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RESEARCH ARTICLE

Comparative study to evaluate in vivo and in vitro antioxidant potential of processed and unprocessed C*urcuma logna L.* using Allxon induced diabetic rats

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ABSTRACT

The present study was aimed to investigate *in vivo*, *in vitro* antioxidant activity of the processed *curcuma Longa L.* turmeric (PT) and unprocessed *curcuma Longa L.* (UPT). Both the extract are subjected to qualitative phytochemical screening. In *in-vitro* antioxidant properties total Phenolic content in each extract was determined using Gallic acid as a standard, calibration curve was prepared. The result is expressed as mg of Gallic acid equivalent per gram of extract. For unprocessed turmeric (UPT) and processed turmeric (PT) the values were 2.4 mg of GAE/g and 2.1mg of GAE/g. respectively. In *in-vitro* antioxidant properties total flavonoid content was determined in each extract using Quercetin as reference standards. Total flavonoid content in methanolic extract of UPT and PT in terms of quercetin equivalent (QE) was 152.25mg/100 g and 37.98mg/ 100 g of dry extract respectively. Total antioxidant activity of UPT and PT extract in terms of ascorbic acid equivalent (AAE) was 6.394 and 3.922 mg/ ml of extract respectively.

In- vivo study involved five groups of each comprising six Wistar rats (250 - 300 kg) and labelled as Hyperglycemic, Normal, Standard drug Glipizide, UPT treated and PT treated. Besides the Normal group Alloxan was administered in all animals as a single dose (50 mg/kg, i.v.) to induce diabetes. A dose of 300 mg of processed and unprocessed turmeric/kg of body weight was evaluated using acute toxicity test. It is orally administered daily for four weeks after induction of diabetic. The fasting blood glucose level was measured. The animal whose glucose level did not rise to more than 250mg/dl was rejected. Only animals that presented with glycemic levels equal to or above 250 mg/dl were submitted to treatments, which consisted of a daily administration of the UPT and PT turmeric for 21days. It was considered as a first day and blood samples (Approximately 0.5ml) were collected from rats by puncturing the retro orbital sinus, under mild ether anesthesia on 1st,7th, 14th 21st day for glucose estimations using GOD-POD method. At the same time urine sugar was estimated using Diastic. At the end of the study all groups rats pancreases were removed,

labelled, were minced and 10% (W/V) tissue homogenate was prepared using 0.1 M Phosphate buffer (PH 7.4) in a Potter- Elvehjem type homogenizer and used to enzyme assay. The comparison using statistical analysis ANOVA was performed. Decrease level of lipid peroxidase as compare to Diabetic, increase in superoxide dismutase (SOD) and catalase (CAT) activity are statistically analysed.($p<0.05$).These findings suggest that the UPT has potent antioxidant and antidiabetic activity, which may be responsible for some of its reported pharmacological activities and can be used as antioxidant supplement. Substances, chemicals rendering anti-oxidative properties can attenuate alloxan toxicity.

Key words: Antioxidant, Unprocessed Turmeric (**UPT**), Processed Turmeric (**PT**), Diabetic Mellitus.(DM) Reactive oxygen species(ROS).

INTRODUCTION

Biological combustion involved in various processes produces harmful products or intermediates called reactive oxygen species (ROS) or free radicals and thus produced continuously in various metabolic processes and exist in biological systems. Superoxide ($O2•−$) is the proximal mitochondrial ROS (Murphy, 2008). Some are important for maintaining normal physiological function.

ROS are produced naturally in cell following stress, respiration and exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, pesticides, bacterial and viral toxin, various smoking, alcohol, and psychological or emotional stress etc. (Adeolu, 2009) Oxidative stress is involved in the development and progression of diabetes- associated complications. In hyperglycaemic condition, continuous generation of ROS occurs and the evidence showed diabetes induced changes in the activities of antioxidant enzymes in various tissues.

ROS are various forms of activated oxygen, free radicals such as superoxide anion radicals (O-2) and hydroxyl radicals (OH-1), as well as non-free radical species (H2O2) and the singlet oxygen (1O2) (Halliwell, 1995; Squadriato and Peyor, 1998; Yildrim, 2001; Gulcin, 2002).

The free radical may cause lipid peroxidation, aggregation of protein, Protein oxidation and the degradation of DNA, protein and polysaccharide (Esra, 2012) inactivation of enzymes and alteration of intracellular redox state destroys cell membranes and kills cells (Kim, 2003). Thus, ROS are involved in the pathogenesis of number of disease and aging process (Mittler, 2007). Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process. (Khalaf, 2008; Patel, 2010). Excess of free radicals in living beings has been known to cause various problems like asthma, liver diseases, muscular degeneration, and other inflammatory processes (Sen, 2010), resulting in the so-called oxidative stress. Oxidative stress is defined as imbalance between oxidants and antioxidants and causes damage in to the cell and cell environment (Dr¨oge, 2002). Free radicals generated in the body can be removed by body's own natural antioxidant defence e.g. glutathione, catalase, SOD etc. but endogenous antioxidant defences are not completely efficient. Therefore, dietary supplement of antioxidant enzymes and free radical scavengers may provide a defensive mechanism against the deleterious acting of ROS, to lower the effect of stress and complications of DM.

Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Shahidi, 1992). Antioxidants act as free radical scavengers, reducing agents, and activators for antioxidative enzyme. These activities are used to suppress the damage induced by free radicals in biological system. It has been found by many researchers that there is an inverse association between the mortality from age related diseases and

the consumption of plant products. Because the plant products contain various antioxidant compounds, phenolic, which are the most reactive compounds, antioxidants present in plant products help in the stimulation of cellular defence system and biological system against oxidative damage.

The antioxidants in biological system can be either enzymatic or non-enzymatic. The enzymatic antioxidants include catalase, superoxide dismutase and glutathione etc. which catalyse neutralization of many types of free radicals (Jacob, 1995). while the non-enzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols etc. These enzymatic and non-enzymatic antioxidants can terminate or prevent the formation of free radicals by donating hydrogen or electrons to reactive radicals or species.

There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and neurological disorder. Plants, herbs, and spice, rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, antiallergenic, antiviral, antiaging, and anti-carcinogenic activities which can be attributed to their antioxidant properties (Ahmad, 2006).

Worldwide the major health problem is Diabetes mellitus (DM) which includes insulin dependent Diabetes mellitus (Type-1) and insulin independent Diabetes mellitus (Type-2). (Gandhi 2017) Numerous animal models have been developed for the past few decades for studying diabetes mellitus and testing antidiabetic agents.

The standard method of inducing diabetes in animal models is with alloxan, a toxic glucose analogue, which selectively destroys insulin-producing (beta) cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus called "alloxan-diabetes" in these animals, with characteristics similar type 1 diabetes in humans. The alloxan model of diabetes was first described in rabbits (Dunn, 1943). Hyperglycaemia increases the production of reactive oxygen species inside cultured bovine aortic endothelial cells. (Nishikawa, 2000) Hyperglycemia can increase oxidative stress through several pathways. A major mechanism appears to be the hyperglycemia-induced intracellular reactive ROS, produced by the proton electromechanical gradient generated by the mitochondrial electron transport chain and resulting in increased production of superoxide (Al-Shamaony, 1994).

Turmeric (*Curcuma longa L.*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native in southeast India, and needs temperatures between 20°C and 30°C. When not used fresh, the rhizomes are boiled for about 30–45 minutes and then dried in hot ovens (Chattopadhyay, 2004). Some farmers use alkaline medium to boil the rhizomes, after which they are dried, polished used to store or used to ground into a deep orange-yellow powder. Turmeric has been used in Asia for thousands of years and is a major part of Siddha medicine (Tayyem *et al*., 2004).

MATERIALS AND METHODS

Material:
In the present study UPT and PT has been purchased from the same farmer. The UPT was washed and cut into a similar size pieces, sun dried and used further as requirement. Alloxan and Nitroblue tetrazolium was purchased from Sigma Chemicals. (St. Louis Mo, USA) and all remaining chemicals are of analytical grade and were purchased from local distributors.

Method, Experimental Design:

Collection and authentication of plant material:
The Fresh Turmeric rhizomes (*Curcuma Longa L.*) were purchased from the Farmer of Rameshwar, District Sangali, Maharashtra, during the month of April. The Sangali district is famous for the cultivation and production of Turmeric. The rhizomes were washed using water. An herbarium of plant *Curcuma Longa L.* was authenticated at the Botany research office (Botanist) from the office of the Botanical survey of India, Pune (MH) where a voucher specimen was submitted.

Preparation of drug and extract:
The fresh rhizomes were cut into small and uniform pieces and air-dried for 3 days in sunlight which are further used for extraction and also subjected to pulverization to get coarse powder. The fine powder was kept in air tight glass containers. The complete

processed turmeric was also cleaned, cut into uniform pieces used for extraction and pulverised and store in air tight glass container. Both containers are labelled properly.

Air dried UPT was extracted in methanol for 72 hours using Soxhlet apparatus. The extract was concentrated under reduced pressure using a rotator evaporator at 40°C until the solvent completely dried. Turmeric is insoluble in water. The yield of the water extract for unprocessed turmeric was 7% and of processed turmeric was 4.5%. The extract was and stored in air tight container at 40C which is used for further studies. Processed and unprocessed turmeric powder mercerized in the carboxy- Methyl Cellulose (CMC) Sodium Salt was used as a drug and administered orally by intra-gastric intubation.

Preliminary phytochemical analysis:
Test solution was prepared by dissolving 1.5 gram of both extract separately in 15 ml distilled water. Each extract was tested for the presence of different phyto-constituents, viz. alkaloids, Carbohydrates, steroids, glycosides, phenols, flavonoids, saponins, triterpenoids, Phlobatanins, cardiac glucosides, Anthraquinone, proteins by usual prescribe methods (Kokte, 2004; Harborne, 2006).

