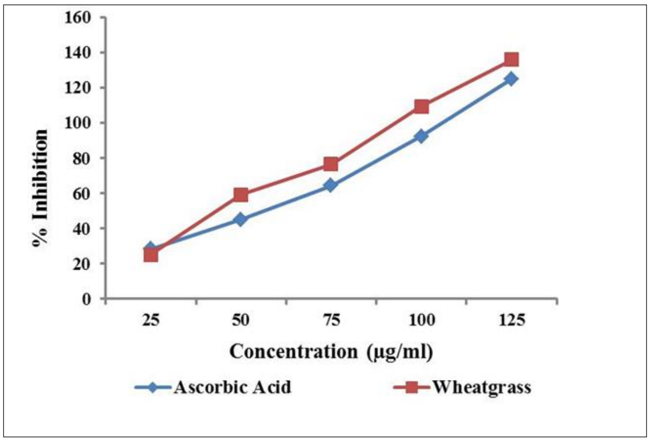

Fig 4: Effect of WG aqueous extract on percentage of cell viability in H9C2 cells by MTT assay

Yellow MTT [3-(4, 5-dimethyl-2-thiaozolyl)-2, 5-diphenyl-2H tetrazolium bromide] is converted to the blue formosan product only by metabolically active mitochondria, and the absorbance is directly proportional to the number of viable cells. Effects of WG aqueous extract on percentage of cytotoxicity in H9C2 cells was determined by MTT assay. H9C2 cells proliferation was significantly inhibited by *WG aqueous extract*at different concentrations 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µM respectively. The inhibitory effect was observed at 24h incubation. We found that *WG aqueous extract*at 20 µM concentrations resulted in a 50% inhibited in cell viability. Hence, we have selected four different concentrations 5, 10, 15 and 20 µM for further study.

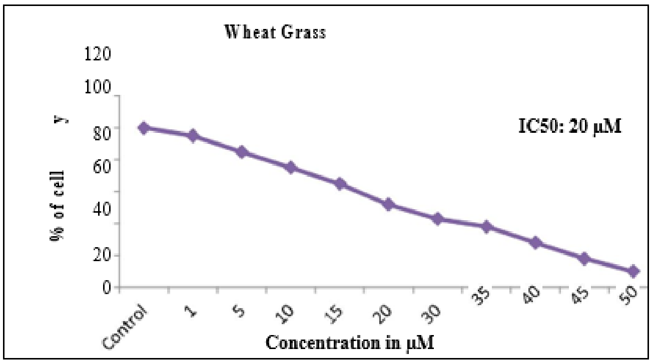

Fig 5: Effect of WG aqueous extract on percentage of cell viability in H9C2 cells by MTT assay

Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

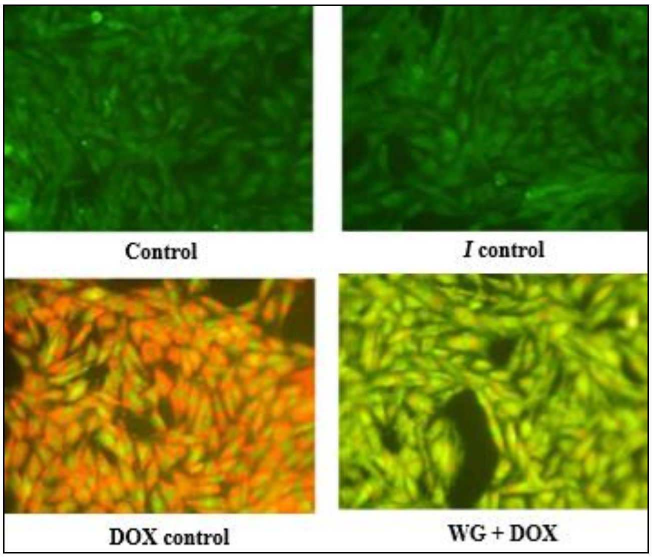

Fig 6: Cellular morphological changes were observed under a fluorescence microscope using OA/EtBr staining (20x)

Effect of WG on ISO-induced Mitochondrial Membrane Potential in H9C2 cells

Fluorescence microscopic images showed that the accumulation of Rh-123 dye in control group (Figure 6) and the dye accumulations were decreased in ISO-induced cells (Figure 6). There was decreased fluorescence intensity in ISO-induced H9C2 cells when compared to control. WG treatment before ISO-induction significantly prevented ISO-induced loss of Δψm in cardiomyocytes (Figure 6).

