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EVALUATION OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF SELECTED MEDICINAL PLANTS USING DIFFERENT MODELS IN SWISS ALBINO MICE BRAIN

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Abstract:

The current research was aimed to estimate and compare the antioxidant effect of selected medicinal plants collected from different areas of Balochistan, Pakistan. The antioxidant activitywas determined by 2, 2-diphenyl-1-picryl hydrazyl(DPPH) a radical scavenging assay, lipid peroxidation assay and total antioxidant assay. Plant extracts demonstratedhangups alongside thiobarbituric acid reactive species (TBARS) tempted by pro-oxidant 10uM ferroussulphate (FeSO4) or sodium nitroprusside in the brain region of mice. Plants extracts showed significantferric reducing and free radical scavenging activity and also showed the higher concntration of both flavonoids and phenols. It was observed that higher thetotal polyphenolicand falavonoidscontentsbetter the antioxidant effect. All the plant samples showed the order of their antioxidant activity as SolanumNigram>Momordicacharantia>SolanumVillosum>TinosporaCordifolia> Ginkgo Biloba>Cuscutareflexa. The data obtained in the present study shows that all selected plants have inhibitory effect and antioxidant activity. The antioxidant effect of the selected plants might be related to the higher concentration of phenolic. Contents,ferric reducing ability and free radical scavenging activity.In this study we tried to provide the scientific proof for the traditional use of the selectedmedicinal plants as antioxidants.

Key Words: *Antioxidant activity, Free radical, DPPH, Medicinal plants, Oxidative stress, Mice brain.*

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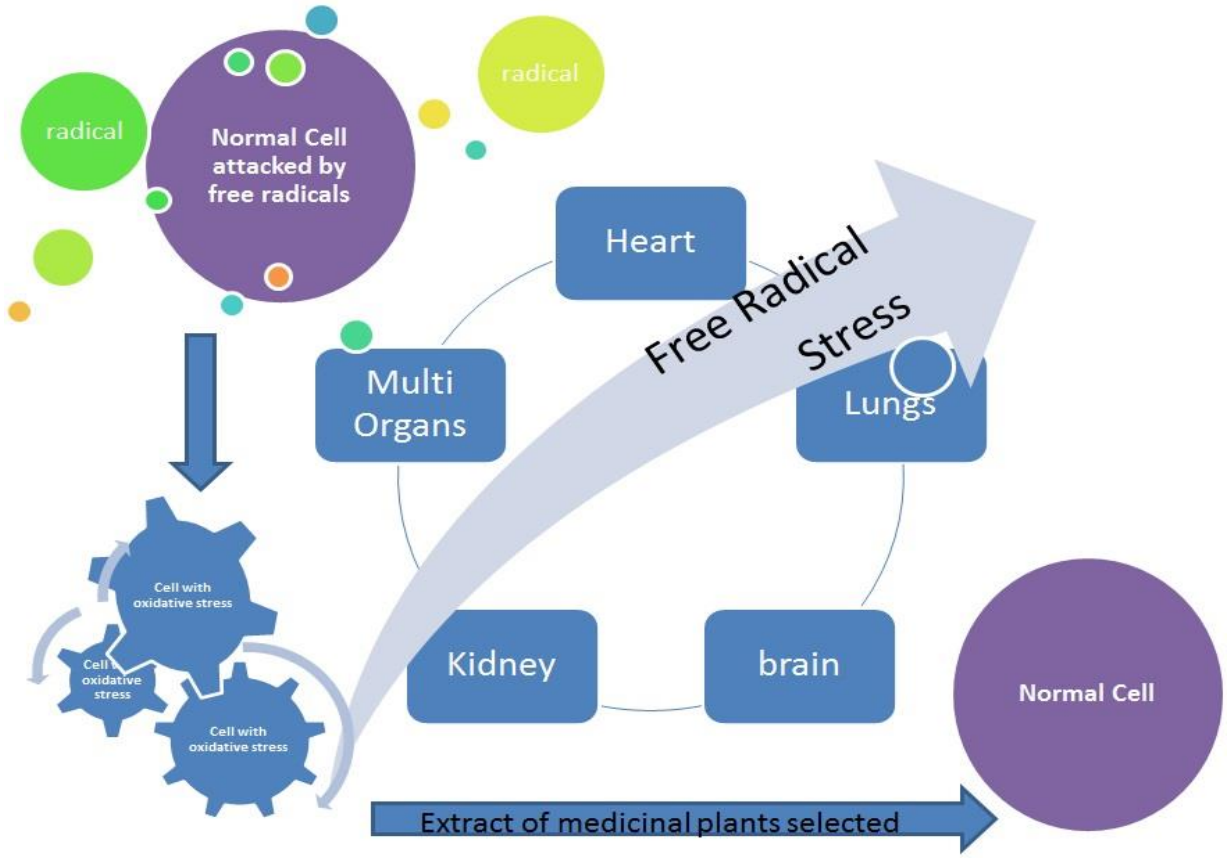

