Antioxidant and Cytotoxicity Profile of the Selected Alcoholic Beverages Widely Consumed in the Maharashtra State of India

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Abstract

The health concern of the alcoholic beverages has remained a key issue to be addressed to the consumers. The aim of the present study was to assess the antioxidant activity and cytotoxicity of the selected 22 bottled commercial alcoholic brands widely consumed in the Maharashtra state of India. The result of the present study shows that the brands of wines followed by whiskies and rums were more effective as free radical scavenging agents, containing considerable amount of polyphenols. Overall, the vodaka brands were observed to be more cytotoxic as compared to other brands. With few exceptions, there exists a positive correlation in the amount of polyphenols and free radical scavenging activity, while the former was negatively correlated with cytotoxicity.

***Key words**: Antioxidant; Alcoholic Beverages; Cytotoxicity; Polyphenol*

Introduction

Drinking of alcoholic beverages (ABs) is a global habit of majority of human beings. The trend of alcohol drinking is increasing all over the world considering its role as a stress or pain killer. The health concern of the ABs has remained a key issue to be addressed to the consumers in the present situation of 'stress rich satisfaction poor' socioeconomic scenario. During the process of production of ABs, manufacturers add variety of additives in the form of preservatives, flavors, coloring agents etc. which ultimately accounts for the final quality of the finished brands. Several studies have shown the benefits as well as adverse effects of the consumption of alcoholic beverages in daily life. The adverse effects of ABs are usually attributed with the type of brand, consumption frequency, amount of dose and the overall physiological status of the consumer; however the health benefits of many ABs are linked with the presence of therapeutically important substances such as flavonoids, phenolic compounds, amino acids etc. and the aforesaid factors. Series of scientific evidences showed that the people who are moderate drinkers of alcoholic beverages have substantially reduced the risk of many degenerative human ailments like coronary heart disease, cancer [1] etc. It has been also described that the flavonoids and other polyphenolic compounds derived from the source material are implicated in free radicals scavenging mechanism[2].

In the present study, 22 different commercial bottled alcoholic brands belonging to wine, whiskey, rum, gin, vodaka, local brand which are commonly consumed in Maharashtra state in particular and other states of India in general, were evaluated for their free radical scavenging activity and cytotoxicity against normal Chang liver cell line.

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Materials and Method

Alcoholic Beverages

The selected Alcoholic beverages were purchased from the local market at Nanded city (MS). The details Alcoholic beverages are shown in Table 1.

Table 1. The details Alcoholic beverages with their percentage of alcohol and cytotoxicity.

Sr. no.	Type of beverage	Brand name	Percent of alcohol (%)v/v	Cytotoxicity (%)
1	Whisky	Bagpiper	42.8	5
2	Whisky	Imperial blue	42.8	ND
3	Whisky	Mc dowell no.1	42.8	2.3
4	Whisky	Royal stag	42.8	ND
5	Whisky	Royal challenge	42.8	15.7
6	Whisky	Signature	42.8	ND
7	Rum	Old monk	42.8	ND
8	Rum	Mc dowell	42.8	0.5
9	Rum	Bacardi	42.8	7.6
10	Vodka	Romanov	42.8	6.7
11	Vodka	White mischief	42.8	23.7
12	Vodka	Magic moments	42.8	13.9
13	Vodka	Shark tooth	42.8	2.59
14	Vodka	Fuel	42.8	18.4
15	Gin	Blue reband	42.8	7.2
16	Gin	Blue reband duet	42.8	6.7
17	Gin	American	37.14	2.3
18	Brandy	Honey bee	42.8	6.3
19	LocalBrand	Bhingri	56.2	ND
20	Wine	Figuria	18	ND
21	Wine	Madira	13	ND
22	Wine	Samara	14	ND
		H2O2		4.34

ND-not determined, H_2O_2- standard used for cytotoxicity

Antioxidant activities:

DPPH radical assay:

DPPH (1, 1-diphenyl-2-picryl hydrazine) radical scavenging assay was performed as per the earlier reported method[3]. The reaction cocktail was prepared by mixing individual sample of ABs with equal volume of DPPH radical (10^{-4} M in absolute ethanol) solution. After 20 min reaction time, the absorbance was recorded at 517 nm using UV-Visible spectrophotometer.

OH Radical scavenging activity

Hydroxyl radical (OH) scavenging activity was measured as per previously published protocol[4]. The reaction mixture contained 60 µl of 1mM, $Fecl_3$, 90 µl of 1mM 1, 10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2 and 1.5 ml of individual brand. The reaction mixture was kept at room temperature for 5 minutes incubation and absorbance was recorded at 560 nm using UV-VIS spectrophotometer.