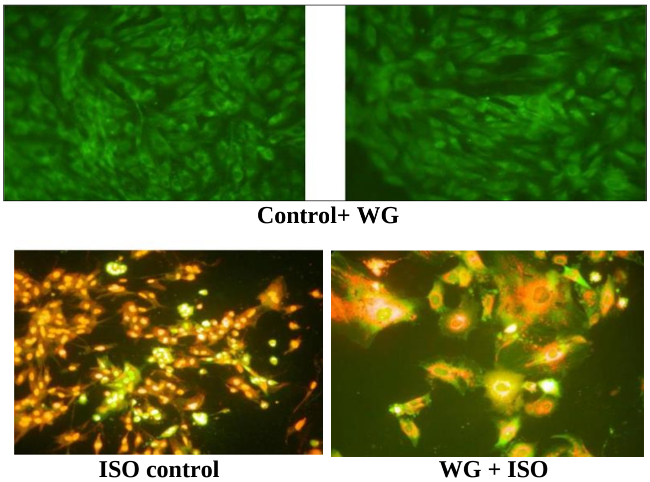

Fig 7: Cellular morphological changes were observed under a fluorescence microscope using OA/EtBr staining

In this study, we used acridine orange (AO) and ethidium bromide (EtBr) to differentiate cells that are apoptotic and/or viable (Figure 7). ISO-induced H9C2 cells showed condensed nuclei, membrane blubbing and apoptotic bodies (EtBr stained cells). In contrast the control cells (AO stained cells) showed intact nuclear architecture. WG pretreated + ISO-induced cells showed decreased % apoptotic cells when compared to ISO-induced cells.

Discussions

Traditionally, WG has been used since ages for treating a number of diseases. However, not enough scientific experiments have been done in order to study the effect and mechanism of WG on various disorders and metabolic parameters. Detailed study of the constituents of the WG and its effects on different pharmacological parameters could help us to screen novel compounds some of which could potentially be lead molecules. Phytomedicines have multi-constituents that give multi-targeting effect and cause minimum side effects compared with synthetic drugs (Briskin, 2000) [7].

The analysis of total flavonoids and phenolic contents showed that significant quantity of polyphenols and other flavonoids are observed in the WG extract. This was confirmed by GC-MS analysis. GC-MS analysis which showed the presence of diverse class of organic compounds in varying percentage in the WG aqueous extract, ranging from fatty acids (eg. n-hexadecanoic acid and octadecatrienoic acid), alcohols (eg.2-methyl benzenediol and phytol), terpenes (such as alpha and beta amyrin, caryophyllene and caryophyllene oxide). Majority of the compounds extracted belong to hydrocarbon class such as octadecene, nonadecene, 2-methyl octacosane, squalene, nonacosane and tricontane. Sterols such as gamma sitosterol are also reported in GC-MS analysis. Each of these compounds influence metabolism in some way or the other.

Gamma sitosterol has been reported to influence cholesterol synthesis in liver and intestinal cell lines (Ho and Pal, 2005) [17]. Squalene is a polyunsaturated hydrocarbon that has been reported to prevent oxidative damage and control the toxicity of 6-hydroxydopamine (Kabuto *et al*., 2003) [19].

Octadecane, another hydrocarbon, is present in significant amount in aqueous extract. It has been reported that it reduces

Pathophysiology consequences in Plasmodium berghei infected animals when treated with extracts that contain this particular compound in significant concentrations (Nahrevanian *et al*., 2009) [25]. Caryophyllene and its oxides are one of the most important biological compounds that influence diverse metabolism. Caryophyllene has been reported to have significant anti-cancer properties. It induces apoptosis through suppression of multiple pathways such as PI3-Kinase, AKT, mTOR and S6K1 (Park *et al.*, 2011) [28]. Many more effects like anti-bacterial, anti-fungal, immunomodulatory and anti-inflammatory have been reported for caryophyllene oxides (Astani *et al*., 2011) [4]. It is also reported to possess anti-platelet aggregation activity (Lin *et al*., 2003) [23].

Alpha and beta amyrins are the two biologically active penta cyclic triterpenes that influence wide physiological parameters such as anti-inflammatory, antioxidant, gastro protective and hepatoprotective effects at non-toxic concentrations (Oliveira *et al*., 2005; Holanda Pinto *et al*., 2008 and Aragao *et al*., 2007) [27, 18, 3]. Anti-hyperglycemic effect and hypolipidemic effect of amyrin have been recently investigated with positive conclusion suggesting that this compound is a potential candidate for diabetes and atherosclerosis (Santos *et al*., 2012) [30]. Thus, the GC-MS analysis of WG aqueous extract clearly shows the presence of some biologically functional active principles were already reported to possess numerous effects in regulating the general physiological and biochemical parameters.