INTRODUCTION:

The ratio of production of the reactive oxygen and nitrogen species play a vital role in Oxidative stress control. When the said balance is disturbed with in the living cell in human or animal bodies, the cell compounds gets started damage leading to ultimate cell death even. These compound may include protein, lipids and nucleic acids etc[1]. Reactive oxygen species are formed indogeniously as well as exogeniously. Mitochondria produce ROS during respiratory chain reactions while exogeniously ROS are produced by different reasons such as UV radiations, chemicals , inflammation etc [2]. Disorders like cancer, diabetes mellitus, asthma, heart diseases, premature aging and neurodegenerative diseases might be due to rapid generation of free radicals that are capable to cause Oxidative damage to human body cells. The chief source of free radicals are reactive oxygen species and they lead to serious damages and cause neurological abnormalities like Parkinson's and Alzheimer`s diseases [3]. Antioxidants provide defence against malfunctioning caused by overproduction of ROS and related lipid peroxidation. Human body antioxidant defence system includes antioxidant enzymes such as super-oxide dismutase and glutathione 2 per-oxidase where as non-enzymatic antioxidants are also present such as glutathione, 9-tocopherol and ascorbic acid, thiolantioxidants , carotenoids, melatonin and some other compounds. Currently existing synthetic antioxidants have been reported to cause several undesirable effects. Therefore, researchers have focused their studiestowards natural substances like plants to neutralize theOxidative stress induced by free radical and further more to minimize the undesirable effects of available conventional anti-oxidants.

Plants have been considered as major source of human medicines. Medicinal plants have been used as a means of relief from sickness for years and have been used as the basis of several traditional medicines around the world such as India and China. In recent times, they occupy a key role in the raw materials used for formulation and preparation of therapeutic products like nutraceuticals food supplements, skin preparations (cosmetics) and pharmaceuticals. A survey done by World Health Organization (WHO), suggested that about 80% of the world population is dependent over traditional way of treatment to meet their health concerns. The demand for therapeutic plants is constantly increasing because the isolated products are nontoxic, available at low prices with no untoward effects. Solamunnigram belongs to family Solanaceae. Berries and leaves are used for medicinal purposes. It's pharmacological activities are antidiabetic, antimicrobial, anti-

ulcer,hepatoprotective, antioxidant, anti-inflammatory, cardioprotective and larvieidal [4]. It is used in fever, pain, skin problems, tumors, freckles, diabetes mellitus, wounds, abdominal upsets and cirrhosis of the liver. SolanumVillosum also belongs to family Solanaceae. It is widely distributed in many parts of Pakistan and India. The plant is an Ayurvedic herb with multiple medicinal properties. SolanumVillosumcontains alkaloids, flavonoids, saponins, tannins and phenols. It is antioxidant, Free radical scavenging, analgesic and antipyretic. It is used in toothache, stomachache, hypertension, swellings and tonsillitis [5]. Momordicacharantia belongs to family Cucurbitaceae and known as Karela in Pakistan. It is used for medicinal purpose and especially for the treatment of diabetes. It contains alkaloids berberine, palmitate, tinospporin, isocolumbin, tembetarine, choline and tetrahydropalmatine; the steroids sitosterol, octacosanol, heptacosanol, nonacosan-15-one, hydroxyecdysone and makisterone. It is antioxidant, antineoplastic, antidiabetic, hypolipidemic, anti-arthritic, antiosteoporotic and anti-microbial activity. It is used in diabetes mellitus and wound healing also TinosporaCordifolia belongs to family Menispermaceae. Stem is sued for medicinal purpose. It is nutritive, digestive, immunomodulant, anticancery and andhypoglycemic [6]. Ginkobiloba belongs to family Ginkgoaceae. Leaves aare used for medicinal purposes. It contains flavonoids, biflavonaides, proanthocyainidins, triactonicditerpines, Ginkogolides A, B & C. It is used in asthma, tuberculosis, chronic cough, bronchitis. It possesses anti-inflammatory and natural antihistamine. Cusentareflexa belongs to family Convlovulanceae. It is alternative, analgesic, anthelmintic, anti-atrabilious, blood purifier, carminative. It is used in intestinal worms, bilious disorders, constipation, fever, body pains, itchy skin, jaundice, muscle pain and coughs [7]. The purpose of this research was to determine the anti- oxidant potential of traditional medicinal plants and to determine the phenol and flavonoid contents in the plant extracts.

