In vitro antioxidant and reducing capability of weight loss tablet formulation

Syed Rashid Ali Jaffary[1]*, Syed Waseemuddin Ahmed[1], Sadia Shakeel[2], Hafiz Muhammad Asif[3] and Khan Usmanghani[4]

[1]Faculty of Pharmacy, University of Karachi, Karachi, Sindh, Pakistan
[2]Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Sindh, Pakistan
[3]University College of Conventional Medicine, Faculty of Pharmacy Alternative Medicine, Islamia University, Bahawalpur, Pakistan
[4]Herbion Pakistan (Pvt.) Limited, Karachi, Sindh, Pakistan

Abstract: Antioxidants are used as an influential counteractive measure in opposing the generation of reactive oxygen species. The current study was carried out to investigate antioxidant potential and reducing capability of developed weight reducing tablet formulation. When tablets were evaluated at concentrations of 50, 100 and 500µg/ml, antioxidant activity improved in a dose depending way just similar to standard Butylated hydroxyl anisol (BHA). For evaluation of reducing ability the formulation under test evaluated at concentrations of 50, 100 and 500µg/ml and it was observed that formulation contain good reducing capability and possess considerable activity to scavenge super oxide radicals. *In-vitro* analysis of weight reducing tablets formulation showed considerable antioxidant and reducing capacity that will be supportive in averting the development of a variety of oxidative stress-related diseases.

Keywords: Antioxidant capability; reducing ability; weight reducing tablet formulation.

INTRODUCTION

Overweight and obesity is commonly defined as 'a weight that is greater than what is healthy for a specific height' (Hidron *et al*., 2008). Numerous health problems for instance cardiac disease, gallbladder disease, diabetes mellitus, osteoarthritis, and several other complications are associated with obesity and it is considered as a main health issue equally in developing and developed part of the world. Since weight perturbations are so common and detrimental, several treatment options have been developed to overcome the obese condition (Calle & Kaaks, 2004). In recent times, very potent drugs have turn out to be a trendy means to overcome excessive weight (Shaikh & Hatcher, 2005). However, severe adverse toxicities may restrict their effectiveness. Natural substances less probable to produce severe toxicity are successful in reducing appetite and promoting considerable weight loss. It is a common perception that herbal medicines are harmless, gentle, unadulterated for self-treatment of mild disorders (Flier, 2004). Since thousands of years in traditional Chinese medicine safe herbal formulas intended for weight loss have been used. According to the World Health Organization, the use of herbal remedies exceeds to that of the conventional drugs by two to three folds all over the world (Pal & Shukla, 2003).

Antioxidants isolated from natural sources are holding contemplation as probable resource owing to important therapeutic actions, cost effectiveness and low level of toxicity (Djeridane *et al*., 2006; Lin & Yin, 2007). The exploration for compounds obtained from natural source and rich in antioxidant potential is increasing since they have magnitude in controlling a variety of unrelieved therapeutic problems (Auddy *et al*., 2003). The current investigation aims to determine the antioxidant ability and reducing potential of herbal weight reducing tablets that integrates an outstanding combination of herbs including *Foeniculum vulgare*, *Trigonella foenum-graecum-seed*, *Thea sinensis-leaf*, *Ephedera vulgaris plant*, *Althaea officinalis root*, *Zingiber officinale rhizome*, *Apium graveolens leaf*, *Moringa oleifera leaf* and *Mallotus philippensis seed.*

MATERIALS AND METHODS

Tablet composition

Each 500mg tablet contains Foeniculum vulgare: 10mg; *Trigonella foenum-graecum*-seed: 10mg; *Thea sinensis*-leaf: 10mg; *Ephedera vulgaris*: 10mg; *Althaea officinalis*: 10mg; *Zingiber officinale*: 10mg; *Apium graveolens*: 10mg; Moringa oleifera: 10mg, *Glycyrrhiza glabra* 10 mg, *Ruta greveolens* 10 mg and *Mallotus philippensis*: 10mg.

