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IN VIVO ANTIOXIDANT ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF POLYHERBAL FORMULATION IN DIABETES INDUCED RATS

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ABSTRACT

Antioxidant activity of polyherbal formulation of aqueous and ethanolic extract of *Ocimum sanctum, Withania somnifera, Gymnema sylvestre*, *Tinospora cordifolia*, *Phyllanthus emblica* and *Coleus forskohlii* were carried out in the present study. Antioxidant potential was investigated using *in vivo* assay like SOD radical scavenging assay, CAT radical scavenging activity and MDA scavenging activity. Various Phyto constituents were identified in the above selected plant (after taxonomical screening) extracts were poly phenols, flavonoids, terpenoids, tannins, alkaloids. It is evident that terpenoids were stated to possess the scavenging properties against the free radicals. The presence of these Phytoconstituents in selected plants stimulated to assess the scavenging property against free radical.. After 7 days of treatment with Glibenclamide, Hot water extract of Polyherbal Formulation (200 mg/kg & 400 mg/kg) and Ethanolic extract of Polyherbal Formulation (200 mg/kg & 400 mg/kg) in treated groups were observed and it showed significant (P< 0.001) reduction in lipid peroxidation in tissue MDA levels and significantly (P< 0.001) increased the levels of SOD and CAT in diabetic rat liver, and this indicate the effective antioxidant property of the polyherbal formulation. This study forms a base of for drug discovery on natural antioxidant drugs.

KEYWORDS: Polyherbal formulation (PHF), Phytochemical, Anti-oxidant activity, SOD, CAT and MDA.

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INTRODUCTION

The Reactive oxygen species, also known as active oxygen species, occurs in various forms of activated oxygen, which include free radicals such as superoxide ions ($O2^-$) and hydroxyl radicals (OH^-), as well as non-free radical species such as hydrogen peroxide (H_2O_2).[1] These ROS plays an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation.[2] In living organism various ROSs were formed in different ways, through normal aerobic respiration lead to the stimulation of polymorphonuclear leukocytes, macrophages and perioxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals includes tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides.[1] Antioxidant refers to the activity of numerous vitamins, minerals and other phytochemical to protect the damage caused by ROS.[3] Antioxidant defence system scavenges and minimizes free radicals formation. The actions of free radicals are counteracted by antioxidants, either endogenous or exogenous.[4] The living system with different antioxidant enzymes such as catalase, superoxide dismutase, and malondialdehyde that play a significant role in scavenging the free radicals and protecting cell membrane from injury.[5] The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease.[1] Synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used in processed food but they have side effects. Plant based antioxidants are now preferred as an alternative to synthetic ones because of safety concerns. Therefore, many researches regarding antioxidant potential from plant source were carried out. In the present study on polyherbal formulation of hot water extract and ethanolic extract of six selected plants was evaluated for the *in vitro* antioxidant properties using various experimental models.[6]

MATERIALS AND METHODS

Collection of plant material

The present study is to screen the antioxidant activity of six selected known medicinal plants. The plant materials were collected from western ghat and Bangalore rural zone and the said plants were authenticated by the taxonomist of Institute of trans-disciplinary health sciences and technology (Transdisciplinary University). In the polyherbal preparation, leaves of *Tinnospora cordifolia (3827), Ocimum tenuiflorum (3828), Gymnema sylvestre (3825)* were selected, whereas in the plants like *Withania somnifera (3829)* and *Coleus forskohlii (3830)*, the root part of the plant were selected, and in the plant *Phyllanthus emblica*, the fruit portion of the plant was selected to prepare the polyherbal formulation to screen antioxidant activity through in vivo studies.[6]

Preparation of plant's extracts

Air dried plant material was crushed, powdered and were mix in equal proportion) to make polyherbal formulation. The polyherbal formulation was extracted using soxhlet apparatus with polar and non-polar solvent. The solvent used were hot water, cold water, ethanol, methanol, petroleum ether and n-hexane. Cold water extracts was obtained by adding distilled water to the crushed material in ratio the 1:4 and kept in rotary shaker for 24h at 30 degree Celsius and at 130 rpm respectively. After 24 h it was filtered and the resulting extract was stored in refrigerator. Hot water extracts was obtained by soxhlet extraction were 400 ml of distilled water to 100 grams of plant powders were added. Similarly medicinal powders were extracted using organic solvent in ratio 1:3 through soxhlet extraction.[7, 8]