MATERIALS AND METHODS:

Chemicals

Sodium Nitro Prusside (SNP), Iron sulphate, Malon, Aldehyde Bis Dimethyl Acetal (MDA), Thiobarbituric Acid (TBA) (Merck, Germany), FelinCiocalteus Reagents, Gallic Acid and 2-2 Diphenyl 1 Pioryl-Hydrazyl (DPPH) (Sigma, St. Louis. MO, USA).

Preparation of plant Extract

The different parts of plant were obtained from the native marketplace of Quetta and we're identifies by a botanist in Botany Department of Balochistan University. The collected parts of plant (0.1 my dried powder) were soaked for fifteen minutes in boiling water. After cooling that soaked material at 25 0C (room temperature) it was clarified (Wattman filter paper 1). After filtration thereminders were removed two times and then get concentrated in a rotary evaporator (Buchi USA). After drying, the filtrates in an oven (VPM, USA) at 38-50 ^{o}C it produced a production of 21-23%. Sequential dilutions of the samples were prepared for further analysis.

Test animals

Swiss Albino Mice (each weighing 200-250g) of both sexes (National Institute of Health, NIH Islamabad, Pakistan) were enrolled for these *in-vivo* studies. The guidance for use and care of animals were strictly followed provided by NIH. All the mice were housed in clean cages (full access to food) water was given ad-libitum, in a room at a constant temperature (22-25^{o}C) with light and dark cycle 12 hours.

In vitro Assays

Fabrication of TBARS

The mice were anaesthetizing by ether and decapitated. The brain and liver tissues were taken in a petri-dish already positioned in cold environment [8]. After that 1 gram of tissues (each from brain and liver) dispointedly were regimented to uniformity in cold blows putting them in Teflon glass homogenizer (Sigma-Aldrich, Germany). The samples were centrifuged (Sigma-Aldrich, Germany) for 10 minutes at 1400 revolution per minute. Precipitate was formed after centrifugation which was rejected and low-slung supernatant were obtained for accomplishment of additional assay. The homogenates (100uL) were then gestated (by incubation) with or without 50 uLof freshly prepared dissimilar oxidants (ferrous sulphate and nitroprusside) and numerous dilutions of aqueous extracts of plants, a suitable volume of deionized water was used to advance a 30 uLof total volume at 37 ^{o}C for about 60 minutes. The reaction (colorreaction) was achieved by pouring 200, 500 and 500 micro liter each of the acetic acid buffer (pH 3.4),0.6% theobarbituric acid (TBA) and 8.1% sodium dodecyl sulphate (SDS) correspondingly. Mixtures were protected at 97 ^{o}C for 60 minutes along with sequential dilutions of 0.3mM standard MDA. The data of absorbance were occupied after refrigeration tubes using a wavelength of 532nm in a spectrophotometer (UV-spectrophotometer, Shimadzu, Japan).

DPPH radical scavengingactivity

The DPPH radical rummaging of steady radicals was accomplished agreeing with technique by Hatano*et al*,[9] with some modifications. The prepared different aqueous extract solutions (25-200 µg/ml) of each plant were mixed with 0.5ml of DPPH (solution 0.25mM in 95% ethanol). After proper shaking the samples having reagents were allowed undisturbed for 30 minutes to prepare far-reaching reaction. Lastly, absorbance (Abs) of each sample was determined at 517nm using UV-spectrophotometer. By using the control of each solution inhibition (%) was calculated.

Phenolic contents

For determination of total phenolic contents, 0.5ml of the aqueous extract, FolinCiocalteau's Reagent (2.5ml 10% v/v) and 2ml of Na_2CO_3.(7.5%) was used. The mixture was gestated for 40min at 45ºC.All the samples were processed and absorbancewas determined at 765nm using UV spectrophotometer. For this study positive control was gallic acid [10,11]. The total phenolic contents were comminuted as mgs of gallic acid counterparts per gram of the extract.

Total antioxidant Assay

The decline of molybendum, from molybendum (V) was the base for the assessment. The extracts of each plant (aqueous extract) were introduced to formulate consequently a green phosphate / Mo (V) multifaced at 6.0 pH. The aquous plant extracts (0.1 mg/ml) were mixed with 3ml of the reagent solution (28 mM Na_2PO_4, 0.6M H_2SO_4 and 4mM ammonium molybdate). For many minutes all the tubes were placed in incubator at a 95 ºC. After incubation the absorbance was taken at 695 nm at room temperature.

