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Screening Of Antioxidant Potentials Of Polyherbal Formulation In Experimental Animal Modules

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ABSTRACT

Poly herbal formulations, the name itself are indicating as multiple ingredients of different herbal origin. The plant ingredients may have wide spectrum of biological activities. Polyherbal formulations are mainly used to enhance the activity or to counteract the toxic effects of compounds used from the other plants. These formulations may be give synergetic effect, due to presence of multiple ingredients and also may be show synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves. These formulations having different active constituents with different mechanism of actions which can produce combined action against various complications of diabetes. The aim of the present study is to formulate a polyherbal formulation and screening of its antioxidant potentials in animal modules. The low dose (200mg/kg) of EPHF produced significant increase in hepatic enzymes SOD, CAT and less significant increase in GSH and high dose produced all significant increase in hepatic anti oxidant enzymes. The DPHF at lower dose produced less significant effect in at CAT and SOD but high dose significantly increases them. As well as high dose of PPHF only less significantly increases the hepatic anti oxidant enzymes**.**

Key Words: Polyherbal formulations,Antioxidant, SOD, CAT, GSH

INTRODUCTION

Free radicals are the substances which are highly reactive in nature generally produced in human body due to several cellular metabolisms. These are electron deficiency or lone pair electron having in their outer most orbital and having short period of half life. These can severely effects human body. (Bagchi K *et al*.,1998). **Eg**: - $^{\cdot}OH^{-}$ (Hydroxyl radical) $^{\cdot}O_2^{-}$ (superoxide radical), NO (Nitric oxide radical) LOO (Lipid peroxide radical). Free radicals and other oxygen species are produced by the normal essential metabolic process. Some internally generated sources of free radicals. Eg:- Mitochondria, Xanthin oxidase, Phagocytes, Arachidonate pathway, Exorcize, Inflammation. Some externally generated sources of free radicals, X-rays, Ozone, cigarette, smoking, Air pollution. (Bagchi K *et al*.,1998). Free radicals, due to their highly reactive nature loss of function or tissue taking place in human body. This dysfunction leads to generation of several diseases Eg: - Aging, Cancer, Atherosclerosis, Parkinsonism……etc. (Jothivel *et al*., 2007 & Bagchi K *et al*., 1998). In the human body there are several mechanisms undergoing to scavenging of free radicals and other reactive oxygen species.

Types of free radicals:-

Enzymes: - These are enzymes which will convert the free radicals into neutral molecules.

Eg: Glutathione Peroxidase (GSH), Superoxide dismutase (SOD) and Catalase (CAT).

$$O2 \xrightarrow{(SOD)} O_2^{-} \xrightarrow{Catalase} H_2O_2+O_2 \longrightarrow H_20$$

These require several essential metals including Selenium, copper, Manganese and Zinc. Nutritional supply of these minerals is inadequate enzymatic defenses against free radicals may be impaired.

Metabolites: - These are the substances produced as byproducts from the different metabolisms of cells. These will neutralize the free radicals by donating an electron. These are also called as antioxidants.

Eg: - Glutathione, Ubiquinol, Uric acid.

Vitamins: - Vitamins also capable of scavenging the free radicals. These will protect us from free radical peroxidation reactions.

E.g.:- Vitamin E (Tocopherol)

Vitamin C (Ascorbic acid). (Bagchi K *et al*.,1998)

Around the world and especially in developing countries about 80% of the people has been using herbal remedies. The products are considered as less toxic, safe, better cultural acceptability, efficacy, potency and less adverse effects. World health organization (WHO) has listed over 21000 plant species used around the world for medicinal purpose. Herbal remedies are largely has been using by India people. Its climate is very favorable to growth of medicinal plants, around 45000 plant species of which 15000 plants are flowering plants having about 1000 species identified as medicinal plants. Due to this reason India is also calling as "Medicinal Garden of The World" Only 40 plant species are currently used by the pharmaceutical companies. **(**Mohamed Bnouham*et al*., 2006; Shipra Guptha et al2007).

There are several treatments have been available for alternative therapy of diabetes. These have ability to decrease the hyperglycemic condition, but not identified the exact mechanism of action. Antioxidants relatively have potential to inhibit the oxidation chain reactions at even small concentrations also. Several plant antioxidants are experimentally proved and used as effective protective agents against oxidative stress. It has been recognized that the anti hyperglycemic effect of these plants either may be due to stimulation of pancreatic β cells or the facilitation of metabolite in insulin dependent process or inhibit the intestinal absorption of glucose. (Sangeethadevi et al,. 2021 & Akanksha *et al.,* 2009).