Quantification of total phenolic:
The concentration of total phenolic content in the both extract was determined by the Folin-Ciocalteu colorimetric method (Singleton, 1965; Sadashivan, 1997). Gallic acid was used as a standard. Calibration curve is prepared using dilution of standard solution of 0.1mg/ml of Gallic acid. Samples of extracts were evaluated at a final concentration of 0.1 mg/ml and were mixed thoroughly with 2 ml of 2% Na_2CO_3. After 2 minutes Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of Gallic equivalents per 100 grams of dry weight (g $100g^{-1}$D.W.) of the plant extract. A blank was prepared using the same procedure but replacing the plant extract with an equal volume of methanol. The total content of phenolic compounds in plant extracts as gallic acid equivalents (GAE) was calculated using the following equation (Demiray, 2009):

$C = (c \times V)/m$ Where; C = total content of phenolic compounds, mg/gm plant extract, in GAE, c = the concentration of gallic acid established from the calibration curve (mg/ml), V = the volume of extract in ml, m = the weight of crude plant extract in gram.

Quantification of total flavonoids:
Total flavonoid content was measured by the Aluminium chloride colorimetric assay (Zhishen, 1999). Quercetin was used as a standard and total flavonoid content of extracts are expressed as mg quercetin equivalents (QE)/100 g of sample. An aliquot of 1 ml of extracts and standard solution of Quercetin (0.1mg /ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml 5 % $NaNO_2$ were added. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. Then at the 5th min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Calibration curve of quercetin was prepared using aliquots of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, and 2 ml standard solution. Accordingly, proportionate additions were done as describe above. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm.The calibration curve was plotted. A blank, which was prepared by the same procedure except replacing the plant extract with an equal volume of methanol. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following equation:

$C = (c \times V)/m$ Where; C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent, c = the concentration of quercetin established from the calibration curve in mg/ml, V = the volume of extract in ml and m = the weight of crude plant extract in gm.

Reducing Power Capacity Assessment:
The reducing power of UPT and PT was determined by the slight modification of the method of Oyaizu (Oyaizu, 1986). Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex, having maximum absorption spectra at 700nm. Various concentrations of the plant extracts dissolved in water were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide.

This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric-chloride solution (0.5 ml). Control was prepared in similar manner excluding samples. Stock standard solution of 1% ascorbic acid was prepared. Serial dilutions of standard ascorbic acid 1% were used and calibration curve was prepared. Increases absorbance of the reaction mixture containing extract at various concentrations indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract. The experiment was performed thrice and results were averaged.

In-vivo studies:

Venues

The whole experimental work was conducted at the NDMVP's College of Pharmacy, the University of Pune.

Animals

Forty albino Wistar rats (190-220 g) of either sex were employed in this study. They were housed in well ventilated cages and maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ±15% and normal photo period [12 h dark/12 h light]. They were used for the experiment. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Hyderabad, India. The rats were acclimatized for seven days in the laboratory and were fed with Commercial pellet rat chow diet (Hindustan lever, Kolkotta, India) and water was provided *ad libitum.* All the animal experiments were conducted according to the ethical norms (OECD/OCDE, OECD Guidelines for the testing of chemicals, revised draft guidelines 423:2001) approved by CPCSEA, Ministry of Social Justice and Empowerment, Government of India, and ethical clearance (IAEC/2014/14) was granted by Institutional Ethical Committee presentations held on 24\02\2014 at NDMVP Samaj's College of Pharmacy, Nashik.

Preliminary toxicological evaluation: Acute toxicity test

Acute toxicity is the adverse effect occurring within a short time of oral or intra peritoneal administration of single dose of any substance given within 24 hours. The acute oral toxicity studies of processed and unprocessed turmeric were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA, Ministry of Social Justice and Empowerment, Government of India. The animals of either sex were segregated into six groups consisting of six rats each and were fasted for 18 hours and then use. There was an orally administration of the stepwise doses in which the starting dose of unprocessed turmeric was 100mg/kg of animal weight. The increasing doses were administered up-to 3000mg/kg of animal weight in a range of 100mg/kg. Additionally, 3 mice were kept as control. Same procedure was repeated for processed turmeric. Since no mortality was observed in both studies, the behavioral pattern was unaffected. Hence, the dose selected for all the extract were as per the OECD guidelines No 423, fixed dose methods for evaluation of anti-diabetic activity was 300mg/kg, body weight (1/10 of 3000 mg/kg body weight.)

Grouping of animals with induction of diabetic mellitus using alloxan:

In this method, experimental rats were fasted in individual cage for 24 hours. Care was taken to avoid carophagy. Forty Wister rats of either sex weighing 190 -220 gm were divided into five groups of six animals each and grouped as follows [OECD/OCDE, OECD Guidelines 2000-2001].

Group I:
Served as normal control and did not receive any treatment.

Group II:
Served as diabetic and received single dose of alloxan monohydrate (50mg/kg,i.p.)

Group III
Alloxan monohydrate + unprocessed turmeric (300 mg/kg, p.o.)