Chemicals

All the analytical grade solvents were used. The standard (Butylated hydroxyanisole) was purchased from Merck, Pakistan. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals were procured from Sigma-Aldrich Chemie (Buchs, Switzerland).

Extract preparation

The herbs used in the preparation were sieved through mesh #60. Each grinded herb was taken into extractor and

**Corresponding author:* e-mail: rashidjaffary1955@gmail.com

water was added as solvent in the proportion of 1:10 (herb: solvent). The decoction was obtained by heating the extractors with steam for 2-3 hours. Filtration was done and the filtered decoction was shifted to evaporators to eradicate the additional solvent.

DPPH radical scavenging activity

1. 2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl in the concentration of 3mM was prepared in ethanol.
2. The well plates were categorized as control compound, blank compound and test compound.
3. 95µl DPPH solution was added in each labelled well.
4. 5µl test compound of concentration 10-1000µM into DMSO was subsequently added in DPPH solution and shaken for few seconds.
5. Micro titre plate was read at the absorbance of 515 nm after 30 minutes. UV visible 1601 Shimadzu double beam spectrophotometer was used to measurement of spectra.

The following equation was used to determine the DPPH radical scavenging activities

$$DPPH\ radical\ scavenging\ effect\ (\%) = Ac - \frac{As}{Ac}\ x\ 100$$

Where
Ac = Control compound's Absorbance
As = Test compound's Absorbance

Determination of the reducing power

1. 100µl: 10-1000µM test compound made in DMSO was mixed up with 250µl phosphate buffer having pH 6.6 and concentration 0.2M.
2. In the test tube 250µl Potassium ferricyanide (1%) was subsequently added.
3. Following centrifugation, 250µl upper layer solution was taken in different test tubes and mixed up with 250 µl DMSO.
4. To this mixture 50µl Ferric chloride (0.1%) was added.
5. An absorbance was measured at 700 nm using a spectrophotometer.

Following equation was used to determine the percent reduction ability

$$Percent\ Reduction\ Activity = \frac{At}{As}\ \ X\ \ 100$$

Where
A_t = Test compound Absorbance
A_s= Standard compound Absorbance

Super oxide scavenging activity by alkaline DMSO method

Nitro-blue tetrazolium (NBT) was made for this process (Shakeel *et al*., 2015) in quantity of 1mg/ml DMSO. For preparation of alkaline DMSO 0.1ml 5mM NaOH solution in water and 0.9ml DMSO was mixed up. Stock solution of 1mM test compounds and their serial dilutions were prepared in the range of 7 to 1000µM in methanol or DMSO. 100ml NBT (1mg/ml) was added in every tube. Compound was added of varied identified concentrations in the quantity of 300µl in tube marked as test. Lastly, 1 ml alkaline DMSO was added into it for making volume 1.4ml. Absorbance was determined at 560nm using a spectrophotometer.

Following equation was used to find out the scavenging activity

$$Percent Super\ oxide\ scavenging activity = \frac{Control - Test}{Control} \times 100$$

RESULTS

In current study, when tablets were evaluated at different concentrations (50, 100 and 500µg/ml), (table 1) ability of DPPH radical scavenging improved in a dose dependent way just similar to standard Butylated hydroxyanisole (BHA). At 50µg/ml tablets possess 23.1%, at 100µg/ml tablets exhibit 54.6% and at 500µg/ml tablets possess 67.3% activity. The standard BHA revealed 40.8%, 76.3% and 87.2% antioxidant activity at concentrations of 50, 100 and 500µg/ml correspondingly. (fig. 1) For evaluation of reducing ability the formulation under test evaluated at different concentrations (50, 100 and 500µg/ml), reducing capacity improved in a dose dependent way similar to standard (table 2). It was observed that formulation contain good reducing capability. At 50µg/ml tablets possess 14.2%, at 100µg/ml tablets exhibit 34.3% and at 500µg/ml tablets possess 56.4% reducing activity. The standard showed 54%, 68.2% and 87.1% reducing activity at concentrations of 50, 100 and 500µg/ml correspondingly. (fig. 2) Formulation was compared at different concentrations (50, 100 and 500µg/ml) (table 3) and it was revealed that weight reducing tablets have moderate activity to scavenge super oxide radicals. At 50µg/ml tablets possess 13.5%, at 100µg/ml tablets exhibit 32.9% and at 500µg/ml tablets possess 54.6% activity. The standard revealed 51.3%, 73.6% and 87.1% activity at concentrations of 50, 100 and 500µg/ml correspondingly. (fig. 3).