Statistical analysis

One way ANOVA test was used through SPSS version 14.0 software for the statistical analysis. The results were measured as mean and standard deviation (SD). Duncan's multiple range test was used to compare the mean of different groups with each other with significant difference of $P<0.05$.

RESULTS AND DISCUSSION:

To base the lipid peroxidation the mice brain homogenates were induced with iron and sodium nitoprusside and the effect of *SolanumNigram*(Plant 1),*SolanumVillosum*(Plant 2), *Momordicacharantia*(Karela) (Plant 3), *TinosporaCordifolia* (Plant 4), *Ginkgo Biloba*(plant 5) and*Cuscutareflexa (Plant 6)* were determined.

Lipid peroxidation in mice brain induced with sodium nitroprusside and iron

The antioxidant potential of *SolanumNigrum*,*SolanumVillosum*, *Momordicacharantia*(Karela) in animal modle (Mice) brain are presented in figure 4.1 (a). The result shows that handling with 5µM SNP produced a significant (P= 0.05) increase in TBA reactive agents as compared to the basal. In experiment 3 controls were used for *Solanumnigrum*, *SolanumVillosum*, *Momordicacharantia*(Karela). Administration of different dilutions of aqueous extracts (25-200 mcg/ml) caused as substantial reduction in lipid peroxidation.*Solanumnigrum* displayed a higher reduction in lipid peroxidation as compared to the extracts of other plants. *SolanumVillosum* displayed a decrease in lipid peroxidation upto concentration 75mcg/ml, above that concentration it exhibited the pro-oxidant effect by producing escalation in lipid peroxidation. Where as, *Momodicacharantia*showed antioxidant activity at all concentrations. Figure 4.1 (b) shows the effect of *Ginkgo Biloba, TinosporaCordifolia*and *Cuscutareflexain* on mice brain. Three separate controls were used *for Ginkgo Biloba, TinosporaCordifolia* and *Cuscutareflexa.* Treatment with *ginkgo biloba* and *TinosporaCordifolia* caused a significant decrease in lipid peroxidation however *Ginkgo Biloba* shows pro-oxidant activity at concentration of 25 mcg/ml. *Cuscutareflexa*showed anti-oxidant activity up to 50mcg/ml while onward it showed pro-oxidant effect on all higher concentrations.