Group IV:
Alloxan monohydrate + processed turmeric (300 mg/kg, p.o.)

Group V:
Alloxan monohydrate + Glipizide (5mg/kg, p.o.) and served as Standard.

Diabetes was induced in the rat by injecting alloxan monohydrate intra-peritoneally in a single dose of 50mg/kg in 0.95% of weight/volume NaCI, to the overnight fasted rat. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 24 hrs-48 hrs (1-2 days) post injection. (Parashare, 2016)

The fasting blood glucose level was measured. The animal whose glucose level did not rise to more than 250mg/dl was rejected. Only animals that presented with glycaemic levels equal to or above 250 mg/dl were submitted to treatments.

Methodology of oral drug administration:
Intraperitoneal route of administration of drug is selected because it is more rapid and predictable absorption than oral administration. This route is also advantageous because the drug is not inactivated or destroyed as may happen in the gastrointestinal tract. Rat oral gavage (16 gauze) needle was used, it was blunted at the tip to avoid any injury to the inner surface of mouth. The feeding needle was attached to the syringe. Required amount of drug solution was withdrawn in the syringe without any air bubble. By adjusting the needle to desire position it was inserted through the intra-dental to space and pushed gently (Book 1) . Study consisted of a daily administration of the PT and UPT for 21days.First drug administration day was considered as a first day.

Collection of Blood and Preparation of tissue homogenate:
The blood samples (Approximately 1ml) were collected on 1st, 7th, 14th, and 21st day by puncturing the retro orbital sinus, under mild ether anaesthesia in a properly labelled EDTA bulb for glucose estimations using GOD-POD method. At the end of the study, 21st day all animals were anesthetized in an ether chamber. The pancreas was removed and placed at 4°C after perfusion with ice cold saline with proper labelling. Pancreas tissues from each animal were minced and 10% (W/V) tissue homogenate was prepared using 0.1 M Phosphate buffer (PH 7.4) in a Potter- Elvehjem type homogenizer (Arulselvan, 2007). All the test tubes were labelled carefully and then subjected to centrifugation at 1000 g, 40C for 10 minutes. A pellet from all the test tube was discarded. The supernatants were subjected for centrifugation at 12.000 g for 20 minutes at 4oC to obtained mitochondrial supernatant (PMS). The supernatants were sieved separately through two layers of muslin cloth and immediately used to estimate the activity of catalase and superoxide dismutase (SOD), and lipid peroxidase.

Estimation of blood and urine glucose:
The EDTA collected blood samples were centrifuge at 5000 RPM for 20 minutes to separates the plasma. Plasma is immediately subjected for the glucose estimation by GOD-POD method (Trinder, 1969). At the same time one drop of urine is used for glucose assessment using Di-strips. Both these studies are carried on 1st, 7th, 14th, and 21st day of the study.

Determination of *in vivo* antioxidant activity:

Estimation of antioxidant enzymes:
Catalase activity was determined according to the method of luck (Luck, 1971). 3ml H_2O_2- phosphate buffer of pH 7 (12.5mM H_2O_2 in 67 mM Phosphate buffer) was pipetted directly in the cuvette. 0.05 ml of tissue homogenate (10%) mitochondrial supernatant was added to the buffer solution. The content was mixed and the decomposition rate of hydrogen peroxide, decrease in the absorbance was followed and noted at 240nm for 5 minutes. The results were expressed as micromole of H_2O_2 decomposed/ min/mg protein using Molar extinction coefficient of H_2O_2 (0.07 mM/cm) at 240nm. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Superoxide dismutase activity was assayed by the method of Kono (Kono, 1978). An aqueous solution of hydroxylamine was prepared daily and its pH was adjusted to 6.0 with 2M sodium hydroxide. Control consist of a mix of 2ml of NBT (Nitroblue tetrazolium 1.5mM)), 0.5ml hydroxylamine hydrochloride. The reduction of NBT was inhibited by SOD and was measured spectrophotometrically at 560nm. In a test, enzymatic reaction, 2ml of NBT was mixed with 0.5ml of hydroxylamine hydrochloride and appropriate amount of mitochondrial supernatant was added the development of blue colour was measured at 560nm for 2 minutes. Enzyme activity was expressed as Units /mg protein where one unit of enzyme is defined as the amount of SOD requires inhibiting rate of reaction by 50%.

Estimation of lipid peroxidation (Niedernhofer *et al*., 2003, Tietz, 1968) in terms of malonaldehyde (MA) thiobarbituric acid reaction was performed. The reaction mixture contained 0.1ml of tissue homogenate, 0.2ml of 8.1% sodium dodecyl sulphate (SDS), 1.5ml of 20% acetic acid and 1.5ml of 0·8% aqueous solution of thiobarbituric acid (TBA). The pH of 20% acetic acid was adjusted with 1 N NaOH to 3.5. The mixture was finally made up to 4·0 ml with

distilled water and heated at 95°C for 60 min. After cooling under tap water, 1ml of distilled water and 5ml of a mixture of n-butanol and pyridine (15:1 by vol.) was added and the mixture was shaken vigorously on a vortex mixer. After centrifugation at 2200 g for 5 minutes the absorbance of the organic layer (upper layer) was measured immediately at 532 nm. Lipid peroxide formation in tissues clearly requires substrates, the unsaturated fatty acids of the tissue lipids, and a catalytic system. Either of these factors may limit or control the extent of peroxide formation. Malonaldehyde, an index of LPO was calculated with extinction coefficient of 1.5 x 105 M-1cm-1.

