J Integral Sci, 2018, Volume 1, Issue 4, 7-11. ISSN: 2581-5679

www.jisciences.com

Research Article

Journal of Integral Sciences

In-vitro antioxidant activity of Wheatgrass powder

Ashya Sk [1,] *, Kishore Naidu K [2], Jagadeesh P[1]

[1] Raghu College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-531 162.

[2] A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530 003.

*Corresponding author: E-mail: shaikaasya@gmail.com; Mobile: +91 7670877889.

Received: 16 Sept 2018; Revised: 28 Sept 2018; Accepted: 01 Oct 2018

Abstract

The present study was undertaken to evaluate the *In-vitro* antioxidant activity of wheatgrass powder with bioenhancer cow urine. Antioxidant activity was evaluated on superoxide, Hydroxyl, DPPH, ABTS and Molybdinum free radicals. The wheat grass power is mixed with cow urine before extraction with water and the extract at different doses tested using standard procedures for antioxidant activity. The in vitro antioxidant study of wheatgrass powder showed good free radical scavenging activity in a dose dependent manner on tested free radicals and its activity is comparable with standard drug ascorbic acid. The results conclude that there is antioxidant capacity enhancement in the *Triticum aestivum* antioxidant capacity. Further research will be worth full, in the study of phytochemical variations between the normal *T. aestivum* grass powder before and after mixing with the Cow urine.

Key words: *Triticum aestivum*, Cow urine, Free radicals and Antioxidant activity.

1. Introduction

Cow urine (CU) has a special importance in the countries using traditional medicine like India, Nigeria, Myanmar. As per Ayurveda, CU used for the treatment of different diseases like leprosy cancer, anaemia (Jain *et al.*, 2010; Sairam, 2008; Salahdeen and Fagbohun, 2005). CU has its own medicinal value and simultaneously it acts as a bioenhancer, for example the other medicinal value plants parts (Barks of *Azadirachta indica*, *Justicia adhatoda* and leaves of *Nerium oleander*) by combing with CU used in the treatment of different diseases (US 6410059).

Triticum aestivum (*T. aestivum*) is belongs to the family Poaceae and producing third most commercially available cereal and its consumption protects us against different diseases such as high blood pressure, obesity, diabetes, gastritis, ulcers, anaemia, asthma, eczema cardiovascular disease, and cancer (Thompson, 1994; Jacobs *et al.*, 1998). There was scientific evidences about *T. aestivum* grass powder nutritional contents (Vitamins, Minerals) and presence of phytochemicals having biological activities (Phenolic compounds, Flavanoids) (Kulkarni *et al.*, 2006; Youngquist *et al.*, 2000; Laurent D *et al.*, 1998). As earlier said that CU is using as bioenhancer, the present study was carried out to know the presence of enhancement in the Antioxidant activity of wheat grass powder mixed with CU before its extraction.

2. Materials and methods

2.1 Drug and chemicals

Ascorbic acid, Riboflavin, Deoxy ribose, Nitroblue tetrazolium, 2, 2-Diphenyl -1-picrylhydrazyl (DPPH), Sodium phosphate, Ammonium molybdate, Potassium persulfate, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma chemicals, USA. All chemicals used were of analytical grade.

2.2 Preparation wheat grass extract

Dried wheat grass powder was directly obtained from market, Visakhapatnam and the dried powder was mixed with CU and dried under shade to obtain dried powder, this procedure repeated three times for the same wheat grass powder. The obtained dried powder was extracted with the distilled water and allowed the extracted solvent for drying to yield a dried extract.

2.3 In vitro antioxidant activity

2.3.1 Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich, (1969) method. This depends on light induced superoxide generation by riboflavin and the

corresponding reduction of nitro blue tetrazolium (Ganga Rao *et al.*, 2013).

2.3.2 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe2+/EDTA/ H2O2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (Elizabeth Kunchandy *et al.*, 1990; Ganga Rao *et al.*, 2013).