Induction of Diabetes

A single intraperitoneal injection of Streptozotocin (STZ) measuring 40 mg/ kg freshly dissolved in 10 mM citrate buffer (pH 4.5) was used for injection. To confirm the diabetes, blood sample were taken from the diabetic rats and were screened for blood glucose level with the help of Glucometer, which showed 300 mg/dl of blood sugar level in rats confirms that all the experimental animals have become diabetes.[18]

Animal ethical committee approval

Male albino Wistar rats around 150-250 g were obtained from CPCSEA approved institution, Acharya & B M Reddy College of Pharmacy, Bangalore, Karnataka and housed . Three animals per cage with paddy husk as bedding were kept. Animals were housed at temperature of 25 ± 2°C, relative humidity of 30-60% and 12:12 h light and dark cycle was followed. The animals had accessed to feed and purified water *ad libitum*. All the experimental procedures were carried out accordance with committee of CPCSEA. The experimental procedures were approved by the institutional animal ethical committee (Ref.: IAEC/ABMRCP/2014-15/21) to carry out the aforesaid research.

Acute toxicity study

The acute toxicity studies were carried out for Ethanolic and hot water extract of Polyherbal Formulation using up and down procedure according to OECD guidelines no. 425.[9] Healthy adult male Swiss albino rats weighing between 150 to 250 g were used for the study. The limit tests of 2000 mg/ kg dosage were carried out, in which a single rat were dose with test sample to confirm its survival. The study is further confirmed by taking four additional animals and dosed sequentially hence a total of five animals were tested to confirm the toxicity and survival rats. Since all animals survived for more than a week then trial was further repeated for the limit test of 4000 mg/ kg dosages were performed, in which a single rat were dose with test sample to confirm its survival, if the animal died after administration than repeat the same procedure with 2 more animals and finally all the animals died. Hence based on the selected concentration 2000 mg/ kg two doses were selected such as $1/10^{th}$ dosages is 200 mg/ kg and $1/5^{th}$

dosages 400 mg/ kg (according to OECD guidelines) were selected to study the antioxidant study.[9]

Preparation of homogenate

Both control and experimental rats were sacrificed by cervical dislocation and dissected out the liver from rats. Liver was removed, collected and washed in ice cold phosphate buffer and weighed 10 % to prepare liver homogenate was used. Liver was homogenated and the homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Finally the supernatant was used for biochemical estimations.

METHODS

Test of extracted material from different part of plant (phytochemical analysis).

Test for the presence of different Bio-components such as alkaloids, flavonoids, glycosides, saponins, phenols, steroids and terpenoids were performed using standard procedures.

1. Superoxide dismutase (sod)

Principle

As we are aware, superoxide dismutase is an enzyme that catalyzes the dismutation superoxide into oxygen and hydrogen peroxide. This method is based upon the ability of SOD to inhibit the reduction of nitrobluetetrazolium to blue colored tetrazolium in presence of phenazinemethosulphate and NADH by superoxide dismutase enzyme. The color intensity was measured in spectrometer at 560 nm.

Reagents preparation

1). Tris-buffer (0.25M) solution: it is prepared by taking 3.0285 gm of Tris-buffer was weighed, transferred to a volumetric flask and the volume was made upto 100 ml with distilled water.

2) Sodium pyrophosphate buffer (0.052 M, pH 8.3) solution

1.38 gm of tetra-sodium pyrophosphate was weighed carefully, which is transferred to a volumetric flask where the volume was made upto 100 ml with distilled water and the pH was adjusted to 8.3.

3) Phenazine methosulphate (186 µM) solution:

In this reagent preparation 5.69 mg of Phenazine methosulphate was weighed transferred to a volumetric flask and the volume was made upto 100 ml with distilled water.

4) Nitroblue tetrazolium (300 µM) solution:

A quantity of 12.25 mg of Nitroblue tetrazolium was weighed transferred to a volumetric flask and the volume was made upto 50 ml with distilled water.

5) NADH (780 µM) solution

13.83 mg of NADH was weighed, which is transferred to a flask to make the volume to 25 ml with distilled water.