MATERIALS AND METHODS

Plant Collection

The plant were collected in Vaikaalmedu, authenticated in the department of Pharmacognosy, Nandha College of Pharmacy. The collected plant material was washed in running water, shade dried and pulverished to get powdered drug. The material was subjected to further experimental purposes.

Experimental Animals

Wistar rats were purchased from The Animal House, Department of Pharmacology, IRT Perundurai Medical College, Erode, Tamilnadu. Animals were housed in the Animal house, Nandha College of pharmacy and Research Institute, Erode, Tamilnadu. The animals were placed randomly in polypropylene cages with paddy husk as bedding and housed at standard condition maintained a temperature of 24±2^{0}C and relative humidity of 30-70%. A 12 hours light and 12 hours dark cycle were strictly followed. The animals had a free access to standard animal pellet diet (Sukumar Agro Industries, Pune) and water. A research proposal were submitted according to the guidelines of CPCSEA and approved by Institutional Animal Ethical Committee (IAEC) of Nandha College of Pharmacy and research Institute, Erode-52.

Drugs and Chemicals

Alloxan monohydrate (Sigma chemicals, Bangalore), Formalin solution (Nice chemicals, Cochin), Carboxy methyl cellulose, Diethyl ether (Nice chemicals, Cochin), Normal saline. Polyherbal formulation, Glibenclamide 5mg/kg

Herbal Formulations

The composition and ratio of herbal ingredients are selected according to the potency of anti diabetic activity which was stated in previous references. Powder form of Polyherbal formulation. Decoction of Polyherbal formulation. Crude extract of Polyherbal formulation.

Table No-1. Composition of Polyherbal Formulation

S.NO	PLANT NAME	PERCENTAGE %
1	*Aegle marmelos*	2.66%
2	*Annona squamosa*	9.32%
3	*Bougain villia*	2.66%
4	*Cassia auriculata*	6.66%
5	*Emblica officinale*	8.00%
6	*Ficus carica*	13.30%
7	*Hibiscus rosa sinensis*	6.66%
8	*Psidium guajava*	6.66%
9	*Stevia rebaudiana*	9.32%
10	*Tea leaves*	2.66%
11	*Tenospora cardifolia*	10.66%
12	*Terminalia chebula*	8.00%
13	*Zingiber officinale*	13.30%

a. Preparation of Powder of Polyherbal Formulation (PPHF)

The coarsely powdered plant materials were sieved through sieve no. 60. It helps to get uniform sized powder and according to the weight of individual powder, which are present in table no. 1 has taken in to a beaker and mixed well by using suitable blender to get homogenous powder formulation. Packed in suitable container and kept in dry place.

b. Preparation of Decoction of Polyherbal Formulation (DPHF)

All individual powders were taken in a beaker and mixed well with suitable blender until to get uniform powder formulation. Then packed the formulation in water diffusible paper to make easily dip in the hot water for making of decoction.

c. Crude Extract of Polyherbal Formulation (EPHF)

The Polyherbal formulation is a mixture of all the individual different parts of plant extracts. It contains *Aegle marmelous* (leaves), *Annona squamosa* (leaves), *Bougan villia*(leaves), *Cassia auriculata* (flowers), *Emblica officinale* (fruits), *Ficus carica* (fruits), *Hibiscus rosa sinensis* (flowers), *Psidium guajava*(leaves), *Stevia rhubidiana*(leaves), *camellia sinensis, Tenospora cardifolia* (leaves), *Terminalia chebula* (fruit), *Zingiber officinale* (rhizome). This extraction is carried out by maceration process for 24 hrs. All the thirteen dried and pulverized plant ingredients were taken in individual conical flasks. Then added some

amount of water up to rinse the powder, gentle agitated for first 6 hrs and kept a side for remaining 18 hrs. After 18 hrs filtered them individually, finally evaporated the solvent at 100^{o}C up to get to get paste like preparation.