Statistical analysis

The experimental results were calculated as mean ± SD of six replicates. The ANOVA test followed by Dunnett's multiple comparison tests was employed for statistical comparison between control and various groups. Significance was considered at $P<0.05$.

RESULTS AND DISCUSSIONS

The phytochemical spot test showed that the extract contained saponins, phenols, terpenes/ sterols, glycol-sides, alkaloids, and flavonoids as given in Table 1.

The total phenolic contents of extract of UPT and PT were found to be 16.5mg GAE/100g and 8.86 mg GAE /100g respectively with reference to standard curve ($y= 0.01284 X - 0.01533$; $R^2 =0.9889$) Fig. 1. The Literature reveals that antioxidant activity of plant extract is mainly due to presence of phenolic compounds, which may exert antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators.

Total flavonoid contents were calculated using the standard curve of quercetin (y=0.5804X - 0.1058; R2= 8654) "Fig. 2", and the result was expressed as quercetin equivalents (QE) per 100 grams of the plant extract. The total flavonoid contents of both unprocessed and processed turmeric extract were found to be 75.625 mg QE/100g and 18.99 mg QE/100g, respectively. According to our study, the high contents of flavonoids in unprocessed turmeric extract can explain its high radical scavenging activity.

These phytochemical compounds are known to provide support for bioactive properties of plant, and thus they are responsible for the antioxidant properties of unprocessed *curcuma Longa L.*

The total reducing power capacity using potassium ferric cyanide reduction method is determined. In this assay, the yellow colour formed in the reaction is significant indicator of antioxidant activity. The UPT extract shows high absorbance of 1.48, then comes the absorbance of PT 0.91**.** Fig. 3. Among the extract the UPT extract exhibited the most reducing power. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power. The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Gordon, 1990) The reducing capacity of UPT is a significant indicator of its potential antioxidant activity.

Results of the effect of unprocessed and processed turmeric on blood glucose level of Alloxan -induced diabetic rats are presented in Table 2. Significant antihyperglycemic effect was observed from the 7th day onwards. The reduction in blood glucose was maximum on the fourth week, receiving 300 mg/kg body weight of unprocessed turmeric and the results were comparable with that of glipizide a standard. Alloxan caused a significant increase in blood glucose level as reported in the Table 2. The results of the present study clearly indicated that the UPT exhibited significant antidiabetic activity in Alloxan induced-diabetic rats, comparable to the effect exhibited by standard drug glipizide. The effect of administration of UPT was found to reduce glucose concentration. (***$P< 0.001$**)**. At the end of study after 21st days of treatment period, it was observed that the animals treated with UPT, PT and glipizide showed significant decrease in diabetes induced urine glucose level Table 3.

In present study the MDA level was found to be significantly decrease in UPT and PT treated group of animals, as compare to diabetic group($P<0.001$). The antioxidants SOD and Catalase were found to be lowest in diabetic rats. The *P* value calculated for the rise in SOD, Catalase and lowering of MDA against the groups as listed in “Table 4” were all significant.

Table 1: Qualitative Phytochemical Screening of methanolic extract of UPT and PT.

Sr. No	Test	Unprocessed turmeric (UPT) extract	Processed turmeric (PT) extract	Inference
1	**Alkaloids**			
a	Mayer's test	Yellow colour with Brownish red ppt. was observed	Red colour with ppt. was observed	Alkaloids present
b	Wagner's test	Yellow colour with Brownish red ppt was observed	Red colour with ppt. was observed	Alkaloids present
c	Hager's test	Dark yellow colour was observes	Red colour with ppt. was observed	Alkaloids present
2	**Glycosides**			
a	Benedict's test	Orange red precipitate was obtained	Orange red precipitate was obtained	Glycosides /reducing sugar present
b	Fehling's test	First yellow and then brick red precipitate was obtained	First yellow and then brick red precipitate was obtained	Glycosides /reducing sugar present
3	**Cardiac glycosides**			
a	For deoxy sugars Keller-killiani test	No brown ring at the junction of two liquid, blackish colours is formed.	No brown ring at the junction of two liquid, blackish colour formed.	Deoxysugars absent
b	Legal's test	Negative, no pink, red, reddish brown colour, Dark yellow colour is formed.	Negative, no pink, red, reddish brown colour, Dark yellow colour is formed	Cardiac glycosides absent
4	**Anthraquinone glucosides**			
a	Borntrager's test	In the lower ammonical layer colour is absent	In the lower ammonical layer colour is absent	Absent
5	**Saponin glycoside**			
a	Foam test	slight foaming	Slight foaming	Saponins present
6	**Flavonoids**			
a	Alkaline reagent test	Dark Yellow colour appear	Yellow colour appear	Flavonoids present
b	Shinoda test	Orange colour appears	Orange colour appears	Flavonoids present
c	Ammonia test	Dark yellowish red color appear	Dark yellow colour appear	Flavonoids present
7	**Phenolic compound**			
a	Lead acetate test	Clear dark yellow colour was observed with colourless precipitate at bottom	Clear yellow colour was observed with colourless precipitate at bottom	Phenols Present
8	**Sterols**			
a	Salkowski reaction	Dark red colour appears in the chloroform layer	Dark yellow colour appear in the chloroform layer	Sterols present
9	**Proteins**			
a	Biuret test	No purple dark purple colour , instead red colour appears	No purple dark purple colour , instead red colour appears	Protein absent
b	Lowry reagent	No purple dark purple colour , instead red colour appears	No purple dark purple colour , instead red colour appears	Protein absent
b	Ninhydrin test	No deep violet colour appears	No deep violet colour appears	Protein absent