Procedure

Procedure for screening the SOD activity was performed by Kakkar *et al* method of 1984, [16] the liver tissue was dissected out and homogenized with polytron homogenizer in ice-cold Tris-HCl buffer to set a 10% w/v homogenate. It was centrifuged at 15,000 rpm at - 4°C for 10 min using cryo centrifuge. The supernatant 0.1 ml was added to 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3) followed by addition of 0.1 ml of 186 µM phenazonium methosulphate, 0.3 ml of 300 µM nitroblue tetrazolium, 0.2 ml of 780 µM NADH. The reaction mixture was incubated for 90 sec at 30°C, and the reaction is stopped by the adding of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min using cryocentrifuge. Which was measured at 560 nm, a control was prepared using 0.1 ml of distilled water devoid of 0.1ml of homogenate. One unit of the enzyme activity is defined, as enzyme concentration required inhibiting the absorbance of chromogen production by 50% in control sample [10].

Formula for estimation of SOD

$$\frac{\text{Control O.D-Experimental O.D}}{\text{(Control O.D/2)}} \times \frac{1}{\text{Protein in mg}} = \text{Units/mg protein}$$

Where: O.D=Optical Density

2. Catalase activity (cat)

Principle

This enzyme measured by the ability of CAT to oxidize hydrogen peroxide (H_2O_2). Results in decomposition of H_2O_2 give water and oxygen.

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The UV light absorption of hydrogen peroxide solution can be measured at 230 to 250 nm. On decomposition of H_2O_2 by CAT, the absorption decreases with time. The enzyme activity could be arrived at this decrease. But this method is applicable only with enzyme solution which do not absorb strongly at 230-250 n

Reagents preparation

1) 0.1 M Tris-HCl buffer (pH 7.4)

1.58 gm of tris buffer was weighed and transferred to volumetric flask to make it up to 100 ml with distilled water and pH was adjusted to 7.4 using HCl.

2) Phosphate buffer (50 mM, pH 7.0)

In this reagent preparation 680 mg of potassium dihydrogen phosphate and 871 mg of dipotassium hydrogen phosphate weighed, transferred to a volumetric flask to make it up to 100 ml with distilled water where the pH was adjusted to 7.0 with using 10% NaOH.

3) Hydrogen peroxide solution (30 mM)

It was prepared using 85 µl of hydrogen peroxide which was transferred to a volumetric flask to make it up to 25 ml using distilled water.

Procedure

Enzyme catalase activity was screened by Aebi*et al*, method of 1974.[17] Liver tissue was dissected and homogenized with polytron homogenizer in ice-cold Tris-HCl buffer to produce a 10% w/v homogenate. It was centrifuged at 15,000 rpm at - 4°C for 10 min using cryocentrifuge from this supernatant 0.1 ml was added to cuvette containing 1.9 ml of 50 mM phosphate buffer. To this mixture, 1.0 ml of freshly prepared 30 mM H_2O_2 was added and changes in absorbance for 3 min was measured at 240 nm with an interval of 30 second. Simultaneously a control was prepared using 0.1 ml of distilled water without 0.1 ml of homogenate [11]. Enzyme activity is calculated as enzyme concentration required inhibiting change in absorbance by 50% in one min in the control sample.

Catalase technique was estimated using the formula

$$\frac{\Delta A \times \text{Vol of R.M}}{43.6 \times \text{Vol of E}} \times \frac{1}{\text{Protein in mg}} = \mu \text{ moles of } H_2O_2 \text{ metabolized/mg protein/min}$$

Where: R.M= Reaction Mixture, E= Enzyme added

3. Estimation through MDA method

Oxygen species from free radicals reacts with membrane lipids to develop lipid peroxides and malondialdehyde (MDA). Since the free radicals have a very short half-life and in clinical studies their activity is usually assessed indirectly by measuring either the level of free radical reaction products such as MDA or free radical scavengers. If free radical activity will increase than the MDA levels will also increases, hence MDA concentration by estimating thiobarbituric acid (TBA) reactivity with MDA. The TBA reaction was first applied to biological materials and has been widely used as a measure of lipid peroxidation in food products, fatty acids and lipid membranes. The reaction of lipid peroxides with TBA has been widely adopted as a sensitive assay method for lipid peroxidation in normal tissues which widely used in the scientific study [12].

Principle

The oxygen generated from free radicals reacts with membrane lipids to form lipid peroxides which are heated with TBA in the presence of acetic acid. The resulting chromogen is extracted with a mixture of n-butanol and pyridine and the absorbance of organic phase is determined at a wavelength of 532 nm. Standard solution-[Malonaldihyde-bis-diethyl-acetal (1,1,3,3-tetraethoxypropane)] was dissolved in distilled water to prepare a 10 µM solution [12].