Then the different weights of the extracts according to the table.1 taken in to a beaker mixed them properly with a little amount of water and evaporated the water up to get a paste like preparation and kept in refrigerator after packed in a suitable container. (Sengottuvelu *et al*., 2008)

SCREENING OF ANTIOXIDANT ACTIVITY OF POLYHERBAL FORMULATION

Fresh healthy Wistar rats of either sex weighing 150-200gm were used and acclimatized in laboratory condition for seven days before starting the experiment. The animals were kept in polypropylene cages, provided with standard animal pellet diet and water ad libitum.

The animals were divided In to nine groups with six of each.
Group I - Normal animals treated with vehicle (0.5%w/v CMC).
Group II - Diabetic animals treated with Alloxan150mg/kg.
Group III - Diabetic animals treated with Glibenclamide 5mg/kg/day P.O).
Group IV - Diabetic animals treated with EPHF, 200mg/kg P.O.
Group V - Diabetic animals treated with EPHF, 400 mg/kg. P.o.
Group VI - Diabetic animals treated with DPHF, 200mg/kg. P.O.
Group VII - Diabetic animals treated with DPHF,400mg/kg.P.O.
Group VIII - Diabetic animals treated with PPHF, 200mg/kg. P.O.
Group IX - Diabetic animals treated with PPHF,400mg/kg.P.O.

Group I and group II animals were daily administered with 2ml of 0.5%w/v of Corboxy Methyl Cellulose suspension P.O, for eight days. All the other animals, group III, IV, V, VI,VII, VIII and IX animals were daily administered with their respective doses P.O., for eight days. On the seventh day all animals, except normal control group were received with equal mixture of carbon tetra chloride and Olive oil (50% w/v) 5ml/kg I.

After seven day's drug administration, all animals were sacrificed on eighth day by euthanasia; the livers were immediately dissected out and washed in ice cold saline. The livers were homogenized with 10ml of 0.1M Triss-HCL buffer using Tissue homogenizer (Remi Industries, Mumbai). The homogenate was centrifuged by using cooling centrifuge (model C24- BL, Remi Industries, Mumbai) and the supernatant was used for determination of antioxidant activity. The hepatic antioxidant enzyme levels of Polyherbal formulation treated animals were compared with that of Toxic control animals.

A. Super oxide dismutase (SOD)Assay:-

The activity of Super Oxide Dismutase was assayed by the method of Kakker et al (1984). In a test tube 0.5ml of supernatant of centrifuged tissue homogenate was taken to the 1.5 ml of carbonate buffer (P_H – 10.2), 0.5 ml 0f EDTA and 0.4µl of Epinephrine was added just before taking OD (Dallak et.,al 2009).

B. Catalase (CAT) assay:-

The Catalase activity was assayed by the method of Simha (1972). The incubation contained in a final volume of 2ml, 0.1ml of diluted homogenate, 1ml of Phosphate buffer and 0.4ml of distilled to which 0.5 ml of Hydrogen peroxide solution was added to initiate the reaction, while the Hydrogen peroxide solution was left out in control tube. After the incubation for 1min at 37$^{o\,C,}$ the reaction was stopped by addition of 2 ml of Potassium Dichromate-Acetic acid reagent. The sample was kept in

boiling water bath for 15 minutes, finally cooled and absorbance was measured at 570 nm against control (Dallak et.,al 2009).

C. Reduced glutathione (GSH) assay :

The method was based on the reaction of reduced glutathione with Di Thio Nitro benzoic Acid (DTNB) to give a compound that absorbs at 412nm.Briefly after centrifugation; 0.5 ml of supernatant was taken and mixed with 2.0 ml of 0.3 mol/L disodium hydrogen phosphate (Na_2HPO4) solution. A 0.2 ml solution of Di Thio Bis Nitro Benzoate (0.4 mg/ml, 1% sodium citrate) was added and the absorbance was measured immediately after mixing. Results were expressed in µmol/GSH/min/mg Protein.

STATISTICAL ANALYSIS

The collected data was subjected to appropriate statistical tests including one way ANOVA (Analysis of Variance), followed by an appropriate Dunnett's post hoc test. P values of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

RESULTS

Effect of Polyherbal formulations in anti oxidant study.

