To Investigate the Antioxidant Property of GINGER-JUICE (ZINGIBER OFFICINALE ROSCOE) Using Established Antioxidant Parameters

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Abstracts: Background & objectives: To investigate the Antioxidant property of ginger-juice (G.J) in rat. Methods: Albino rats (n=6-12) were administered G.J at two doses (4ml/rat, p.o) as a chronic treatment over period of 21 days. The liquid portion which was obtained by the course of filtration, looked like yellowish hazy opalescent liquid. ANTIOXIDANT STATUS (FREE RADICALS): Blood samples were used for following antioxidant parameters. 1. Glutathione peroxidise 2. Glutathione reductase 3. Total antioxidant status Results: The chronic administration of G.J (4ml/rat, p.o) over a period of 21 days did not alter any of these parameters except glutathione reductase. Conclusion: G.J rules out the Antioxidants property in form of rise reduced glutathione level was noted.[Prasad S S NJIRM 2012; 3(2): 31-35]

Key Words: Glutathione peroxidise, Glutathione reductase, Total antioxidant status, ginger-juice

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Introduction: Ginger is one of the most important and oldest spices, consisting of the prepared and sun-dried rhizomes of Zingiber officinale (Zingiberaceae). It is cultivated in many tropical countries. It is produced all over India from ancient times. It has a good commercial value and is claimed to have many medicinal uses. Because of differences in cultivation pattern, harvesting technique and climatic conditions it's commercial value differs and so also the medicinal actions and uses. It is referred by different names in the languages of different regions and countries.

It is widely consumed almost all over the world however in tropical countries or warm regions like Asia, it is more popular[1]. Because of its typical taste and a pleasant odor it's widely used as flavoring agent in numerous food recipes, beverages, pickles, many popular soft drinks etc[2].

From the ancient times it is included in many traditional medicinal systems for treatment of number of diseases. It is widely claimed as a Stomachic, aromatic, carminative, aphrodisiacs, diaphoretic, antiemetic, allergic rhinitis and gastric stimulant and for treating migrane headache. It is also used an antispastic against intestinal colic. Ginger oil is used in mouthwashes and liquors[3].

Many varieties of ginger are found such as processed, coated or unscraped, unbleached (natural) and bleached ginger. There are different types of active principles present in the ginger. Ginger oil is isolated by distillation of dried ginger. Many scientists have investigated the ginger oil and found about 50 constituents, mainly aroma, Starch, Volatile oil, Zingiberene, Gingerol, Oleoresin (Gingerin), Zingiberol, Zingerone, Shagaol etc. The acetone extract of ginger contains Zingerberone and ether extract contain Zingerone (Pungent principles).

In view of the available literature, we have tried to screen some actions of ginger-juice; as crude form of ginger. We presume that crude form contains majority of active principles, may be in very low concentrations. Keeping in mined some of its potential therapeutic applications we have carried out animal experiments to investigate the effects of ginger-juice on antioxidant status.

Chemical Constituents: Gingerin contains approximately 18-35% of volatile oil of ginger. It contains pungent, as well as, non- pungent principles of ginger. Fresh samples of Gingerin may contain 30.0% of gingerly (the main pungent substance of ginger), shogaol and zingerone[3]. "Gingerol" and "Zingerone". About 70 years ago, [26] isolated the pungent principles from ginger named the mixture of substances "Gingerol" (very pungent yellow oil) . He encountered considerable difficulty in extracting in pure form, because the

compound is easily affected by various reagents (on heating with alkaline hydroxides, 2% KOH, pungency is destroyed)[2].

Ether extract is treated with alkali, a very pungent flavor is obtained to be a ketone called "Zingerone" found zingerone in ginger not as free ketone, but in the form of compounds in which the ketone is a product of condensation (in molecular proportions) with saturated aliphatic aldehydes, principally enanthaldehydes (n-heptanal). Gingerol to be a mixture of homologues of this type:[2]

Material and Methods: A keeping in view the aims and objectives, experiments were planned to study the effects of ginger on antioxidant status as a physiological function.

Preparation of ginger-juice: The commercially available ginger was obtained from the local market. It was confirmed from the botanist that it was Zingiber officinale. The rhizome of ginger after cleaning and scrapping the superficial skin was cut into small pieces. With the help of mixer-grinder the pieces were made in to paste. The paste was taken on a white clean cloth and the liquid was squeezed out. The juice so obtained was used in the experiments. The stock of juice was kept in a refrigerator for maximum period of 15 days and the required quantity was used for the experiments after removing particulate matter from it. 500 gm ginger rhizomes yielded about 250ml juice. 250ml juice was filtered which yielded about 120 - 150ml filtrate.The liquid portion which was obtained in the course of filtration, looked like yellowish hazy opalescent liquid. It was administered orally in chronic experiments. The doses were either 2 ml to 4 ml per rat.