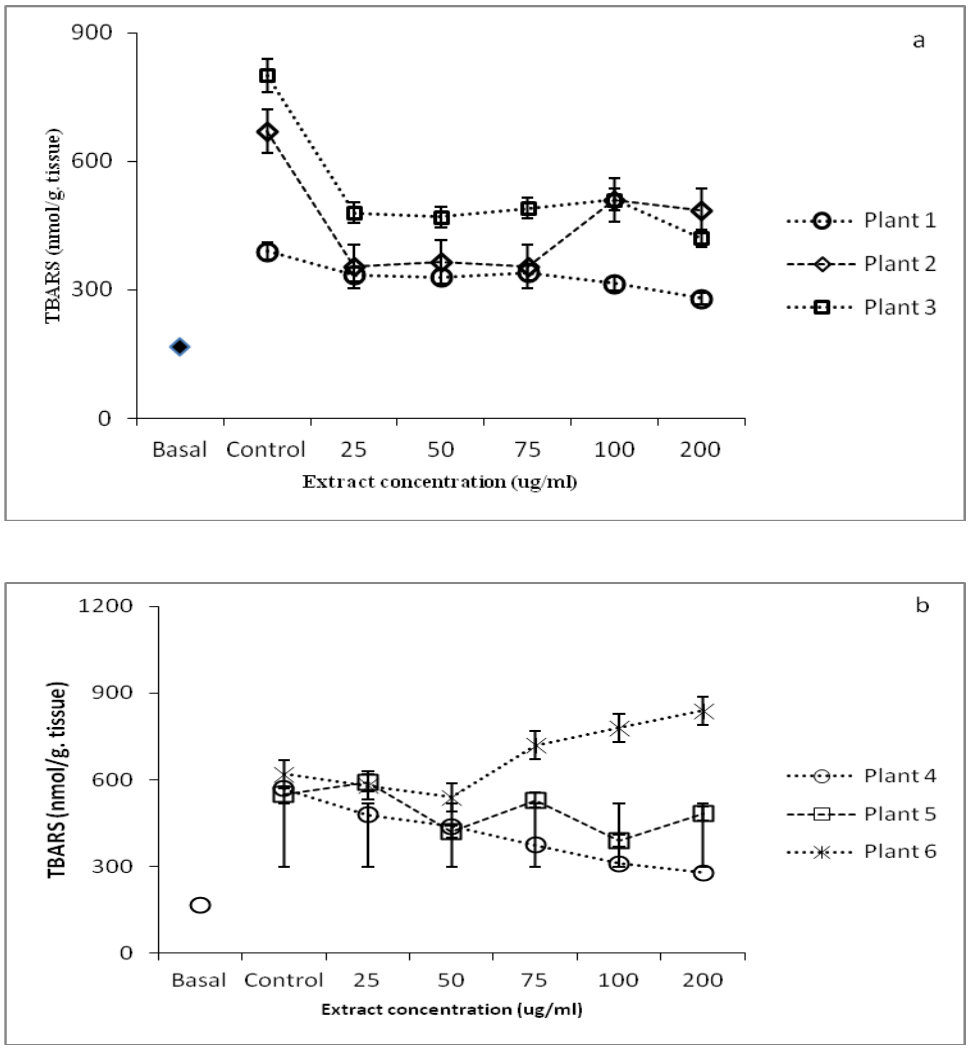

Fig 1:The effect of aqueous extracts of medicinal plants against lipid peroxidation in mice brain induced by sodium nitroprusside. (a) The effect of *Solanumnigram*(Plant 1), *SolanumVillosum*(Plant 2), *Momordicacharantia*(Karela) (Plant 3), lipid peroxidation in mice brain. (b) The effect of *TinosporaCordifolia*(Plant 4), *Ginkgo Biloba*(Plant 5) and *Cuscutareflexa*(Plant 6) against lipid peroxidation in mice brain

Figure 4.2 (a) shows the anti-oxidant effect of *SolanumNigrum, SolanumVillosa* and *Momordicacharantia*in mice brain. In this experiment, TBARS were included with 10µM iron. The results proved that by treating with ferrous (II) caused a significant ($P < 0.05$) elevation in TBARS compared to the basal. In experiment 3, separate controls were used for of *SolanumNigrum, SolanumVillosum*and *Momordicacharantia.* Treatment with different concentrations of*SolanumNigrum, SolanumVillosa* and *Momordicacharantia*caused a noticeable decrease in lipid peroxidation. The results reveled these extracts could be more effective in decreasing lipid peroxidation against iron compared to sodium nitroprusside in mice brain. *SolanumNigrum* and*Momordicacharantia*showed a higher percentage decrease in lipid peroxidation compared *SolanumVillosa.* Figure 4.2 (b) shows the anti-oxidant effects of with *ginkgo biloba, tinosporacordifolia*and *cuscutareflexa*in mice brain was persuade with ferrous. Administration with Ferrous (II) stimulated the TBARS synthesis. However, the lesser concentration of *ginkgo biloba, tinosporacordifolia*were found to be effective in decreasing lipid peroxidation however *Ginkgo Biloba* was found to be very potential anti- oxidant at high dose. *Cuscutareflexa* did not show the anti-oxidant effect.