Superoxide anion scavenging activity

Superoxide anion radical (SOR) scavenging activity of the selected ABs was measured by generating them in a non enzymatic phenanzinemethosulfate- nicotinamide adenine dinucleotide (PMS-NADH) system though the reduction of nitro-bluetetrazolium (NBT)[5]. SORs were generated from the reaction mixture containing 3ml of tris-HCl buffer (100 mM, pH 7.4), 0.75 ml of NBT (300 mM), 0.75 ml of NADH and 0.3 ml of selected ABs.

Reducing Power activity

The reducing power (RP) of the selected brands was determined as per the previously described method[6]. The reaction cocktail contained 0.75 ml of individual alcoholic sample, 0.75 ml of phosphate buffer (0.2 N, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1% w/v). The mixture was incubated at 50^0 C in water bath for 20 min. The reaction was terminated by adding 0.75 ml of trichloroacetic acid (10%) and centrifuged for 10 minutes at 800 rpm. The supernatant (1.5 ml) of the individual reaction mixture was collected in different clean tubes and was mixed with 1.5 ml of distilled water followed by addition of 0.1ml of ferric chloride (0.1% w/v) and kept for 10 min. The absorbance of reaction mixture was measured at 700 nm.

Estimation of polyphenols:

The estimation of polyphenolic content from the selected ABs was determined by earlier reported method[7]. The phenolic compound undergoes reaction with an oxidizing agent phosphomolybdate present in the Folin-Ciocalteau reagent, the resultant reaction product is a blue coloured complex having maximum extinction at 660 nm. The amount of phenolics was calculated by using a standard curve using serial dilutions of catechol (500 µg/ml). The total amount of polyphenol was estimated as µg/ml of samples.

Evaluation of Cytotoxicity

The MTT cytotoxicity assay was performed as per the reported method[8]. Normal human Chang liver cell line was purchased from NCCS (National Center for Cell Science), Pune (MS). The cells were harvested and inoculated in 96 well (4×10^4 cells/well) microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the selected ABs. After 72 h incubation, the medium was aspirated followed by addition of 150 µL of MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution (5 mg mL^{-1} In PBS, pH 7.2) to each well and the plates were incubated for 4 h at 37° C. After incubation, 800 µL of DMSO was added to the wells followed by gentle shaking to solubalize the formazan dye for 15 min. Absorbance was read at 540 nm and the cytotoxicity (%) was calculated.

The DPPH, OH and SOR radical scavenging activity, RP potential and cytoxicity (%) was calculated using the following formula and the results were compared with the respective reference compounds.

$$\text{Activity (\%)} = 1 - \frac{T}{C} \times 100$$

T= Absorbance of the test sample & C= Absorbance of the control sample

Results and Discussion

The results shows the efficacy of the selected brands of wines such as Madira (99.24%), Sumara (98.92%), Figuria (98.59%) and brands of rums like Mc Dowell (98.54%), Old Monk (98.54%) followed by whisky brands like Royal Challenge (96.47%), Signature (95.78%) and Royal Stag (95.56%) as excellent DPPH radical scavenging agents. The brandy, Honey Bee (99.24%) was also observed to possess excellent DPPH radical scavenging activity. While the remaining brands showed good DPPH radical scavenging activity in the range of 74.02 to 94.26 % as compared to ascorbic acid (95.31 %). DPPH radical is a stable free radical and it has been widely used to evaluate the free radical scavenging ability of different dietary antioxidants. The principle of the assay is that the antioxidant reacts with DPPH radical and converts into corresponding hydrazines. The fall in extinction is correlated with the potential of antioxidant to scavenge free radicals. Greater the DPPH reducing ability higher is the antioxidant potential[9].

Among the selected ABs, again the wine brands like Figuria (78.9%) and Samara (98.92%) followed by rums like Mc Dowell (77.3%) and Bacardi (73.6%) demonstrated significant OH radical scavenging activity, all other brands showed the activity in a range of 20.8-72.9 % as compared to reference compound α-Tocopherol (76.79 %). OH radicals are generated through Fenton reaction. These radicals are the most reactive radicals in the biological systems. The hyper reactivity of OH radicals paralyses variety of cellular functions by indiscriminate reactions with biologically important molecules like proteins, enzymes, nucleic acids etc. and excess production of OH radicals induces several degenerative diseases in humans [10].

The profile of the SOR scavenging activity indicates the effectiveness of wines like Madira (70.2%), Samara (69.5%) and Figuria (67.8%) as significant SOR scavenging agents. The other selected AB samples showed considerable SOR scavenging activity in the range of (48.71-65.5%) as compared to ascorbic acid (53.3 %). SORs are capable of damaging cellular membranes (through peroxidation reactions), protein and other macromolecules[11]. The cellular damage caused by SORs has been implicated in aging process and in initiation of numerous age related diseases such as cancer, heart disease, Parkinson's disease etc [12].