Reagents preparation

1) 0.1M Tris-HCl buffer (pH 7.4)

This reagent was prepared by weighing 1.58 gm of tris buffer and transferred to volumetric flask to make it up to 100 ml with distilled water and pH was adjusted to 7.4 using HCl.

2) 8.1% Sodium dodecyl sulphate (SDS)

Prepared 8.1 ml of SDS transferred to volumetric flask to make it to 100 ml with distilled water.

3) 20% Acetic acid solution

Glacial acetic acid measuring 20 ml was transferred to volumetric flask to make it to 100 ml using distilled water. The pH was adjusted to 3.5 by adding N/10 Sodium hydroxide drop wise.

4) 0.8% aqueous solution of TBA

For preparing this reagent 0.8 gm of TBA was weighed, transferred to a standard volumetric flask to make it up to 100 ml using distilled water.

5) n-butanol and pyridine (15:1 v/ v)

It was prepare by the mixture of solution, n-butanol and pyridine in the ratio of 15:1.

6) Standard solution [Malondialdehyde – bis – diethyl – acetal (1,1,3,3-tetraethoxy propane, TEP)] (10 nM)

Stock I was prepared by: 12 µl of TEP diluted to 50 ml with distilled water (10 µM).
Stock II: 1 ml of stock I diluted to 10 ml with distilled water (1000 nM).
Stock III: 1 ml of stock II diluted to 100 ml with distilled water (10 nM).

Procedure

The lipid peroxidation end product Malondialdehyde (MDA) was measured by the method of Okhawa*et al*. 0.2 ml of tissue homogenate was treated with 0.2 ml sodium dodecyl sulphate (8.1%), 20% of 1.5 ml of acetic acid (pH 3.5), 1.5 ml TBA (0.8%). The above mixture was made up to 5 ml using distilled water followed by heating at 95°C in oil bath for 60 min. The

mixture was cooled and 5 ml of n-butanol and pyridine mixture (15:1 v/v) was added. Then it was shaken vigorously. After this the mixture was centrifuge at 4000 rpm for 10 min, the organic layer was taken and the absorbance was measured at 532 nm. The concentration of MDA formed is expressed as nmol/g wet tissue [12].

Standard procedure

Stock solution of 1, 1, 3, 3-tetraethoxypropane (TEP) was prepared by taking 22 mg of TEP in the volumetric flask and it was made up to 10 ml using distilled water in a standard volumetric flask. From this stock solution 1 ml was taken in a volumetric flask and it was made up to100 ml using distilled water to obtain a 10 µM solution. From this working standard solution, 0.2 ml, 0.4 ml, 0.6 ml 0.8 ml and 1ml quantities which representing 2 nmol, 4 nmol, 6 nmol 8 nmol and 10 nmol of TEP respectively, this is transferred into centrifuge tubes. Then the 0.2 ml sodium dodecyl sulphate (8.1%), (20% of) 1.5 ml of acetic acid (pH 3.5), 1.5 ml TBA (0.8%). This mixture was made up to 5 ml using distilled water and then heated at 95°C in oil bath for 60 min. The mixture was cooled and 5 ml of n-butanol and pyridine mixture (15:1 v/v) was added. The mixture was shaken vigorously. And centrifuge at 4000 rpm for 10 min, the organic layer was taken and the absorbance was measured using wavelength 532 nm. The concentration of MDA formed is expressed as nmol/g wet tissue. The standard graph was plotted by taking concentration of MDA on X-axis and the corresponding absorbance on Y-axis [13]

Statistical analysis

All the values were expressed as mean ± SEM. The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett's comparison test and $P < 0.05$ which was considered as significant.

RESULTS

Phytochemical screening of the polyherbal formulation

From the first part of phytochemical analysis study, it is evident that both hot water and ethanolic solvent shows the presence of carbohydrates, glycoside, alkaloids, sterioids, triterpenoids, flavonoids, phenol, saponins and tannins. Out of the all the phytochemical, presence of tannins was maximum. To find the type of tannins, the sample was screened further and was found to be hydrolysable tannin.

In vivo antioxidant activity

In the present study, in diabetic group there was significant decrease in SOD and CAT levels in liver, whereas a significant increase in lipid Peroxidation (increase in tissue MDA level) was observed when compared to the normal group. After 7 days of treatment with Glibenclamide, Hot water extract of Polyherbal Formulation(200 mg/kg & 400 mg/kg) and Ethanolic extract of Polyherbal Formulation(200 mg/kg & 400 mg/kg) in treated groups, there was significant ($P< 0.001$) reduction in lipid peroxidation in tissue MDA levels and significantly ($P< 0.001$) increased the levels of SOD and CAT in diabetic rat liver (Table-2 to 5, Graph- 2-7), which indicate the effective antioxidant property of the selected polyherbal formulation on the liver extract.