Free radicals such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide and other reactive oxygen species are highly reactive and effectively destroying transient radicals generated in metabolic process. They are known for playing a double role as both harmful and helpful species, in view of the fact that they can be either deleterious or helpful to living organism (Halliwell and Gutteridge, 1999) [14, 15].

The *in vitro* radical scavenging activities of WG were estimated by a panel of antioxidant assays including $OH^{\bullet}$, metal chelation, hydrogen peroxide radicals, NO radical scavenging assays as well as assay for reducing power. In all the assays WG effectively scavenged the free radicals almost in a all doses in increasing order. The greater radical trapping efficacy of WG may be attributed to various effective compounds like flavonoids, phenols, phytol, sitosterol, squalene that are present in the WG aqueous extract.

Oxidative stress, which is usually associated with increased formation of reactive oxygen species (ROS), modifies phospholipids and proteins leading to lipid peroxidation and oxidation of thiol groups; these changes alter membrane permeability and configuration in addition to producing functional modification of various cellular proteins (Griendling and Alexander, 1997) [12]. Lipid peroxidation is an important pathogenic event in myocardial necrosis and accumulation of lipid hydroperoxides reflects damage of the cardiac constituents (Hamberg *et al*., 1974) [16].

In the present study, increased intracellular ROS levels were observed in ISO-induced H9C2 cells as evidenced by increased DCF flurescence (Figure 8a.). Whereas WG (10 µM) treatment prior to ISO-induction significantly reduced intracellular ROS levels, this is due to the possible scavenging of ROS during ISO-induced cells. Chen *et al*., (2006) have also shown that quercetin reduced the OH radical-induced plasmid DNA damage and inhibits ROS-dependent and independent apoptosis in rat glioma C6 cells. Based on our results and along with previous reports we state that the WG could scavenge ISO-induced free radicals.

As mitochondria play a major role in the induction of apoptosis, we examined the involvement of mitochondrial damage in ISO-induced apoptosis. To investigate the effect on mitochondria, we performed Rh-123 staining in H9C2 cells. The present findings showed that ISO-induced loss of $\Delta\psi m$ have been restored by WG pretreatment.

It suggests that WG protects cells from apoptosis by inhibition of the mitochondrial alteration. Most estimates suggest that the majority of intracellular ROS production is derived from radiolysis of water in the cellular milieu (El-Missiry *et al*., 2007) [10]. We therefore examined intracellular ROS levels by DCF method and apoptotic cells by EtBr/Ao staining.

References

1. Akram M, Aftab F. Effect of Cytokinins on *In vitro* seed Germination and Changes in Chlorophyll and Soluble Protein Contents of Teak (Tectona grandis L.). Biochem Physiol. 2015; 4:166.
2. Amare P, Jain H, Kabre S, Deshpande Y, Pawar P, Banavali S, Menon H, *et al*. Cytogenetic Profile in 7209 Indian Patients with de novo Acute Leukemia: A Single Centre Study from India. Journal of Cancer Therapy. 2016; 7:530-544.
3. Aragao L, Malhi Y, Roman-Cuesta RM, Saatchi S, Anderson LO, Shimabukuro YE. Spatial patterns and fire response of recent Amazonian droughts, Geophys. Res. Lett, 2007, 34.
4. Astani A, Reichling J, Schnitzler P. Screening for antiviral activities of isolated compounds from essential oils. Evid Based Complement Alternat Med, 2011.
5. Bharali B, Haloi B, Chutia J, Chack S, Hazarika Kl. Susceptibility of Some Wheat (*Triticum aestivum* L.) Varieties to Aerosols of Oxidised and Reduced Nitrogen. Adv Crop Sci Tech. 2015; 3:182.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology. 1995; 28:25-30.
7. Briskin D. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Update on phytomedicines. Plant Physiol. 2000; 124:507-514.
8. Chen YG, Xu F, Zhang Y, Ji QS, Sun Y, Lü RJ, Li RJ. Effect of aspirin plus clopidogrel on inflammatory markers in patients with non-ST-segment elevation acute coronary syndrome. Chin Med J. 2006; 119(1)32-36.
9. Dey S, Sarkar R, Ghosh P, Khatun R, Ghorai K, Choudhury R, Ahmed R. Effect of wheat grass juice in supportive care of terminally ill cancer patients-A tertiary cancer centre experience from India. Journal of Clinical Oncology. 2006; 24:8634.
10. El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol Environ Saf. 2007; 66:278-86.
11. Ghani A, Khan I, Umer S, Ahmed I, Mustafa I, Mohammad N. Response of Wheat (*Triticum aestivum*)