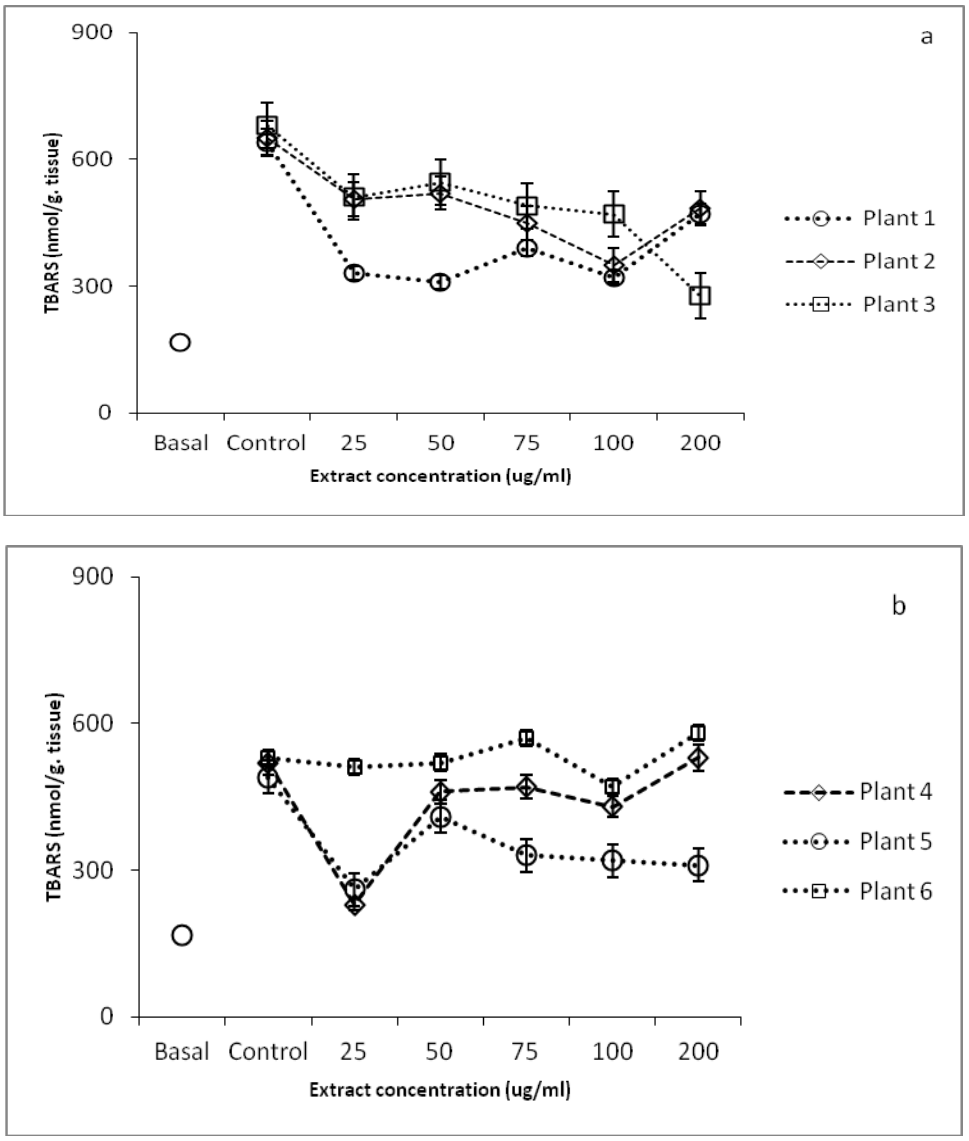

Fig 2: The effect of aqueous extracts of medicinal plants against lipid peroxidation in mice brain induced by iron. (a) The effect of *Solanumnigram*, SolanumVillosum, *Momordicacharantia* against lipid peroxidation in mice brain. (b) The effect of *TinosporaCordifolia* , *Ginkgo Biloba, Cuscutareflexa*against lipid peroxidation in mice brain

DPPH radical scavenging assay

Figure 3shows the DPPH free radical scavenging activity of selected medicinal plants. High anti-oxidant neither then 50%. Still the order of their activity is *SolanumNigrum, SolanumVillosa,Momordicacharantia,TinosporaCordifolia, Ginkgo Biloba,Cuscutareflexa.*

*Momordicacharantia*showed higher percentage of scavenging then *SolanumNigrum*at diluted concentrations.

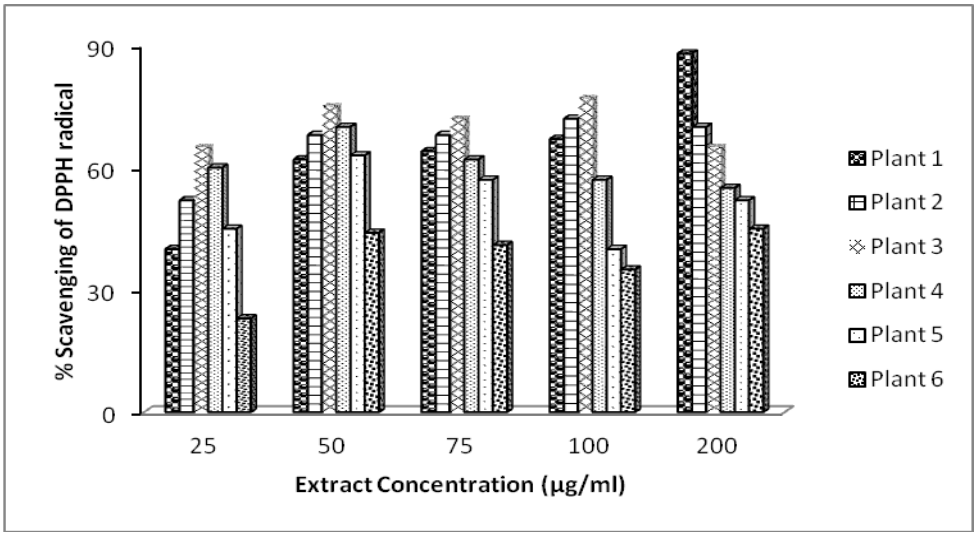

Fig 3:Antioxidant activity of aqueous extract of medicinal plant leaves. DPPH radical scavenging activity of different plants. Values are means ± SD (n=3)