Table 1

Calibration curve for the estimation of Malondialdehyde in tissues

Sl.No	Tetraethoxy propane (nmol.)	OPTICAL DENSITY (O.D)							
		Trial-1	Trial-2	Trial-3	Trial-4	Trial-5	Trial-6	Mean	±SEM
1	0	0	0	0	0	0	0	0	0
2	2	0.496	0.156	0.138	0.126	0.165	0.265	0.145	0.05796
3	4	0.290	0.310	0.255	0.424	0.312	0.320	0.308	0.02316
4	6	0.522	0.356	0.362	0.432	0.391	0.440	0.411	0.02530
5	8	0.571	0.521	0.523	0.530	0.510	0.433	0.521	0.01845
6	10	0.650	0.657	0.626	0.581	0.657	0.632	0.649	0.01182

Graph 1

Calibration curve for MDA

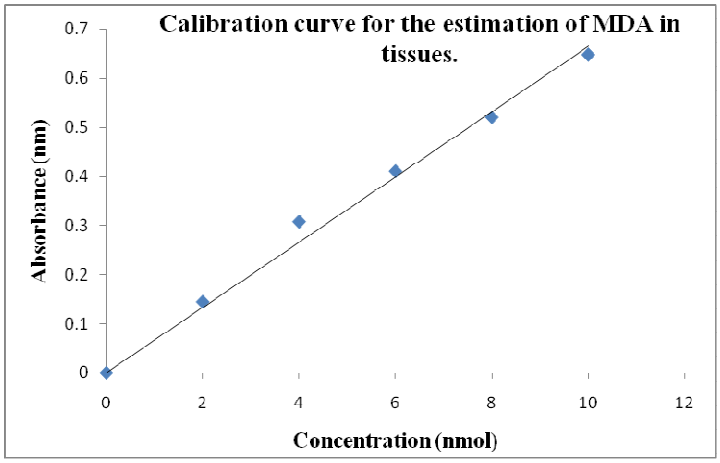


Table 2
Effect of 200 mg/kg and 400 mg/kg of HWEP and EEP on SOD estimation

No.	Healthy control	Diabetic control	Glibenclamide	HWEPF (200 mg/kg)	HWEPF (400 mg/kg)	EEPF (200 mg/kg)	EEPF (400 mg/kg)
1.	1.485	0.635	1.595	0.640	1.003	1.485	1.289
2.	1.346	0.760	1.272	0.704	1.200	1.346	1.119
3.	1.267	0.721	1.439	0.832	0.991	1.267	1.445
4.	1.400	0.847	1.051	0.770	1.015	1.400	1.103
5.	1.482	0.456	1.173	0.621	0.963	1.482	1.300
6.	1.468	0.583	1.243	0.634	1.340	1.468	1.163
Mean	1.408	0.667	1.296	0.700	1.084	0.815	1.236
SEM	± 0.035	± 0.056	± 0.079	± 0.034	±0.061	±0.052	±0.053

Table 3
Effect of 200 mg/kg and 400 mg/kg of HWEP and EEPon MDA estimation

No.	Healthy control	Diabetic control	Glibenclamide	HWEPF (200 mg/kg)	HWEPF (400 mg/kg)	EEPF (200 mg/kg)	EEPF (400 mg/kg)
1.	122.041300	176.035300	110.207000	232.987900	170.118100	110.946600	76.183330
2.	110.207000	305.473000	93.195140	190.088500	196.005700	144.970200	130.177300
3.	111.686200	102.070900	93.934790	190.828200	196.745300	153.845900	116.863800
4.	110.946600	247.041100	70.266180	218.934600	167.159500	159.763100	147.189200
5.	105.769100	250.739300	99.851940	286.242200	190.828200	164.940600	99.851940
6.	110.946600	164.940600	93.195140	215.976000	198.964200	147.189200	136.094500
Mean	**111.9**	**207.7**	**93.44**	**222.4**	**186.5**	**146.8**	**117.6**
SEM	**±2.200**	**±29.97**	**±5.35**	**±14.47**	**±5.807**	**±7.822**	**±10.66**