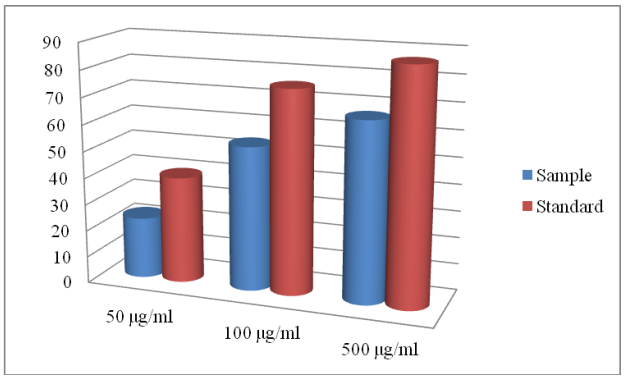

Fig. 1: Comparison of Antioxidant potential of Formulation with Standard

DISCUSSION

The generation of reactive oxygen species (ROS) by ionizing radiation, ultraviolet light, metabolic processes,

sunlight and chemical reactions away from the antioxidant capability of a biological system confers to oxidative stress (Shakeel *et al*., 2015). ROS comprised of singlet oxygen, hydrogen peroxide, hydroxyl radicals and super oxide radicals have been implicated in the pathogenesis including carcinogenesis, DNA damage and different degenerative disorders for instance diabetes mellitus, atherosclerosis, hypertension, inflammation, cancer and AIDS (Finkel & Holbrook, 2000). In view of the fact that ROS have probable harmful effects, unnecessary ROS must be rapidly eradicated from the cells. Antioxidants comprises of both hydrophilic and lipophilic molecules in favor of metabolizing ROS (de Souza *et al*., 2007; Gutteridge & Halliwell, 2000). It is evident from the literature that the antioxidants can play a vital role in the pathogenesis of a variety of diseases (Preethi *et al*., 2010). Several artificial additives, such as BHA and BHT, are used to protect oxidation of food for extensive period of time, with no perceptible harmful effects. Nevertheless, their consumption is limited in view of the fact that they are supposed to be carcinogenic(Basniwal *et al*., 2009).