2.3.3 DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al.*, (2003) (Ganga Rao *et al.*, 2013). This assay is based on the measurement of the ability of antioxidants to scavenge the stable radical 2, 2-diphenyl-1-picrylhydrazil (DPPH). The free radical DPPH is reduced to corresponding hydrazine when it reacts with hydrogen donors.

2.3.4 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) scavenging activity

ABTS scavenging activity was performed as per the method described by Re *et al.*, (1999) (Aoxue Luo *et al.*, 2011). ABTS reacts with potassium persulfate produce the ABTS radical cations on incubation in dark. At time of experiment, ABTS solution mixed with ethanol to obtain the absorbance 0.70±0.02. The test samples antioxidant capacities were measured as absorbance of test sample with ABTS solution in ethanol at 734nm.

The percentage inhibition was calculated as

Scavenging effect or Percentage inhibition (%):

Control absorbance-Sample absorbance/Control absorbance×100.

2.3.5 Phosphomolybdate assay

Antioxidant capacity of test samples was measured as per standard method Umamaheswari and Chatterjee, (2008). The test sample (100µL) mixed with reagent solution (1mL) (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) and closed tubes were incubated in water bath at 95°C for 90 min. The incubated samples were cooled to room temperature; the absorbance was measured at 765nm against blank. The blank contains reagent solution and appropriate volume of distilled water. The assays were carried out in triplicate and expressed as mean ± SD. The antioxidant activity was expressed as the absorbance of the sample.

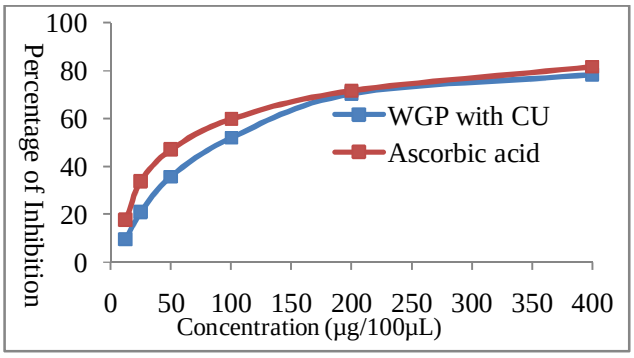

Fig 1. Percentage scavenging effect of wheat grass powder with cow urine and ascorbic acid on superoxide free radical.

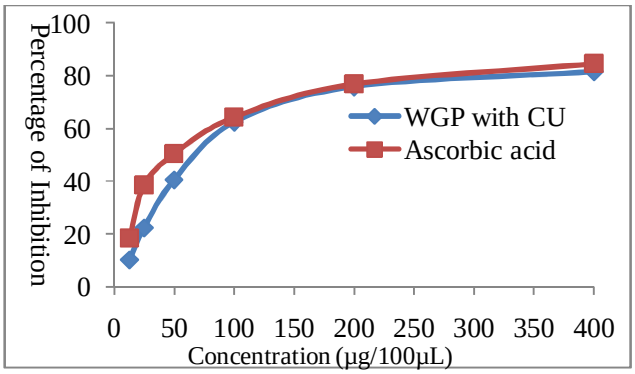

Fig 2. Percentage scavenging effect of wheat grass powder with cow urine and ascorbic acid on hydroxyl free radical.

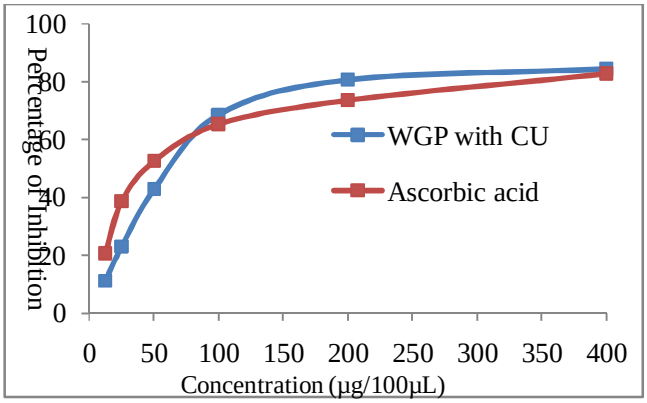

Fig 3. Percentage scavenging effect of wheat grass powder with cow urine and ascorbic acid on DPPH free radical.