Table 4
Effect of 200 mg/kg and 400 mg/kg of HWEP and EEP on CAT estimation

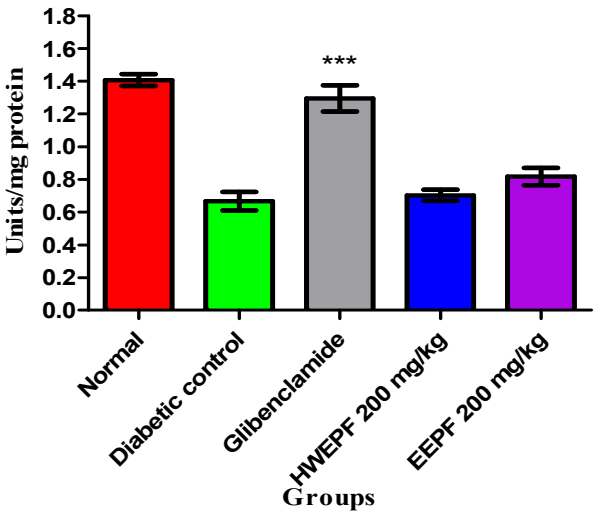
No.	Healthy control	Diabetic control	Glibenclamide	HWEPF (200 mg/kg)	HWEPF (400 mg/kg)	EEPF (200 mg/kg)	EEPF (400 mg/kg)
1.	0.01488608	0.007028704	0.01348593	0.007415575	0.009434749	0.008475541	0.0123871
2.	0.01487342	0.00699913	0.01392556	0.00755897	0.009667444	0.009845222	0.0130088
3.	0.01492405	0.006979414	0.0130463	0.007610182	0.01082035	0.009402393	0.0144868
4.	0.01521519	0.006792114	0.01309383	0.00780479	0.01149728	0.009247918	0.01276246
5.	0.01368354	0.006910409	0.01264232	0.008030125	0.01055592	0.008228381	0.01252786
6.	0.0136962	0.00681183	0.01335523	0.006995632	0.01305211	0.009711343	0.01260997
Mean	**0.014**	**0.006**	**0.013**	**0.007**	**0.010**	**0.009**	**0.012**
SEM	**±0.0002**	**±4.072e-005**	**±0.0001**	**±0.0001 ns**	**±0.0005**	**±0.0002**	**±0.0003**

Table 5
Effect of 200 mg/kg and 400 mg/kg of HWEP and EEP

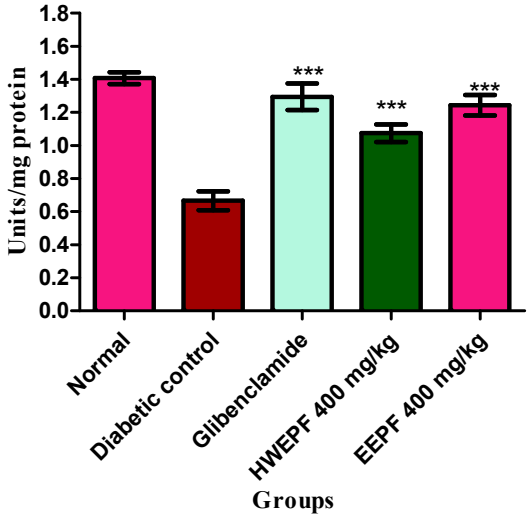
Group	SOD (Units/mg protein)	CAT (µmoles of H_2O_2 metabolized /mg protein/min)	MDA (nmol/gm wet tissue)
Normal	1.408±0.035	0.014±0.0002	111.9±2.200
Diabetes control	0.667±0.056	0.006±4.072e-005	207.7±29.97
Reference drug(Glibenclamide 5 mg/kg)	1.296±0.079 ***	0.013±0.0001 ***	93.44±5.35 ***
HWEPF (200 mg/kg)	0.703±0.034 ns	0.007±0.0001 ns	222.0±14.47 ns
HWEPF (400 mg/kg)	1.074±0.061 ***	0.011±0.0005 ***	185.9±5.807 ns
EEPF (200 mg/kg)	0.817±0.052 ns	0.009±0.0002 ***	146.4±7.822 *
EEPF (400 mg/kg)	1.244±0.053 ***	0.013±0.0003 ***	117.0±10.66 ***

The values are mean ± SEM; n=6, *** P<0.001, * P< 0.05 are significant as compared to diabetic control group.