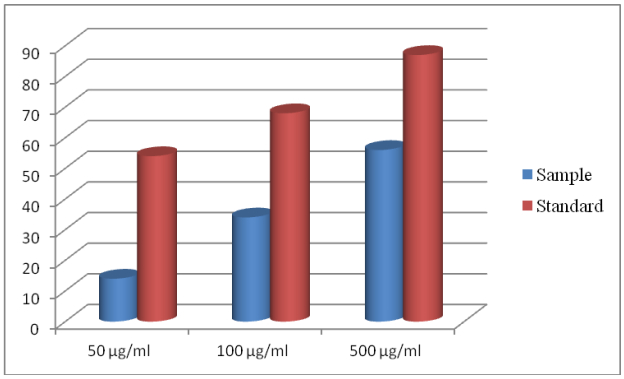

Fig. 2: Comparison of reducing ability of Formulation with Standard

Plants enclose a numeral compounds with antioxidant action for instance anthocyanins, phenolic acids, carotenoids, tannins and flavonoids. Phenolic components present in plants have showed their action like scavengers and inhibitors of lipid per oxidation (Pham-Huy *et al*., 2008) (Farzaneh & Carvalho, 2015). Therefore, the researchers are focusing on antioxidants obtained from natural sources (Luximon-Ramma *et al*., 2002). The current study determined the antioxidant ability and reducing potential of herbal weight loss tablets that integrates an outstanding combination of herbs including *Foeniculum vulgare, Trigonella foenum-graecum-seed, Thea sinensis-leaf, Ephedera vulgaris, Althaea officinalis, Zingiber officinale, Apium graveolens, Moringa oleifera Glycyrrhiza glabra, Ruta greveolens* and *Mallotus philippensis.* Oktay evaluated the antioxidant activity of *Foeniculum vulgare* seed by different antioxidant assays and indicated that the *F. vulgare* seed is a prospective resource of natural antioxidant (Oktay *et al*., 2003). *I. Stoilova* observed that properties of the ginger extract, can be compared with the synthetic antioxidant, thereby reported its potential as a natural preservative, applicable in the industries(Stoilova *et al*., 2007). Moringa leaves are found to have distinct antioxidant activity (Siddhuraju & Becker, 2003). In current study, when tablets were evaluated at different concentrations (50, 100 and 500µg/ml), ability of DPPH radical scavenging improved in a dose dependent way just similar to standard BHA. It was observed that formulation contain reducing capability.

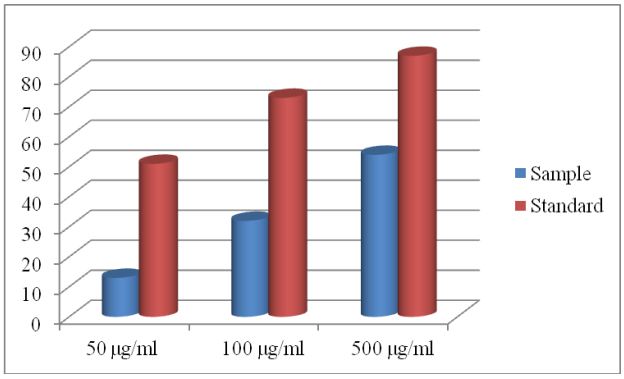

Fig. 3: Comparison of Super oxide scavenging activity of Formulation with Standard

DPPH is a stable free radical possessing a distinctive absorption in between 515 and 517nm, that is reduced due to the compound that is capable enough to reduce it to its hydrazine form by hydrogen/electron transfer (Huang *et al*., 2005). The reducing powers are typically associated with reductones, that show antioxidant action by breaching the free radical chain by contributing an atom of hydrogen (Elmastaş *et al*., 2006). Increased muscle activity can be capable of starting metabolic pathways that form free radicals, including increased electron transport chain activity and conversion of hypoxanthine to urate. Active skeletal muscle may elicit a 100-fold increase in O_2 flux through the aerobic metabolic pathways. Rapid electron transfer with increased respiration may cause some electrons to leak from the electron transport chain and partially reduce oxygen. Partial reduction of oxygen forms $O_2^{\bullet-}$ and subsequently H_2O_2. Among obese persons, high cell respiration rates and oxygen consumption may be exacerbated in muscle tissue during physical activity due to the additive mechanical load of carrying excessive body weight. For instance, during the same absolute load-bearing walking activity, obese persons have 38% higher oxygen consumption values than nonobese persons, and these values were found to be correlated with post exercise lipid hydro peroxide values. Acceleration of mitochondrial respiration for energy production is associated with increased lipid hydro peroxide production in the obese. Obesity exacerbates oxidative stress despite weight support in exercise and identical relative workloads owed to the reason that obesity accelerates the formation of

Table 1: Antioxidant activity of weight reducing tablets and Standard

	Concentration tested	Percent activity sample	Percent activity standard
1	50µg/ml	23.1%	40.8%
2	100µg/ml	54.6%	76.3%
3	500µg/ml	67.3%	87.2%

Table 2: Reducing Ability of Weight reducing tablets and Standard

	Concentration tested	Percent activity sample	Percent activity standard
1	50µg/ml	14.2%	54%
2	100µg/ml	34.3%	68.2%
3	500µg/ml	56.4%	87.1%