The effect of Polyherbal formulations (EPHF, DPHF, and PPHF) on hepatic antioxidant enzymes were given in table. No-6. The low dose (200mg/kg) of EPHF produced significant increase in hepatic enzymes SOD, CAT and less significant increase in GSH and high dose produced all significant increase in hepatic anti oxidant enzymes. The DPHF at lower dose produced less significant effect in at CAT and SOD but high dose significantly increases them. As well as high dose of PPHF only less significantly increases the hepatic anti oxidant enzymes**.**

Table 2. The effect of Polyherbal formulation on liver anti oxidant enzymes in Alloxan intoxicated rats.

No of Groups	Treatment	SOD (units/minutes/mg protein)	CAT N mol H_2O_2 (consumed/min/mg protein)	GSH (mg/dl)
I	CMC (0.5%)	14.8 ± 0.45	8.9 ± 0.3	21.9 ± 0.4
II	Alloxan treated 120mg/kg	08.4 ± 0.20 **	4.4 ± 0.15 **	16.5 ± 0.25 **
III	Glibenclamide 5 mg/kg	12.5 ± 0.25 **	8.2 ± 0.30 **	20.7 ± 0.35 **
IV	Extract of PHF 200mg/kg. P.O	09.4 ± 0.15 **	6.6 ± 0.22 **	18.6 ± 0.22 *
V	Extract of PHF 400mg/kg.P.O	11.8 ± 0.15 **	7.5 ± 0.26 **	21.1 ± 0.25 **
VI	Decoction of PHF 200mg/kg.P.O	08.6 ± 0.30 *	5.7 ± 0.10 *	16.3 ± 0.05

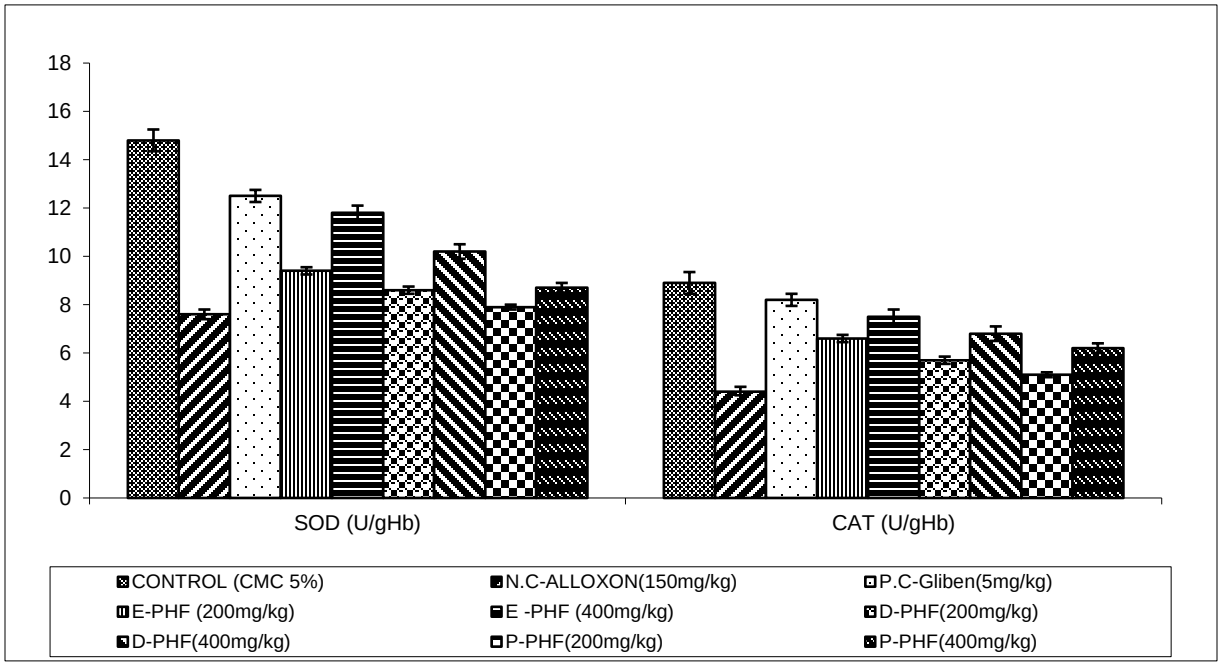
VII	Decoction of PHF 400mg/kg.P.O	10.3 ± 0.10 **	6.8 ± 0.05 **	18.9 ± 0.15 *
VIII	Powder of PHF 200mg/kg.P.O	07.9 ±0.15	5.4 ± 0.21 *	16.2 ± 0.45
IX	Powder of PHF 400mg/kg.P.O	08.1 ± 0.10 *	6.2 ± 0.15 *	18.4 ± 0.20 *

