

Screening of Antioxidant & Antidiabetic Potential of Polyphenol rich fraction from *Cichorium intybus*

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ABSTRACT

Introduction: *Chicory* (*Cichoriumintybus*) commonly known as kasni has health benefits such as improving liver health, digestion, boosting immune system possibly because of its water soluble dietary fibres and polyphenols. A diet rich in polyphenols and fibers helps in lowering cholesterol leading to less plaque formation, preventing blockage and risk of stroke. Owing to the fact that these polyphenols may reduce the elevated glucose the present research work was undertaken to extract the polyphenols from *Chicory* roots and evaluate their antioxidant and hypoglycemic potential. **Materials and Methods:** Polyphenol rich fraction of root extract was subjected to estimation of total phenolic estimation and free radical scavenging activity using various antioxidant assays. The antidiabetic potential of the polyphenol rich fraction was also estimated using Alloxan Induced Diabetic Rat model. **Results:** The total phenolic content of the polyphenolic fraction was found to be 30.23µg/ml. The IC₅₀ values for DPPH and ABTS assay for the polyphenolic fraction were found to be 60µg/ml and 4.2mg/ml respectively. The TEAC value was found to be 1.178 mM. Administration of polyphenol rich fraction (30 mg/kg, p.o.) decreased elevated SG from 122mg/dl to 78.66mg/dl on 14th day (**p<0.001). Also a significant reduction in triglycerides (**P<0.01), total cholesterol (**P<0.01), serum LDL (**P<0.01) and serum alkaline phosphatase (**P<0.01) was observed at 30 mg/kg, p.o. However, serum HDL was found to be elevated with no change in serum insulin (*P<0.05). **Conclusion:** It can be concluded that the polyphenol rich fraction of Chicory roots possess a strong hypoglycemic potential probably due to their antioxidant activity.

Keywords: *Cichoriumintybus*, polyphenols, antioxidant, antidiabetic, total cholesterol.

INTRODUCTION

Diabetes is one of the most common non-communicable diseases found globally (2.8%) and is the fourth leading cause of death in most developed countries. Almost 3.2 million people die of diabetes across the world every year^[1]. World Health Organization (WHO) has estimated approximately 160,000 diabetics worldwide which would double in the year 2025^[2]. Ageing, high caloric rich diet, obesity and stressful life style have further severed diabetes patients^[3,4]. The modern treatment includes administration of insulin or oral hypoglycemic agents like sulphonylureas, biguanides etc. However these oral

hypoglycemic agents are found to be less effective in insulin-dependent diabetes and their side effects such as allergic skin reactions (photosensitivity), gastrointestinal disturbances, blood dyscrasis, hepatic dysfunction and hypoglycemia etc further limit their use^[5]. Thus despite considerable progress in the treatment of diabetes in modern and traditional systems of medicine search for newer drugs continues^[6-8].

The present research work is one such attempt to investigate the antidiabetic potential of Chicory, a leafy vegetable with a broad therapeutic potential. Hydroalcoholic seeds extract of the plant acts as hepatoprotectant. Its root extracts is found to lower liver lipids, triglycerides and cholesterol content. Chicory is reported to contain more than 10% of total polyphenols, the most dominant being dicaffeoylquinic acids (71% of total polyphenols) with marked antioxidant and anti-hyperlipidemic activity^[9,10].

The plant also contains Chicoric acid which was found to stimulate insulin release from INS-1 E insulin secreting

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cell lines and rats islet of Langerhans as well as glucose uptake^[11].

Two new anthocyanin have been isolated from flowers of *Cichoriumintybus* and identified as delphinidin 3, 5-di-O-(6-O-malonyl_beta glucoside) and delphinidin 3-O_(6-O-malonyl-beta-D-glucoside)-5-O-beta-D-glucoside^[12].

The Leaves contain coumarines, esculetin, cichoriin and sesquiterpene lactones. A new coumaringlucoside ester Cichoriin-6'-*p*-hydroxyphenyl acetate, was isolated from chicory leaves along with cichoriin. New sesquiterpene lactone like cichoridiol, Cichosterol (seco-sterol), Lactucin, Lactucopicrin, 11,13-dihydro-lactuciprin were also reported in roots and leaves of *Cichoriumintybus*^[13,14].

The plant is reported to possess anti-hyperlipidemic, anti-oxidant, Hypoglycemic, anti-inflammatory, Antibacterial-anti-hepatotoxicity^[15-20].