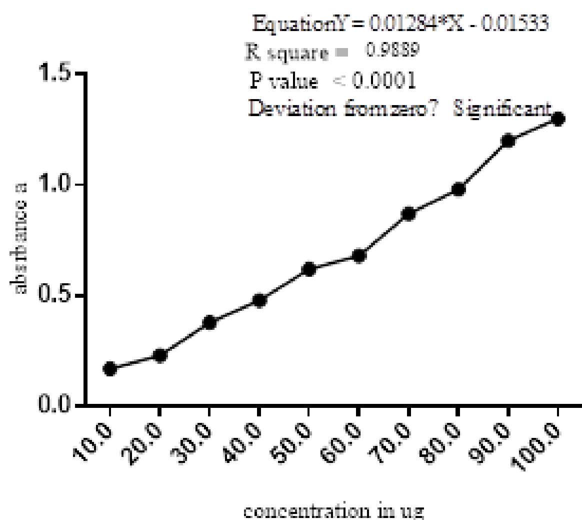

Fig. 1 : Calibration curve of phenolic content using gallic acid as a standard

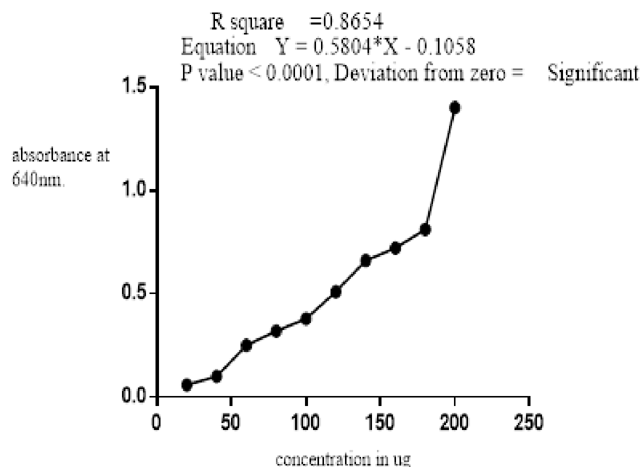

Fig. 2 : Calibration curve of Total flavonoid using quercetin as a standard

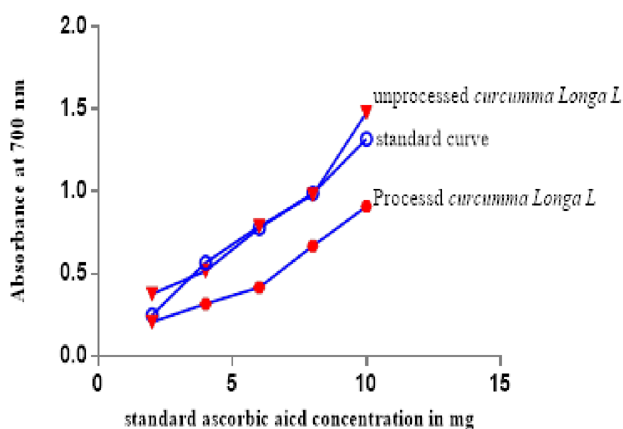

Fig. 3: Comparative reducing power capacity of standards ascorbic acid, unprocessed and processed *curcuma longa* L

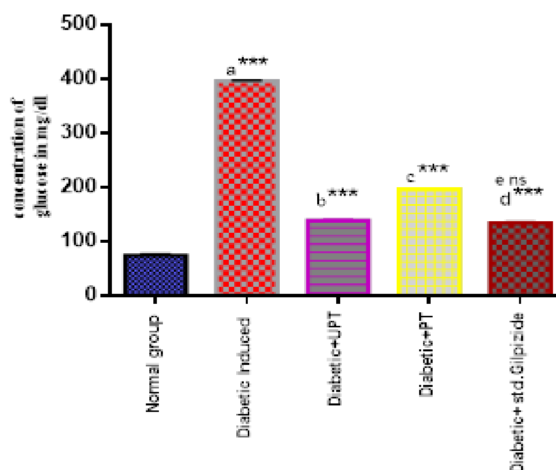

Fig. 4: Effect of unprocessed and processed turmeric on blood glucose on different groups: *a) Group II compare with group b) Group III compare with group II c) Group IV compare with group II d) Group V compare with group II e) Group V compare with group III ns= Non significant. ***P< 0.001, **P< 0.001, *P< 0.05*

Table 2: Effect of unprocessed and processed turmeric on the blood glucose level in Alloxan induced diabetic rats.