3. Results and Discussion

In a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. Nevertheless, when this balance is disrupted towards an excess of reactive oxygen species, oxidative stress occurs (Moreira da Silva *et al.*, 2010).

Table 1. Percentage scavenging effect of wheat grass powder with cow urine on different free radicals.

Concentration of extract/Standard (µg/100µL)	% of scavenging activity on free radicals			
	Superoxide	Hydroxyl	DPPH	ABTS
12.5	09.54±0.65	10.27±0.44	11.25±0.32	9.64±0.12
25	20.68±0.09	22.4±0.76	23.08±0.88	20.22±0.18
50	35.72±1.54	40.64±0.24	42.74±0.92	38.67±0.42
100	52.04±0.44	62.48±0.79	68.62±0.34	59.42±0.06
200	70.22±0.82	75.77±1.04	80.54±0.48	72.88±0.33
400	78.45±0.28	81.66±0.76	84.37±0.92	82.42±0.63

Table 2. Percentage scavenging effect of Ascorbic acid on different free radicals.

Ascorbic acid (µg/100µL)	% of scavenging activity on free radicals			
	Superoxide	Hydroxyl	DPPH	ABTS
12.5	17.54±0.42	18.36±1.23	20.54±0.68	19.34±0.18
25	33.57±0.34	38.47±0.88	38.66±0.44	37.47±0.28
50	46.84±0.98	50.32±0.68	52.48±0.27	49.66±0.92
100	59.82±1.36	64.28±0.64	65.26±1.04	58.88±0.24
200	71.64±0.26	76.88±0.72	73.48±0.77	69.07±1.08
400	81.68±0.47	84.56±0.48	82.64±0.82	80.62±0.82

Recently an intensive search for novel types of antioxidants has been carrying out from numerous plant materials (Medicinal plants, crops) (Kochhar, 2008; Saxena *et al.*, 2007). Simultaneously, the research is going on identification of bio-enhancers, which are responsible in increase the activity of drug and its availability to the body in its dosage form (Navin and Bedi, 2010; Kritika Kesarwani and Rajiv Gupta, 2013). In this point of view, present was carried out on wheatgrass powder with CU, as earlier said which is using as bioenhancer, screened for free radical scavenging activity.

The in vitro antioxidant study of wheatgrass powder with CU showed good free radical scavenging activity in a dose dependent manner (12.5, 25, 50, 100, 200, 400 mg/100µL) and in turn the results were comparable to the standard drug Ascorbic acid.

The wheat grass extract showed the dose dependent scavenging activity on tested free radicals (Table 1; Fig 1-5) and its activity is comparable with the standard drug ascorbic acid (Table 1 and Table 2). The scavenging activity on superoxide, hydroxyl, DPPH, and ABTS free radicals was calculated in percentage inhibition, the scavenging activity in phosphomolybdate assay was measured by absorbance of samples.

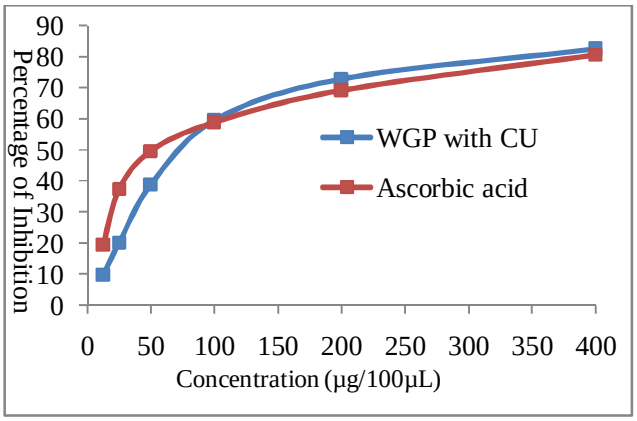

Fig 4. Percentage scavenging effect of wheat grass powder with cow urine and ascorbic acid on ABTS free radical.