Antioxidant Status (Free Radicals): Blood samples were used for following antioxidant parameters.

1. Glutathione peroxidase
2. Glutathione reductase
3. Total antioxidant status

1. Glutathione peroxidase (GPx)

This enzyme GPx was assayed in each sample by using Randox (U.K) Kit. The kits used the method described by [3]. The method worked on following principle. GPx catalyses the oxidation of glutathione (GSH) by cumene hydro peroxide. In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced from with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340nm is measured on Photometer (BTS-320 Photometer, Biosystems).

2. Glutathione Reductase (GSSG - reductase or GR): This enzyme glutathione reductase (GR) was assayed in each sample by using Randox (U.K) Kit. Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in the absorbance is measured at 340nm is measured on Photometer (BTS-320 Photometer, Biosystems).

3. Total Antioxidant Status: Total antioxidant status is the overall capacity of an individual to fight against the oxidative stress. This was measured by using RANDOX (U.K) kit.

Statistical Analysis: Various parameters were expressed in suitable units. The group values are expressed as the Standard Error of Mean (SEM). Statistical analysis was done using unpaired students t- Test and $P < 0.05$ was considered the cut off point for statistical significance.

Result: Antioxidant Status (Free Radicals): A. Effect of ginger-juice 4ml/rat administered over 21 days.

1. Glutathione peroxidase (GPx): The mean serum enzyme glutathione peroxidase level in this vehicle treated control group was 902.06 $\pm$ 30.47 U/L and in the ginger-juice treated group the glutathione peroxidase level was 907.87 $\pm$ 32.95 U/L. The results are shown in the table-1. This shows that there is no significant difference in two groups indicating that there is no effect of ginger juice treatment for 21 days on enzyme glutathione peroxidase.

2. Glutathione reductase (GR): In the vehicle treated control group the mean serum enzyme glutathione reductase level was 64.22 $\pm$ 6.41 U/L, while in ginger-juice treated test group was 100.48 $\pm$ 2.93 U/L. It is evident that 4ml/rat for 21 days ginger-juice treatment significantly alter the

enzyme glutathione reductase level. The results are illustrated in table-1.

3. Total antioxidant status: The mean total antioxidant status in vehicle treated control group was 1.19 ± 0.10 mmol/L, while in ginger-juice treated group the mean total antioxidant status was 1.33 ± 0.09 mmol/L. This shows that there is no significant alteration in total antioxidant status between two groups indicating there is no effect of ginger-juice (4ml/rat for 21 days). The results are portrayed in table-1.

Table-1: The Effects Of Ginger-Juice Treatment (21 Days') On Glutathione Peroxidase, Glutathione Reductase And Total Antioxidant Status In Rats.

Parameters	n	Control	Ginger juice(4ml/rat)
Glutathione peroxidase	12	902.06± 30.4U/L	907.87 ± 32.95 U/L
Glutathione reductase	12	64.22 ± 6.41 U/L	100.48 ± 2.93 U/L ***
TAS	10	1.19± 0.10 mmol/L	1.33 ± 0.09 mmol/L

Table-1 shows the effect of ginger-juice (4ml/rat for 21 days) on various parameters like glutathione peroxidase (GPx), glutathione reductase (GR) and total antioxidant status in the rats. The statistical significance vis-à-vis the vehicle treated control is presented as *p<0.05 **P<0.01 ***P<0.001. Looking to the overall results related to the various parameters of antioxidant activity, it is clear that ginger-juice treatment over a period of 21 days' did not alter any of these parameters except glutathione reductase.

Discussion: Oxidative mechanisms play role in ageing process and many human diseases. The possibility of utilizing the antioxidants for their ability to preventing various disorders is becoming matter of study presently. The present study included the screening for antioxidant effect of ginger-juice using some biochemical marker parameter like glutathione peroxidase (GPx), glutathione reductase (GR) and total antioxidant status (TAS).