Group	Fasting blood glucose concentration Mg/dl			
	Day 1	**Day 7**	**Day 14**	**Day 21**
Normal Group I	75.00 ± 0.706	77.49 ±1.600	75.11 ±1.716	74.40 ± 0.988
Diabetic induced Group II	378.29 ± 0.3898	355.9 ± 0.2367	392.2 ± 0.825	396.1 ± 0.5345
Diabetic + UPT Group III	358.00 ± 0.3122	242.41 ±1.736	185.5 ± 0.9521	139.16 ± 0.4771
Diabetic +PT Group IV	359.2 ± 0.1983	317.4 ± 0.7783	275.00 ± 0.670	196.4 ± 0.4666
Diabetic control Group V	359.5 ± 0.1611	218.85 ± 0.485	172.21 ± 0.761	134.6 ± 0.667

Values are expressed as Mean ± SD (n =6 rats). The data of 21 days in all group of rats was subjected to ANNOVA.

Table 3: Effect of processed and unprocessed turmeric on the urine glucose level in Alloxan induced diabetic rats.

Treatment	Intensity of glucose in the urine before treamnent and from the 1st day of treatment				
	0 day	1 day	7 day	14 day	21 day
Normal group I	Nil	Nil	Nil	Nil	Nil
Diabetic induced group II	Nil	+++	++++	++++	++++
Diabetic +UPT	Nil	+++	++++	++	++
Diabetic +PT	Nil	+++	++++	+++	++
Diabetic + gilpizide	Nil	+++	++++	++	+

Intensity of glucose in urine, +: mild, ++: moderate, +++: higher, ++++: severe

Table 4: The antioxidant enzymes Superoxide dismutase (SOD), Lipid peroxidase(LP) , Catalase studied in the pancrease tissue of all group animals at end of 21st day.

Group	Catalase	SOD	Lipid peroxidase
Normal Group I	12.02± 0.2635	5.7650 ±0.2645	9.400 ±0.2978
Diabetic induced Group II	4.0855 ± 0.1287#	1.810± 0.03011#	26.84±0.4229 #
Diabetic + UPT Group III	9.970 ± 0.04655a *** f ***	3.978± 0.04527b ***e ***	17.742±0.412b*** d ***
Diabetic + PT Group IV	7.82 ± 0.09878a ***	2.667± 1.535b ***	22.42± 0.3555 b***
Diabetic+ Glipizide Group V	4.048 ± 0.03414 b a ***	1.978± 0.003 b***	19.66±0.477 b***

*Values are expressed as mean ± SD. (n=6) & are statistically significant at *P < 0.05, **P < 0.001 and ***P < 0.01*

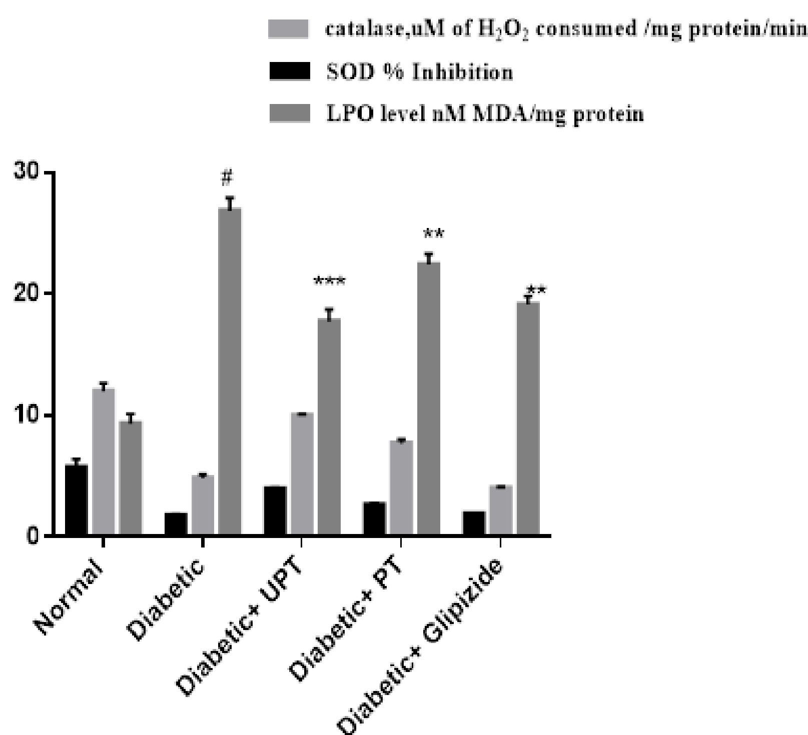

Fig. 5 Effect of UPT and PT on the SOD, Catalase, LPO level in all groups of studies. Values are the mean ± SD for 6 animals in each group. Values are statistically significant at* P<0.05, **P<0.01, P<0.001. Statistically significant was compared within the groups as:
[a]Diabetic rats compared with Normal rats,
[b]Glipizide, UP and PT treated data compared with Diabetic data.