The selected extract showed variations in scavenging activity on different free radicals. The percentage inhibition on superoxide free radical varies from 09.54±0.65 to 78.45±0.28 depends on extracts concentration respectively. The percentage inhibition on hydroxyl free radical varies from 10.27±0.44 to 81.66±0.76 depends on extracts concentration respectively.

The percentage inhibition on DPPH free radical varies from 11.25±0.32 to 84.37±0.92 depends on extracts concentration respectively.

The percentage inhibition on ABTS free radical varies from 9.64±0.12 to 82.42±0.63 depends on extracts concentration respectively.

The extract also showed the antioxidant activity in phosphomolybdate assay. Phosphomolybdate assay is based on absorbance, because the antioxidants present in the extract convert the molybdenum (VI) to molybdenum (V) forming a green colour in reagent solution. The increase in absorbance at 765nm shows the presence of antioxidant capacity of selected extract in dose dependent manner (Fig 5). The extract showed the scavenging activity as standard drug ascorbic acid on tested free radicals (Table 2).

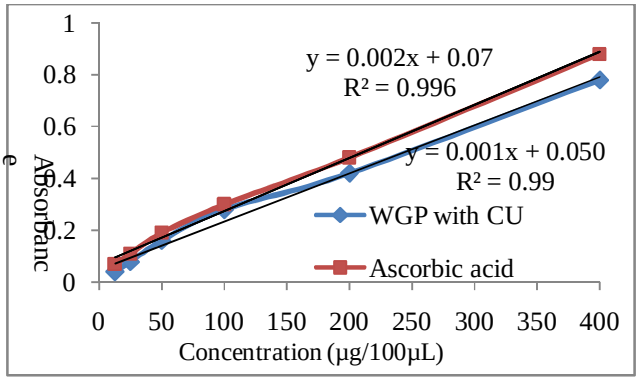

Fig 5. Correlation between different concentrations of extract and their antioxidant capacity determined by the formation of phosphomolybdenum complex assay.

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. The over production of ROS, result in damage to biomolecules (e.g., lipid, protein, amino acids, and DNA) hence the cell tissue are deprive of their normal function (Moreira da Silva *et al.*, 2010). So, the researchers searching for a new antioxidant molecules and identified some herbal drugs with antioxidant activity (Valko *et al.*, 2007; Nunes *et al.*, 2012; Krishnaiah *et al.*, 2011). But, the identified antioxidant molecules are poorly available in the body when they used in dosage forms. So, the present study carried on the enhancement of antioxidant activity of the *T. aestivum* grass powder reported by some researchers about its free radical scavenging activity (Kulakarni *et al.*, 2006) mixed with bioenhancer cow urine (CU) before extraction which was used as bioenhancer (Jain *et al.*, 2010; Sairam, 2008; Salahdeen and Fagbohun, 2005). In this process, we identified the antioxidant capacity enhancement of the *T. aestivum* antioxidant capacity.

4. Conclusion

From the above results, it could be conclude that the CU is acting as bioenhancer for the active ingredients present in the *T. aestivum*. Further research will be worth full, i.e. the direct extraction of *T. aestivum* grass powder will have more effective in antioxidant activity and the observation of any phytochemical alteration will occur between the normal *T. aestivum* grass powder before and after mixing with the CU.

Acknowledgments

The authors are thankful to authorities of A.U. College of Pharmaceutical Sciences for providing the necessary facilities for completes the present research work.

Conflict of Interest

We have none to declare.

References

Barbara L, Miriana D, Fiorenza M, Antonella G, Angela C *et al.*, 2015. Phytochemical Composition and Anti-Inflammatory activity of Extracts from the Whole-Meal Flour of Italian Durum Wheat Cultivars. *Int J Mol Sci*, 16, 3512-3527.