It is now a worldwide accepted truth that diet rich in polyphenols helps in lowering cholesterol levels in the blood leading to less plaque formation, preventing the blockage and hence reducing the risk of hypertension, stroke^[10] Owing to the fact that these polyphenols may also reduce the elevated glucose concentration and since very few research papers pertaining to their hypoglycemic potential are reported, the present research work was undertaken to extract the polyphenols from *Chicory* roots and evaluate their antioxidant and hypoglycemic potential. Various pathophysiological parameters such as lipid profile (HDL, LDL), body weight, serum alkaline phosphatase level were also monitored which are generally elevated with the onset of diabetes.

MATERIAL AND METHODS

Plant material collection, Authentication and extraction

The dried roots of *Cichoriumintybus* family *Asteraceae* was collected from Ayurvedic store and authenticated from Botanical Survey of India, Koregaon park, Pune (BSI/WRC/Tech/2010/1008).

Air dried roots of *Cichoriumintybus* were coarsely pulverized and passed through 120 sieves to remove fines. The coarse root powder was exhaustively extracted with ethanol, filtered and the filtrate was concentrated in a rotary evaporator. The ethanolic extract was redissolved in

water, filtered and the filtrate was concentrated to get a polyphenol rich aqueous fraction^[10,11].

Determination of Total Phenolic Content

A 100µl of extract was mixed with 0.5ml FC reagent (diluted 10 times with distilled water). The solution was mixed with 7ml of distilled water and allowed to stand at room temperature for 5 minutes. 1.5ml sodium bicarbonate (60mg/ml) solution was added to the mixture and left at room temperature in dark for 2 hours. Absorbance was read at 725nm against blank using UV-Visible spectrophotometer (Perkin Elmer Lambda 35, USA). A calibration curve was prepared, using a standard solution of gallic acid (0.2, 0.4, 0.6, 0.8 and 1mg/ml). Results were expressed as gallic acid equivalents (GAE) mg /ml^[17].

In - Vitro Antioxidant Activity

1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

A solution of 3.3mg DPPH in 100ml methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02 - 0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at 25°C for 30 min. The absorbance of the mixture was measured at 517 nm. BHT was used as reference. The ability to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{A_{(\text{control})} - A_{(\text{test})}}{A_{(\text{control})}} \times 100$$

where $A_{(\text{control})}$ is the absorbance of DPPH radical + methanol; $A_{(\text{test})}$ is the absorbance of DPPH radical + test sample.

IC₅₀ value i.e. the half maximal (50%) inhibitory concentration was calculated using the % Inhibition values graphically^[18].

2. ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution

was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS⁺ scavenging capacity of the extract was compared with that of BHT and percentage inhibition was calculated using the same equation as that for DPPH method^[19].

3. FRAP (Ferric Reducing Antioxidant Potential) Assay:

The stock solutions included 300 mM acetate buffer (3.1 g sodium acetate and 16 ml acetic acid), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM FeCl₃ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl₃. The temperature of the solution was raised to 37°C before using. Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM Trolox. The FRAP scavenging capacity of the extract was compared with that of BHT and the percentage inhibition was calculated using the same equation as that for DPPH method^[19].

Biological Screening

Healthy adult Wistar albino rats aging between 2 and 3 months and weighing 150–200 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25±30°C, 35–60% relative humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Acute Oral toxicity

Acute toxicity test was performed according to the OECD guideline for testing of chemicals 425 (OECD 2001) on healthy Wistar albino rats of either sex. They were divided into two groups, of five rats each and were orally fed with the polyphenol rich fraction in increasing dose levels of body weight as follows 100, 500, 1000, 3000 and 5000 mg/kg. The animals were observed continuously for 24 h for their behavioral and Psychological response^[20].

After a period of 24 h, 72 h and 14 days they were observed for any lethality or death. Approval number: (ACP/2551(G)/2009)

Oral Glucose Tolerance Test

The rats were fasted for 18 hours and later fed with distilled water. Rats were divided into four groups as follows:

Group I- Glucose (2.5 g/kg, p.o.)

Group II- Glibenclamide (0.4 mg/kg)

Group III- polyphenol rich fraction (15 mg/kg)

Group IV- polyphenol rich fraction (30 mg/kg)

All the groups were glucose loaded (2.5 g/kg, p.o.), 30 minute after test and standard drug administration. Blood samples were withdrawn from all animals at 0, 30 and 60 minutes after glucose loading and glucose levels were estimated using Glucose oxidase–peroxidase reactive strips and a Glucometer (Arkay Piramel Medical Pvt. Ltd. INDIA)^[19,20].