Once again the wine brands such as Figuria (88.6 %), Samara (79.7%) and Madira (78.26%) followed by rum, Mc Dowell (77.9%) have demonstrated good reducing ability. Remaining AB samples also showed considerable reducing activity in a range of 48.71-65.5%. In a general pharmacological notion compounds possessing reducing ability are considered good candidates for developing them as antioxidant agents. Higher reducing capability indicates greater antioxidant activity[13].

The maximum amount of polyphenols content was estimated in the wine brands like Figuria and Madira (2000 µg/ml), Samara (1596 µg/ml) followed by the rums such as Mc Dowell (1718 µg/ml) and Old Monk (1031 µg/ml). Other brands of the selected ABs were found to contain <370 µg/ml of polyphenols. ABs like wine has been reported to contain complex mixture of phenolic compounds possessing therapeutically important activities and free radical scavenging capabilities. The diverse biological activities of polyphenols are described to be health ameliorative and involved in preventing many degenerative diseases of humans [14]. It is this reputation of the polyphenols (especially in the wines) that minimizes the risk of heart disease and intake of wines in limited proportions acts as cardio protective agent[15]. Nevertheless many physicians recommend the intake of cardio protective wines to minimize the risk of cardio failure. In general it can be stated that the amount of polyphenol contents is positively correlated with antioxidant activity. To a greater extent this also holds true in case of the results of the present studies.

MTT colorimetric cell viability assay is one of the widely used methods to test the cytotoxicity of the different samples. Cell's survival is checked on the basis of the mitochondrial enzyme's ability to reduce the soluble yellow tetrazolium salt (MTT) to an insoluble formazan dye[16]. The selected ABs were evaluated for cytotoxicity against normal Chang liver cells.

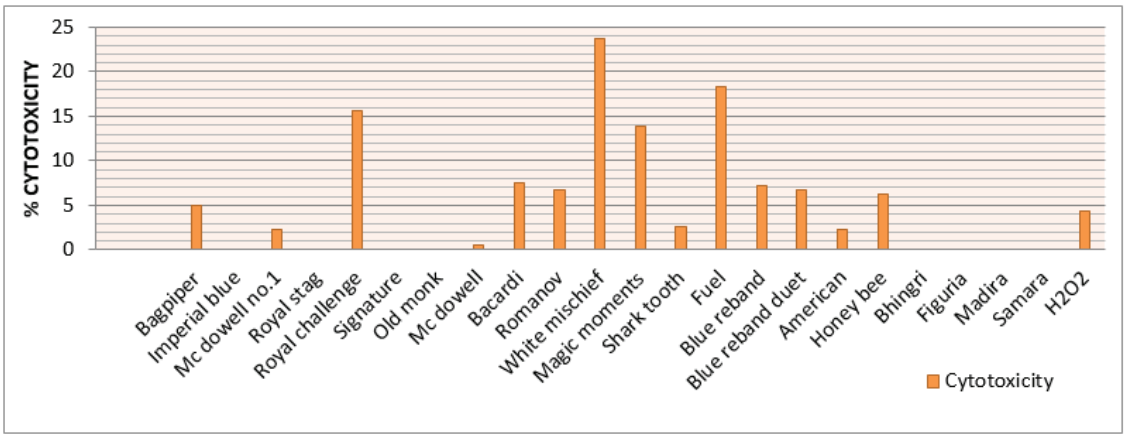

Figure 1. Profile of cytotoxicity (%) of the selected alcoholic beverages against human Chang liver cell line. The assay was performed using micro culture MTT method. The results are compared with hydrogen peroxide (20 mM). The results summarized are mean values of two parallel experiments.

The result summarized in Figure 1, indicates that all the vodaka brands (6.7-25.9 %) and Gins (6.7-18.4 %) have shown moderate cytotoxicity against Chang liver cells. Interestingly, wine brands were observed to be nontoxic towards Chang liver cells. No significant cytotoxicity was observed with whiskeys, rums and brandy. In general the cytotoxic effects of the ABs are linked with alcohol percentage and the types of additives used in the finished samples. Many times in *in vitro* cytotoxicity methods, it is difficult to ascertain the correct levels of toxicity of ABs due to volatilization of many toxic agents along with alcohol. One more important aspect of polyphenols especially flavonoids present in the ABs is linked with antitoxic, hepatoprotective, and cytoprotective effects. Moreover in several countries, plant flavonoids are widely used in the treatment of liver diseases and diseases associated with increased vascular permeability and capillary fragility[17]. The nontoxic nature of wines selected in the present study might be due to presence of the cytoprotective flavonoids derived from the source material.

It is concluded from the present study that the selected wines can be considered as effective antioxidant and free radical scavenging agents with no toxicity. The results of the present study may serve people a cursory ready reference for the health concerns of the selected ABs widely consumed in the Maharashtra state in particular and many other states of India in general. The results may also influence the manufacturers in reorienting their production strategies.

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