Ganga Rao B, Venkateswara Rao Y, Mallikarjuna Rao T. 2013. Hepatoprotective and antioxidant capacity of *Melochia corchorifolia* Extracts, *Asian Pac J Trop Med*, 6 (7), 412-420.

Harman D. 1998. Free radical theory of aging. Current status. Amster-dam: Elsevier, 3-7.

Jain NK, Gupta VB, Rajesh Garg, Ilawat N. 2010. Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India-A survey. *IJGP*, 4 (1), 29–35.

Krishnaiah D, Sarbatly R, Nithyanandam RR. 2011. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process*, 89, 217-233.

Kritika Kesarwani, Rajiv Gupta. 2013. Bioavailability enhancers of herbal origin: An

overview. *Asian Pac J Trop Biomed,* 3(4), 253–266.

Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TP, *et al.,* 2006. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. *Phytother Res,* 20(3)**,** 218-27.

Navin A, Bedi KL. 2010. Bioenhancers: Revolutionary concept to market. *J Ayurveda Integr Med,* 1(2), 96–99.

Nunes PX, Silva SF, Guedes RJ, Almeida S. 2012. Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M *et al.,* 1999. Antioxidant activity applying an improved ABTS radical cation decolourisation assay. *Free Rad Biol Med,* 26, 1231-1237.

Sairam TV. 2008. The Penguin Dictionary of Alternative Medicine. Penguin Books Limited. pp. 316.

Salahdeen HM, Fagbohun TR. 2005. Effects of cow urine concoction and nicotine on the nerve-muscle preparation in common African toad Bufo regularis. *Biomed Res,* 16 (3), 205–211.

Umamaheswari M and Chatterjee TK. 2008. In vitro antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *Afr J Trad Compl Altern Med,* 5, 61-73.

US 6410059. Pharmaceutical composition containing cow urine distillate and an antibiotic.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M *et al.,* 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol,* 39, 44–84.

Mc Cord JM, Fridovich I. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprien). *J Biol Chem,* 244, 6049-6055.

Thompson LU. 1994. Antioxidants and hormone-mediated health benefi ts of whole grains. *Critv Food Sci Nutr,* 34, 473-97.

Elizabeth K and Rao MNA. 1990. Oxygen radical scavenging activity of curcumin. *Int J Pharm,* 58, 237-2340.

Braca A, Fico G, Morelli I, DeSimone F, Tome F *et al.,* 2003. Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. *J Ethnopharmacol,* 86, 63-67.

Jacobs DR Jr, Andersen LF, Blomhoff R. 1998. Wholegrain consumption is associated with a reduced risk of noncardiovascular, noncancer death attributed to inflammatory diseases in the Iowa Women's Health Study. *Am J Clin Nutr,* 68, 248-57.

Youngquist JA, Hamilton TE. 2000. A look at the world's timber resources and processing facilities. In: Proceedings of the XXI IUFRO World Congress. 2000, Sub-plenary sessions Vol. 1, Kuala Lumpur, Malaysia, 183–190.

Laurent D, Costa R, Modesto J, Kohler F, Le Bars P *et al.,* 1998. Fumonisin: a major problem in New-Caledonia. *Rev Med Vet (Toulouse),* 149, 702.

Luo A, Ge Z, Fan Y, Luo A, Chun Z *et al.,* 2011. *In Vitro* and *In Vivo* Antioxidant Activity of a Water-Soluble Polysaccharide from *Dendrobium denneanum. Molecules,* 16, 1579-1592.

Kochhar KP. 2008. Dietary spices in health and diseases: I. *Indian J Physiol Pharmacol,* 52, 106-1022.

Saxena R, Venkaiah K, Anitha P, Venu L, Raghunath M. 2007. Antioxidant activity of commonly consumed plant foods of India: contribution of their phenolic content. *Int J Food Sci Nutr,* 58, 250-2560.

Moreira da Silva F, Marques A, Chaveiro A. 2010. Reactive oxygen species: A double-edged sword in reproduction. *The Open Vet Sci, J* 4, 127-133.