to Exogenously Applied Chromium: Effect on Growth, Chlorophyll and Mineral Composition. J Environ Anal Toxicol. 2015; 5:273.
12. Griendling KK, Alexander RW. Oxidative stress and cardiovascular damage during ischaemia and reperfusion: role of the cellular disease. Circulation. 1997; 96(10):3264-3265.
13. Hafer CL, Olson JM, Peterson AA. Extreme harmdoing: A view from the social psychology of justice. In V. M. Esses & R. A. Vernon (Eds.), Explaining the breakdown of ethnic relations:Why neighbors kill Malden, MA: Blackwell, 2008,17-40.
14. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford, U.K.: Oxford University Press, 1999.
15. Halliwell B, Guttridge JMC, Aruoma OI. Analytical biochemistry. 1987; 165:215-219.
16. Hamberg M, Svensson J, Wakabayashi T, Samuelsson B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc Natl Acad Sci. 1974; 71(2):345-349.
17. Ho SS, Pal S. Margarine Phytosterols Decrease the Secretion of Atherogenic Lipoproteins from Hepg2 Liver and Caco2 Intestinal Cells. Atherosclerosis. 2005; 182(1):29-36.
18. Holanda Pinto SA, Pinto LM, Guedes MA, Cunha GM, Chaves MH, Santos FA, Rao VS. Antinociceptive effect of triterpenoid alpha, beta-amyrin in rats on orofacial pain induced by formalin and capsaicin. Phytomedicine. 2008; 15:630-634.
19. Kabuto H, Hasuike S, Minagawa N, Shishibori T. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. Environ. Res. 2003; 93:31-35.
20. Kothari S, Jain AK, Mehta SC, Tonpay SD. Effect of fresh *Triticum aestivum* grass juice on lipid profile of normal rats. Indian J Pharmacol. 2008; 40(5):235-236.
21. Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TP, Reddy AV. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. Phytother Res. 2006; 20:218-27.
22. Lakshmi V, Neeraja M, Subbalaxmi MV, Parida MM, Dash PK, Santhosh SR, Rao PV.Clinical features and molecular diagnosis of Chikungunya fever from South India.Clin Infect Dis. 2008; 46(9):1436-42.
23. Lin Q, Zou X, Fang L, Willis WD. Sympathetic modulation of acute cutaneous flare induced by intradermal injection of capsaicin in anesthetized rats. J Neurophysiol. 2003; 89:853-861.
24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Method. 1983; 65(1-2):55-63.
25. Nahrevanian H, Hajihosseini R, Arjmand M, Farahmand M, Ghasemi F. Evaluation of anti-leishmanial activity by induction of nitric oxide and inhibition of prostaglandin in Balb/c mice infected with leishmania major. Southeast Asian Journal of Tropical Medicine and Public Health. 2009; 40(6):1188-1198.
26. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972; 46:849-853.
27. Oliveira FA, Chaves MH, Almeida FR, Lima RC Jr, Silva RM, Maia JL. Protective effect of alpha- and beta-amyrin, a triterpene mixture from Protium heptaphyllum (Aubl.) March.trunk wood resin, against acetaminophen-induced liver injury in mice. J Ethnopharmacol 2005; 98(1-2):103-108.
28. Park SC, Park YH, Kim JC, Jeong CY, Kim HS, Lee JW, *et al*. Kwak Sweet potato late embryogenesis abundant 14 (IbLEA14) gene influences lignification and increases osmotic- and salt stress-tolerance of transgenic calli Planta. 2011; 233(3):621-634.
29. Rana S, Kamboj JK, Gandhi V. Living life the natural way - Wheatgrass and Health. Functional Foods in Health and Disease. 2011; 1(11):444-456.
30. Santos FA, Frota JT, Arruda BR, Melo TS, Silva AACA, Brito GAC. Antihyperglycemic and hipolidemic effects of α,β-amyrin, a triterpenoid mixture from Protium heptaphyllum in mice. Lipids in Health and Disease. 2012; 11:98-105.
31. Sofi F, Ghiselli L, Dinu M, Whittaker A, Pagliai G, Cesari F, *et al*. Consumption of buckwheat products and cardiovascular risk profile: a randomized, single-blinded crossover trial. J. Nutr. Food Sci. 2016; 6(3):501-509.