Presently, interest is increasing towards the role of free radicals and oxidative damage caused by them in different pathological conditions. Free radicals generated by the reactive metabolites of drugs and chemical agents induce per oxidation of membrane[6.]. The effect of oxidative stress damages membrane lipids in a process known as lipid peroxidation[7]. Lipid peroxidation is set into motion whenever conditions of increased oxidative stress occur in cell. Free radicals are chemical species or atoms possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive[8]. Free radical production in animal cells can either be accidental or deliberate, and are implicated in a large number of human diseases as reported by [9, 10.] Free radicals have the potential to oxidize biomolecules including proteins, lipid and DNA. Increased quantity of oxidised metabolites of these molecules have been detected in different diseases like ischaemic reperfusion injury of myocardium, diabetes, atherosclerosis, inflammatory diseases, cancer or toxicity caused by drugs like cisplastin, cyclosporin or p-aminophenol, isoproterenol. Beta-adrenergic agonists cause oxidative stress in the myocardium resulting in to myocardial necrosis [11,] During isoproterenol-induced myocardial infarction, enhanced free radical formation of and lipid peroxide accumulation have been proposed as one of the possible biochemical mechanism of myocardial damage [12]. There are numerous[12] sites for generating a large number of free radicals. Free radical generation in a system can be measured by many ways. The production of free radicals like H2O2, superoxide ($O2^-$), hydroxyl (OH^-), Peroxynitrite ($ONOO^-$) and lipid alcoxyl radical (Loo^-) can be measured but that poses a great difficulty. Moreover, by measuring level of antioxidant enzymes like glutathione peroxidase (GPx) [25], glutathione reductase (GR), total antioxidant status (TAS), superoxide dismutase (SOD)[13], glutathione-S-transferase [24] and also Catalase [27], one can have an idea regarding free radicals generated in the system, as they are in inverse relationship. The measurement of antioxidant enzymes is a better way to study the protection offered by drugs. The drugs might

induce synthesis of the antioxidant enzymes by interfering with oxidant mechanisms.

The GPx is a selenium dependent enzyme. Selenium is an important trace element, deficiency of it is said to be involved in a variety of the diseases [16,17]. At normal concentration, selenium may have a protective effect against several diseases[17]. GPx is a repair enzyme and its main function is removal of H2O2 and it prevents formation of highly reactive hydroxyl (OH^-) radical.

GR is a "repair enzyme". The function of this enzyme is restoration of cellular glutathione level by reducing oxidised disulfide glutathione (GSSG). Catalase is an intracelluar haem enzyme, which can also breakdown H2O2 into water and oxygen. SOD causes dismutation of O2 and scavenges this radical. Individulas' ability to stand against oxidative stress is judged by TAS study. It is worth drawing attention to a review by [18]. "How to characterize a biological antioxidant?". The review addresses the criteria necessary to evaluate a proposed antioxidant activity. The review throws light on possibilities of various simple methods for assessing of physiologically feasible scavenging of important biological oxidants (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, haem-associated ferryl species, radicals derived from activated phagocytes and peroxyl radicals, both lipid soluble and water soluble. It is considered worth to see if ginger affords some protection from point of view of antioxidant mechanism or enhancing antioxidant status of the body.

Protection against oxidative stress is achieved by several endogenous systems, among which an important one is glutathione. Glutathione is a major intracellular tripeptide thiole and plays an important role in the regulation of variety of cell functions and in cell protection from oxidant injury[19]. This protection occurs through different ways, one of which is through the enzymatic detoxification of hydroperoxides and H2O2 by glutathione peroxidase/reductase redox cycle[20]. Reduced glutathione plays a pivotal role in the defense system against oxidative damage. The enzymes glutathione reductase and glutathione peroxidase (GSH-Px) are involved in maintaining the balance between GSH and oxidised glutathione (GSSG) [21].

As reported by [22] that 6-Gingerol and Zingerone have antioxidant activity. They inhibit the peroxidation of phospholipid liposomes although Zingerone only exhibited a week effect. These compounds were good scavengers of peroxyl radicals generated by pulse radiolysis. It is concluded that 6-Gingerol may be useful as a potential antioxidant in food.

The mechanism of maintaining H2O2 below toxic levels is largely the function of glutathione and glutathione redox cycle. Glutathione is synthesised by two enzymes of which ϒ-glutamylcysteine synthetase is the rate limiting enzyme. Riboflavin acts as an antioxidant and is needed as a co-factor (flavin adenine dinucleotide, FAD) for glutathione reductase. FAD is the prosthetic group of the enzyme glutathione reductase (GR), which catalyses the reduction of GSG to GSH [14]. Most of the antioxidant enzymes are glutathione dependant, so glutathione suplementation restored the deficiency.

The present study shows that chronic administration of ginger-juice caused significant increase of glutathione reductase (GR) but similar rise was not observed for glutathione peroxidase (GPx) and total antioxidant status (TAS). Rise in glutathione reductase (GR) by ginger may be potentially important as antioxidant. Of course this requires further exploration in disease models. Presently further speculation cannot be made.

The levels of various antioxidant parameters have been reported as glutathione peroxidase 1010 U/L 24, glutathione reductase 77 U/l 24 and total antioxidant status 1.28 mmol/l 24. The levels of various parameter noted to antioxidant profile in the present experiment are comparable to that reported by [24,]. Various authors expressed activity in different units like U/h/g, micromoles etc that matches it difficult to compare the data from various laboratories.

Conclusion: According to the present study Antioxidants property in form of rise reduced glutathione level was noted.

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