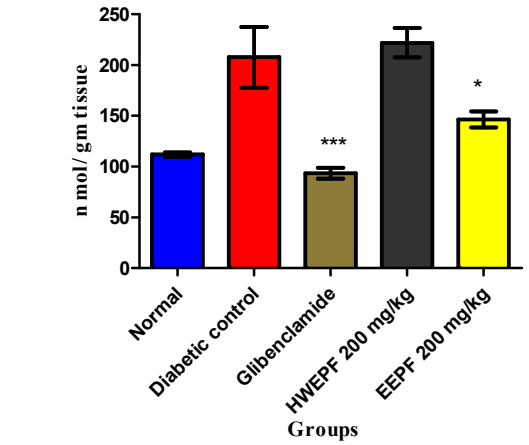
Graph 2
Effect of 200 mg/kg of HWEPF and EEPF on SOD.



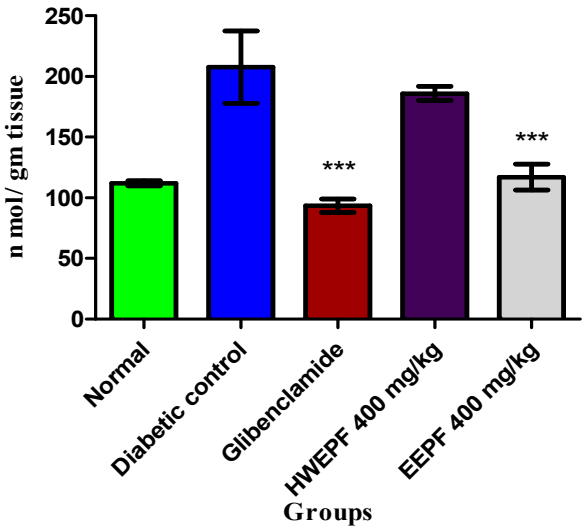
Graph 3
Effect of 400 mg/kg of HWEPF and EEPF on SOD.



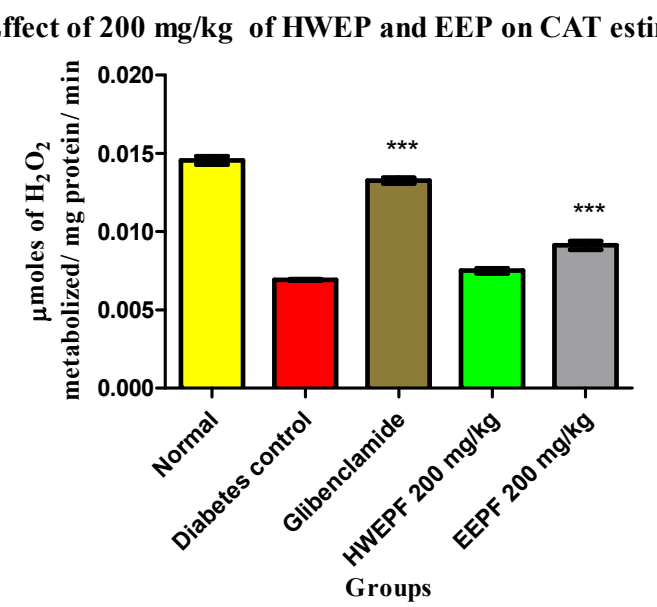
Graph 4
Effect of 200 mg/kg of HWEPF and EEPF on MDA.



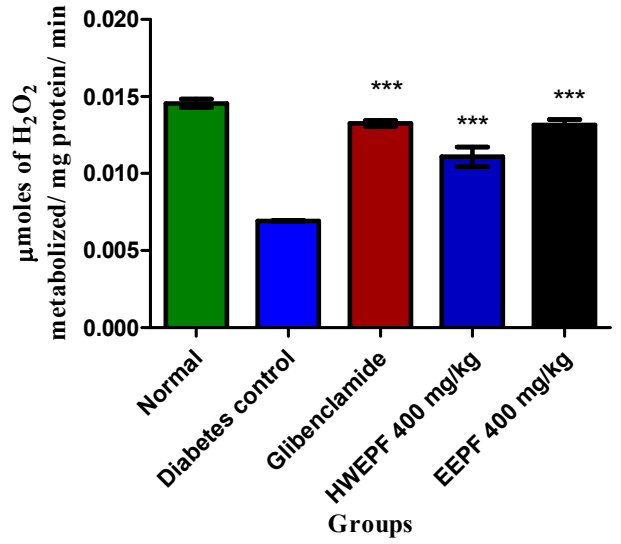
Graph 5
Effect of 400 mg/kg of HWEPF and EEPF on MDA



Graph 6
Effect of 200 mg/kg of HWEPF and EEPF on CAT.