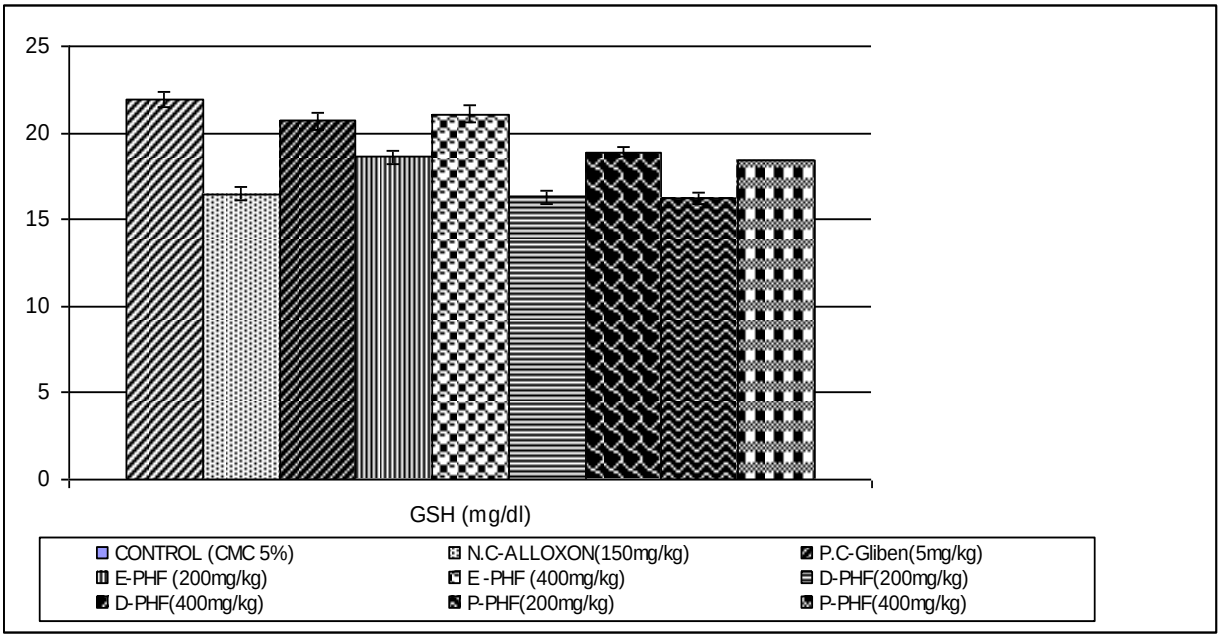
DISCUSSION

Alloxan induces a wide variety of animal species by damaging the insulin secreting pancreatic b-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues **(**Singh, *et al*., 2003). β cells destruction is happens by the free radicals which are generated in Fenton reaction of alloxan metabolism. Formation of Hydroxyl radicals ($\cdot OH^-$) from the Hydrogen peroxide H_2O_2 is called Fenton. Some of free radicals actively destroy β cells of pancreas, it leads to diabetes. (Umamahaswari et.,al 2010)

The enzymatic antioxidant system CAT (Catalase) and SOD (Super Oxide Dismutase) counteract the free radicals generation and reduce the oxidative stress. The anti oxidant activity of Polyherbal formulation is found with a significant growth of CAT and SOD levels and significant decrease in LPO and compared with normal control and diabetic control animals.(Chandra Shekhar Joshi et,.al 2007) In alloxan treated animals the levels of SOD and CAT were significantly reduced. After treatment with Polyherbal formulations the levels of SOD and CAT activities brought near to normal. (Sabu .MC *et al*., (2009).

Figure 1. Effect of polyherbal formulations on hepatic antioxidant enzymes, SOD, CAT and GSH in alloxon treated rats.





CONCLUSION

On the basis of above results it could be concluded that extract of Polyherbal formulation exerts significant anti oxidant activities. Decoction of Polyherbal formulation having less significance of anti oxidant activity compared with that of extract formulation. Powder formulation having less potency compared to decoction and extract Polyherbal formulation.

Further investigations in-depth has to be carried to find out the exact mechanism present behind the aqueous extract's of Polyherbal formulations and also the exact active ingredient responsible for the anti oxidant activities.

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