Table 3: Super oxide scavenging activity of weight reducing tablets and Standard

	Concentration tested	Percent activity sample	Percent activity standard
1	50µg/ml	13.5%	51.3%
2	100µg/ml	32.9%	73.6%
3	500µg/ml	54.6%	87.1%

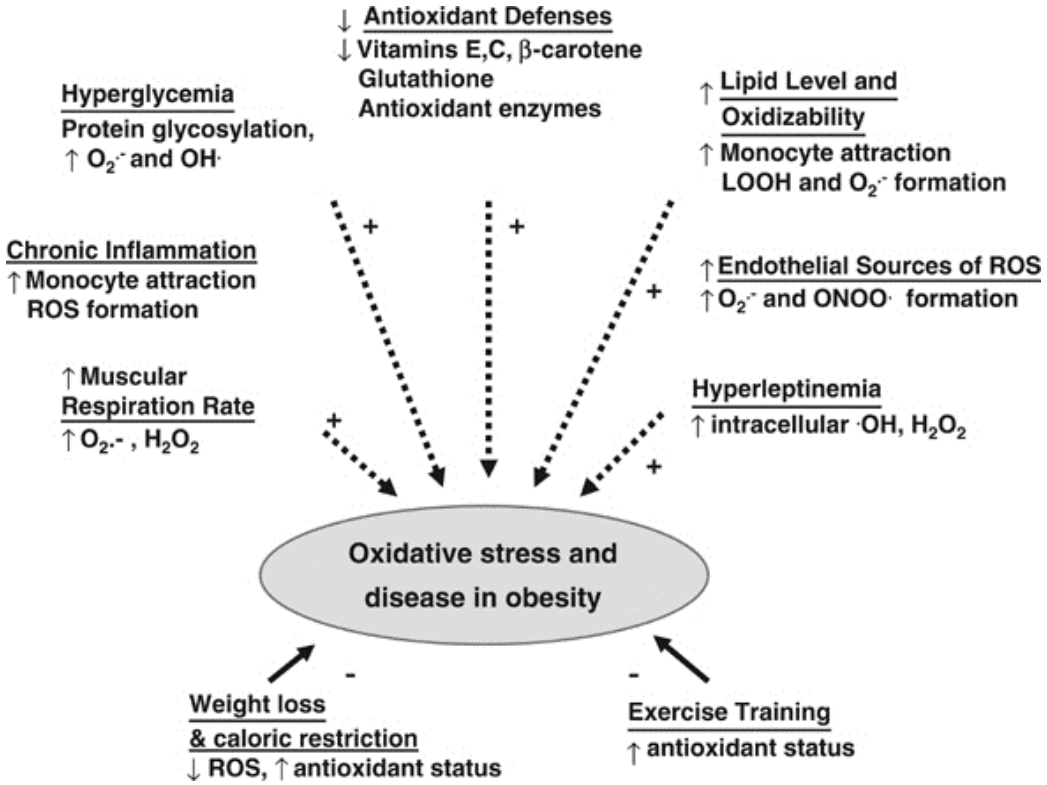

Fig. 4: Relationships between pro-oxidant and antioxidant influences in obesity-induced oxidative stress. + symbol represents an increase on oxidative stress, - symbol represents an attenuation effect on oxidative stress. LOOH, lipid hydro per oxides.

ROS or excessively tax the available antioxidant pool, leading to lipid per oxidation (Vincent & Taylor, 2006). Significant relationships between these antioxidant processes in obesity are shown in fig. 4.

Formulation was compared at different concentrations of 50, 100 and 500µg/ml and it was revealed that weight reducing tablets have moderate activity to scavenge super oxide radicals. In this study, the developed weight reducing formulation showed antioxidant ability due to the polyphenolic compound components of the herbs used in the formulation. This potential will be helpful in weight reducing activity.

CONCLUSION

In–vitro antioxidant investigation of the weight reducing drug showed considerable antioxidant and reducing capability. It will be supportive in averting the development of a variety of oxidative stress-related diseases.

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