Graph 7
Effect of 400 mg/kg of HWEPF and EEPF on CAT.



LD50
LD50 Experiment was carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was reported up to 10 and 15 times of the effective dose as no mortality was observed in any of the groups indicates its safe in administration.

DISCUSSION

In pathogenesis of type-2 diabetes, oxidative stress plays a vital role in the glucose oxidation, protein glycation and free radical. In the normal animals the antioxidant enzyme level increases through free radical scavenging to avoid cell damaged hence the oxidative stress can lead to remarkable diabetes complication. It is also true that diabetes was accompanied by the increase by the free radical due to impaired antioxidant defence as indicated by Cercllo., 2000.[14] The study further extended to screen the effect of antioxidant activity in vivo method obtained through homogenisation of liver of diabetes induced rats. The antioxidant activity was carried out by selected biomarkers such as SOD, CAT and MDA, were estimated in the sample. In the present study it is clear from the result (Table no.2, 3 and 4) that the remarkable increases in SOD and CAT in both hot water and ethanolic extract treated animals, indicates it potential antioxidant property through tissue damage in the vital organ in the body. When we critically analyse the MDA level it is quite apparently exhibiting its decrease in the activity both in hot water and ethanolic extract. When we compared the hot water and ethanolic extract of polyherbal formulation it is quite significant in

the hot water extract than the ethanolic extract. The outcome of the present study indicates the strong antioxidant property of the selected polyherbal formulation. The preclinical study can be further extended trough chemical trials to obtain a safe eco friendly herbal antioxidant drug.

REFERENCES

1. Yildrim A, Oktay M, Bilaloglu V. The antioxidant activity of leaves of Cydonia vulgaris. Turk J Med Sci 2001; 31: 23-27.
2. Huang DH, Chen C, Lin C, Lin Y. Antioxidant and antiproliferative activities of water spinach (Ipomoea aquatic Forsk.) constituents. Bot Bull Acad Sci 2005; 46: 99-106.
3. Khilifi S, El-Hachimi Y, Khalil A, Es-Safi N, Belahyan A, Tellal R, El-Abbouyi A. In-vitro antioxidant properties of Salvia verbenaca L. hydromethanolic extract. Indian J Pharmacol 2006; 38(4): 276-280.
4. Jain A, Singhai AK, Dixit VK. In-vitro evaluation of Tephrosia purpurea Pers. For antioxidant activity. J Nat Rem 2006; 6/2: 162-164.
5. Poongothai K, Ponmurugan P, Ahmed KSZ, Kumar BS, Sheriff SA. Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extract on alloxan induced diabetic rats. Asian Pac J Trop Med 2011; 4(10): 778-785.
6. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM. Farombi EM. Chemical constituents and antioxidant activity of Alstonia boonei. Afr J Biotechnol 2007; 6(10): 1197-1201.
7. Fennell, C.W, Lindsey,K.L, McGaw,L.J, Sparg, S.G, Stafford, G.I, Elgorashi, E.E, Grace, O.M & Van Staden, J.Assessing Aferican medicinal plants for efficacy and safety. Pharmacological screening and toxicology. Journal of Ethanopharmocology. 1994; 205-217.
8. Jakkhetia, V.V, Patel, R, Khatri, P, Pahuja, N, Garg, S, pandey, A. & Sharma, S.A cinnamon: a pharmacological review. Journal of advance scientific research, 2010; 1(2): 19-23.
9. Organization for Economic Cooperation and Development (OECD) guidelines for acute toxicity of chemicals. No 425 (Adopted: 3 October 2008).
10. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase.Ind J Biochem Biophys. 21: 130-2, 1984.
11. Aebi H. Catalase: Methods of Enzymatic Analysis. 2nded. New York: Academic Press;.673-7, 1974.
12. Ohkawa H, Ohoshi N, Tagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95:351-8, 1975. 332.
13. Ohkawa H, Ohoshi N, Tagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.; 95: 351-8, 1975
14. Ceriello A. Oxidative stress and glycemic regulation.Metab. 49:27-29, 2000.
15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase.Ind J Biochem Biophys. 21: 130-2, 1984.
16. Ohkawa H, Ohoshi N, Tagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95:351-8, 1975.
17. Aebi H. Catalase: Methods of Enzymatic Analysis. 2nded. New York: Academic Press;.673-7, 1974.

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