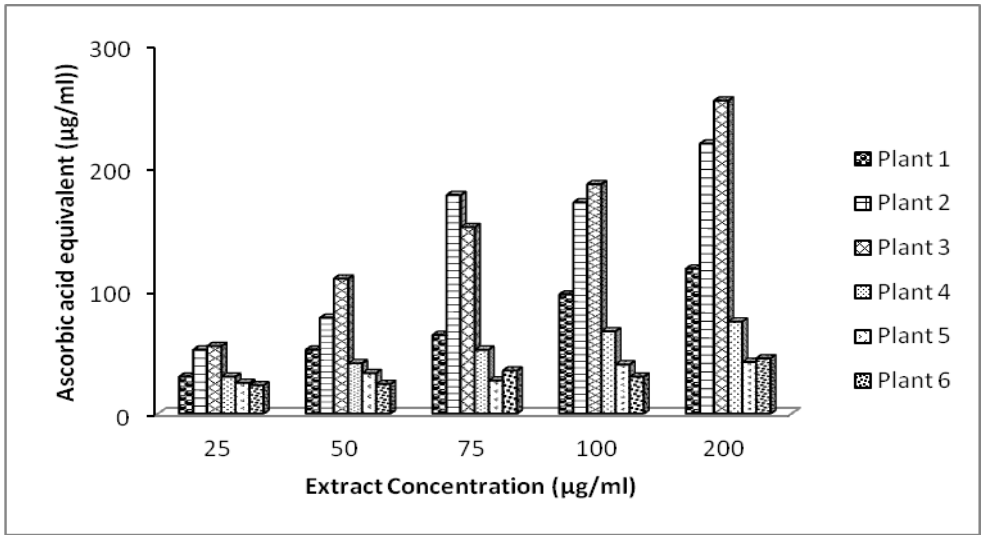

Fig 4: Total antioxidant activity measured by phosphomolybdenum reduction method. Values are mean ± SD (n=3)

Total antioxidant assay throughphosphomolybdenum assay:

The total anti-oxidant assay of different plant samples showed as ascorbic acid equivalent are presented in figure 4. Reducing activity was shown by all plants extracts. The order of their reactivity was *Momordicacharantia>SolanumVillosum>SolanumNigrum>tinosporacordifolia>cuscutareflexa>Ginkgo Biloba.*

Total phenolics content

Mean values for total phenolic content were determined. The highest phenolic contents were found in *Momordicacharantia*(266 ± 1.63mg/g) followed *by SolanumVillosum*(203.38± 0.64mg/g) and *SolanumNigrum* (143.78±1.32mg/g), while Tinosporacordifolia (97.4 ± 0.9mg/gm).*Ginkgo Biloba.*(45.48± 0.61mg/g and Cuscutareflexa (10.3± 0.21 mg/g) were found to contain comparatively low phenolic contents.

DISCUSSION:

This research was performed to evaluate the antioxidant activity, of some selected medicinal plants from different areas of Pakistan. The overall results attribute to a connection between the products tat cause oxidative damage to DNA and TBARS as aindicator of lipid per-oxidation. It is clear from the previous studies that the generation of ROS not just

attack on DNA and proteins but even on other parts of cell which involve poly unsaturated fatty acid residues of phospholipids and are thought to be extremely prone to oxidation. Inhibition of lipid per-oxidation in homogenate in thebrain of rabbit is parallel to neuro-protection [11]. Due to high oxygen utilization the cells as well as tissues of brain are prone to oxidative destruction, due to high concentration of poly-unsaturated fatty acids and occurrence of copper and iron. The antioxidants in plant extracts may beentities to have a strongcurative activity in oxidative hassle produced by free radicals [12]. Production of TBARS by Iron Sulphate (10µM) stimulated oxidation, suggests the possible destruction of cells and tissues with an iron burden. Iron occurred in two organelles i.e., mitochondria as well ascytoso,l may cause significant cellular oxidative damage by enhancing the formation of superoxide, which in response produces Ferrous (II) from Ferric (III) that contribute in Fenton reaction. Rate when burdened with Ferrous cause toxic reactions at various organs like hepatocellular hypertrophy, splenic white pulp, cardio myopathy and pancreatic atrophy and hemosiderosis in the heart, liver, spleen, pancreas and endocrine glands respectively [13]. The ferrous toxicity is triggered by the mechanisms which contain free radical-mediated per oxidative reactions, which are freely catalyzed by ferrous. The protections against the free radicals offered by the aqueous extract of is *SolanumNigrum, SolanumVillosum, Momordicacharantia, tinosporacordifolia, Ginkgo Biloba, Cuscutareflexa*recommend that they will be beneficial to treat brain ailment. Sodium nitroprusside is a drug used to low blood pressur but it has also been reported that sodium nitroprusside (SNP) causes cytotoxicity by the generation of cyanide and nitro oxide. The protection offered by aqueous extracts of is *SolanumNigrum, SolanumVillosum, Momordicacharantia, tinosporacordifolia, Ginkgo Biloba, Cuscutareflexa*on tissues displays the in vitro anti-oxidant activity of above mentioned selected medicinal plants and suggest that they may be helpful in avoiding ailments happening from the increase of iron and sodium nitroprusside. Results of this study also showed that those selected plants show protection at very lesses concentrations (200µg/ml) and majorly has the ability to reduce the fabrication of TBARS lesser than the basal level.