Statistical significance was compared within the group as follows,

a= catalase: Group II compare with the groups of UPT (III), PT (IV) and Glipizide (V) treatment.

b= SOD: Group II compare with the groups of UPT (III), PT (IV) and Glipizide (V) treatment.

c = LPO: Group II compare with the groups of UPT (III), PT (IV) and Glipizide (V) treatment

d= LPO: Group III (UPT) compare with group IV (PT)

e = SOD: Group III (UPT) compare with group IV (PT)

f=Catalase: Group III (UPT) compare with group IV (PT).

Fig. 5 shows the level of catalase, SOD and LPO. It was observed that due to diabetes there was an increase in the MDA (LPO) and decrease in SOD and Catalase enzyme level. In diabetic glipizide treated rats the level of LPO is significantly decreases as compare to diabetic group. Treatment with UPT and PT reversed the MDA (LPO) level which is significantly decrease ($P < 0.001$) and increase in SOD and catalase HDL level were observed after the treatment with UPT and PT. ($P < 0.01$). There is also a significant difference in the comparison of UPT and PT values. ($P < 0.001$).

DISCUSSION

Ethnopharmacological surveys have shed light on the fact that the therapeutic use of even 80% of 122 plant derived drugs may have a link with their recommendations in traditional medicine (Fabricant, 2001). Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs. (Ramratnam *et al*., 1995) Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system (Diplock, *et al*., 1998). Besides endogenous antioxidants such as SOD, catalase, Glutathione etc. the body also relies on exogenous the dietary antioxidants, to obtain the rest of the antioxidants it needs such as Vit. C, Vit. E etc. (Valko *et al*., 2007). The data obtained on the basis of calibration curve highlighted the antioxidant potential of UPT. UPT is a best, and UPT is cheap source of exogenous dietary antioxidant. In the Indian kitchen Daily use of UPT instead of PT may found to be beneficial. These exogenous natural antioxidants have a diversity of biochemical activities, some of which include the inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (Ekrem, 2008). Antioxidants have functioned to inhibit apoptosis because apoptosis was at first thought to be mediated by oxidative stress (Hockenbery, 1993). Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolic are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituent of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Lo-liger, 1991). By taking into consideration, the important of antioxidant UPT is a good source.

In the present study the total phenolic content of UPT was 16.5mg GAE/100g and of PT was 8.86 mg GAE/100g. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavengers. The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The phenolic compounds has been shown to scavenge free radicals, including hydroxyl and superoxide anions (Noda *et al*., 1999). This reduces the further complications of diabetic mellitus (Da Silva, 2010)

Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids are correlating with their antioxidant activities as radical scavenging activities and are very important due to the deleterious role of free radicals in biological systems (Mbaebie, 2012). In this study, it is evident that the extract of the UPT possess more effective antioxidant activity than the PT. The increased activities of catalase and SOD as observed in this study suggest that the UPT extract has an *in vivo* antioxidant activity. The radical scavenging activity of SOD is effective only when it is followed by increase in activity of catalase and other peroxidases. In our study,

the level of MDA in the extract treated groups decreased in a dose dependent manner when compared to control. This decrease in the MDA levels may indicate increase in the activities of glutathione peroxidase and hence inactivation of LPO reactions (Ugochukwu, 2003). UPT dose level 300mg/kg of body weight shows significant low levels of blood glucose which simultaneously proved by urine glucose level. Superoxide dismutase, catalase and non-enzymatic scavengers of hydroxyl radicals were found to protect against alloxan toxicity.

CONCLUSION

The results obtained demonstrated that UPT had the highest total phenolic content and antioxidant activity compared to PT after *in vivo* and *in vitro* study. Administration of UPT and PT in diabetic rats significantly reduced Blood glucose level with urine glucose level, significantly increases SOD and catalase level and decreases LPO level. These enzymes remove the ROS directly or sequentially, preventing their excessive accumulation and consequent adverse effects. The total flavonoids, phenolic and total reducing potential of UPT is significantly more as compare to PT. There was a good correlation between total phenol content and antioxidant activity thus it supports the idea of phenols as contributor of the antioxidant power of plants extracts. A marked reduction in blood glucose level and urine glucose level toward normal level also suggested antidiabetic potential of the UPT extract. Thus, UPT consist of higher antioxidant potential and antihyperglycemic activity and contribute to lower the other complications of DM in the body.

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