The plant extract was studied by using DPPH radical scavenging assay and ferric reduction assay to check the antioxidant activity. Different methods are used to evaluate the antioxidant properties of plants because a single universal method is not enough. The most popular method to check the antioxidant activity of plants is DPPH free radical scavenging activity which is most simple and acceptable among all the methods. Aqueous extracts of *SolanumNigrum, SolanumVillosum, Momordicacharantia* showed plant radical scavenging activity. The medicinal plants extracts have ability to scavenge DPPH radicals, this ability may be due to electron denaturing capacity and due to this capacity they react with free radical and made them inert and also stop radical chain reactions. Reducing power of an extract is used to measure the anti oxidant activity and is assessed by the conversion of Ferric (III) to ferric (II) in the existence of the sample extracts. The reducing capacity of the Ferric (III) to Ferrous (II) transformation assay is easy to execute and this reaction is linked to the most concentration of the antioxidants [14,15]. The plants have many constituents but phenols are very important among these. Phenolic compounds are good antioxidants because they donate hydrogen ions [16,17]. There is a true relationship among total phenol and antioxidant capacity of plant extracts due to the scavenging capacity of hydroxyl groups of their phenolic [18]. Thus, it was very important to determine the amount of extract of selected plant ranging from *Momordicacharantia*(266± 1.63mg/g) to *Cuscutareflexa*(58.3 ± 0.21mg/g). from the selected six plants those plant extract which have higher total phenol content showed better antioxidant activity such as *SolanumNigrum, SolanumVillosum, Momordicacharantia, TinosporaCordifolia* which shows that there is some correlation between total phenolic contents and free radical scavenging assay.

Flavonoids are polyphenols which are naturally occurring in nearly all plant parts. Flavonoids have therapeutic and scientific value. The important role of flavonoids is antioxidant action because they have ability to scavenge dangerous free radicals which are harmful to the body cells and also reactive oxygen species that are formed within the cells and tissues during different metabolic processes lead to oxidation. Flavonoids work by different mechanism including inhibition of enzymes , involved in the production of ROS, chelation of copper and iron and by providing hydrogen ions to free radicals [19], and by the way humans defined from diseases like cancer and myocardial infarction.

High antioxidant activity of *SolanumNigrum,TerminaliaChebula, TinosporaCordifolia* is due to the high quantity of phenolic contents. Whereas the *Cuscutaflexa* and *Ginkgo Biloba*contain less amount of phenolics showed less antioxidant activity. In addition *SolanumNigrum,,* and *Cuscutareflexa*also show antioxidant activity which contain high amount of ascorbic acid. Similar studies were reported by

Vadivel [20] the antioxidant activity of *SolanumNigrum*fruit extract in rats , which showed the protection against ethanol induced toxicity. The mimicking findings were also reported by Kyung-Sun heo and KyeTaek Lim [21] and GokselSener [22]

List of abbreviation

TBARS: thiobarbituric acid reactive species
DPPH: 2,2-diphenyl-1-picrylhdrazyl
ROS: reactive oxygen species

Ethical approval

National Institute of Health (NIH) Islamabad, Pakistan

CONCLUSION:

All of the collected plant extract showed higher anti oxidant activity which must be credited to the presence of higher flavonoid and phenol contents. Out of six plants selected in this current study, some plants indicated high antioxidant activity but there is limited scientific evidence about the exact mechanism. A further research study also required to isolate and identify the components which are responsible for antioxidant activity which are presently unclear.

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