Evaluation of Serum glucose in Alloxan induced Diabetic rats

The acclimatized animals were fasted for 24 hours after which they were administered with water ad libitum and injected subcutaneously with 120 mg/kg b.w. of alloxan monohydrate freshly prepared in 0.9% normal saline solution. After 72 hrs blood samples was withdrawn by retro orbital puncture and serum glucose (SG) was estimated by enzymatic colorimetric method. The animals which showed SG more than 200 mg/dl were selected for study.

Group I: Normal Control (Saline solution, 10 ml/kg, p.o.)

Group II: Glibenclamide (0.4 mg/kg)

Group III: polyphenol rich root extract (15 mg/kg)

Group IV: polyphenol rich root extract (30 mg/kg)

The drug solutions were prepared and administered orally according to the body weight of the animals. Blood samples were withdrawn from all animals at baseline, 7th and 14th day by retro orbital puncture method and were subjected to estimation of various biochemical parameters using enzymatic colorimetric method. The effects of test and standard drugs in normal and diabetic rats were observed by evaluating fasting blood glucose levels using the BIO LAB Diagnostics Kit (505nm) on CHEM-7 semi auto-analyzer along with measure of serum insulin at 7th and 14th day of the study^[21].

Analysis of Biochemical Parameters

The biochemical parameters associated with the diabetic disease were determined on 14th day of treatment on CHEM-7 semi auto-analyzer at specific wavelengths (λ)^[22, 23].

- i. Serum Triglycerides: ERBADiagnostic kit (λ : 505 nm)
- ii. Serum Total Cholesterol: ERBADiagnostic kit (λ : 546 nm)
- iii. Serum HDL: ERBADiagnostic kit (λ : 600 nm)
- iv. Serum LDL: ERBADiagnostic kit (λ : 600 nm)
- v. Serum Alkaline Phosphatase: MBK SPAM Diagnostics Kit(λ : 510 nm)
- vi. Serum Insulin: ELISA reader

Statistical analysis

Data were statistically evaluated using one-way ANOVA, followed by Dunnett test. The values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The Total Phenolic content of *Cichoriumintybus* root extract was found to be 30.23 $\mu\text{g/ml}$

2. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay:

The 50% Inhibition of DPPH radical for standard antioxidant was found at the concentration of 60 $\mu\text{g/ml}$ (IC_{50} 60 $\mu\text{g/ml}$). The polyphenol rich fraction showed a

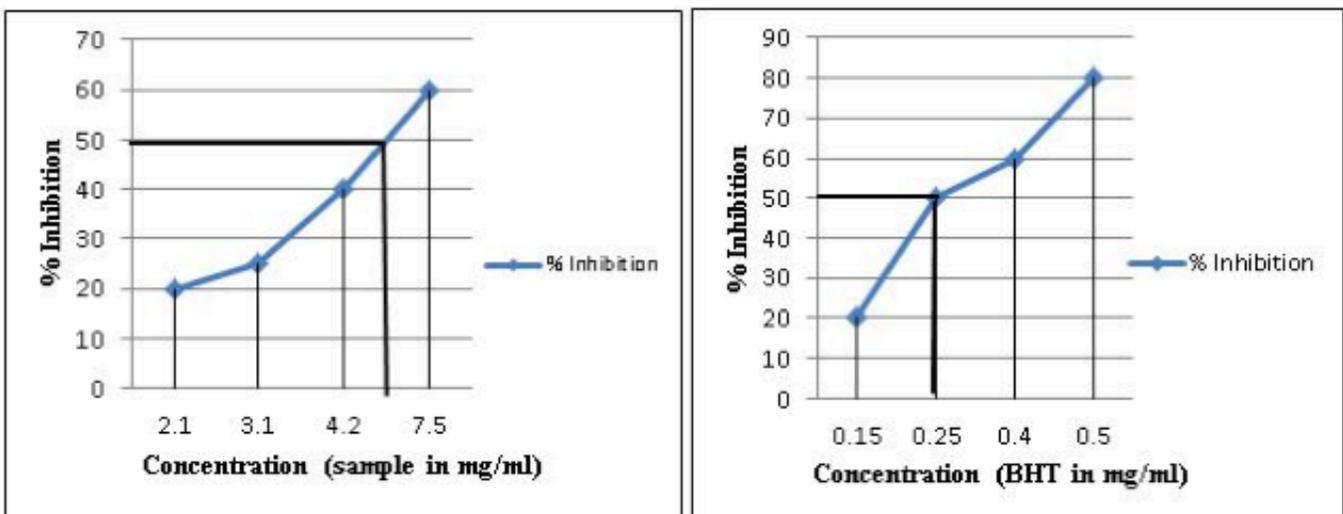


Figure 1: IC_{50} values for DPPH Assay

potential antioxidant capacity with This was comparatively similar to the standard antioxidant compound BHT which showed IC_{50} value of 60 $\mu\text{g/ml}$. (Table 1).

3. ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity:

The 50% inhibition of the reactive oxygen species was found for the isolated polyphenols at 4.5 $\mu\text{g/ml}$ ($***P < 0.001$) concentration. The 50% inhibition of standard antioxidant compound BHT was found at the concentration of 0.25mg/ml ($***P < 0.001$).

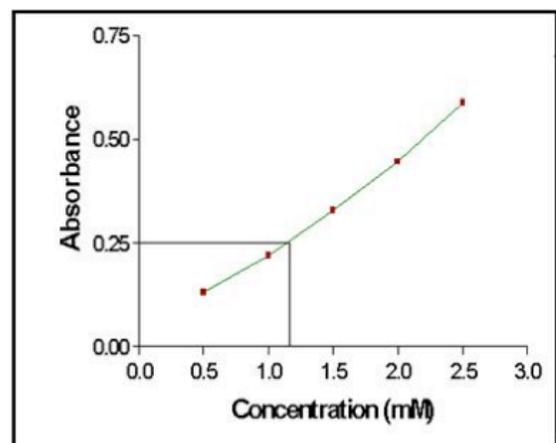


Figure 2: IC_{50} values for ABTS Assay

Table 1: IC₅₀ value for Antioxidant Assays

Sr. No.	Sample	IC ₅₀ value for DPPH Assay	IC ₅₀ value for ABTS Assay
1	Test	60µg/ml	4.2mg/ml
2	Standard (BHT)	50 µg/ml	0.25mg/ml

3. FRAP Antioxidant Assay:

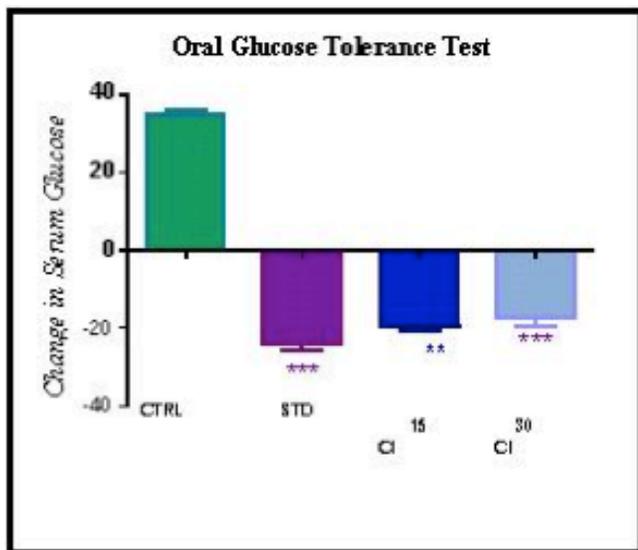


Figure 3: Trolox Equivalent curve

The TEAC value was found to be 1.178 mM for the polyphenol rich fraction of the root extract of *Cichoriumintybus*.

Biological Screening

Acute Oral Toxicity

The LD₅₀ value was found to be greater than 5000 mg/kg with no mortality.

Oral Glucose Tolerance Test

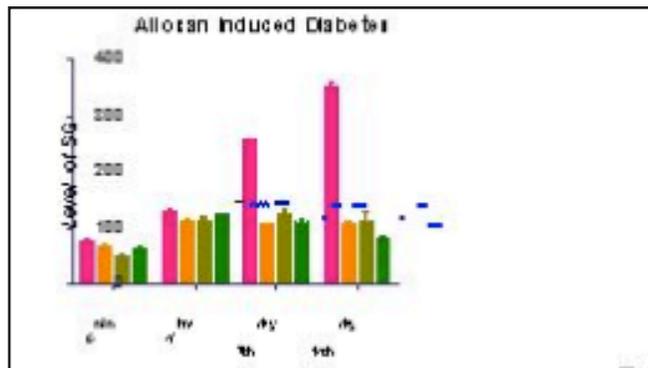


Figure 4: Effect of Drug treatment on glucose loaded rats after 90 min

In the present study glucose (2.5 g/kg) was administered to rats after pretreatment with polyphenol rich fraction. Administration of 15 mg/kg, p.o. dose caused change in SG by 19.33, 16 and 9.5 at 30, 60 and 90 minutes respectively. Peak reduction was observed at 90 minutes. Administration of 30 mg/kg of polyphenol rich fraction caused a significant change in SG by 24, 13.5 and 7 at 30, 60 and 90 minutes respectively. Peak reduction was observed at 90 minutes.

Alloxan Induced Diabetes in Rats

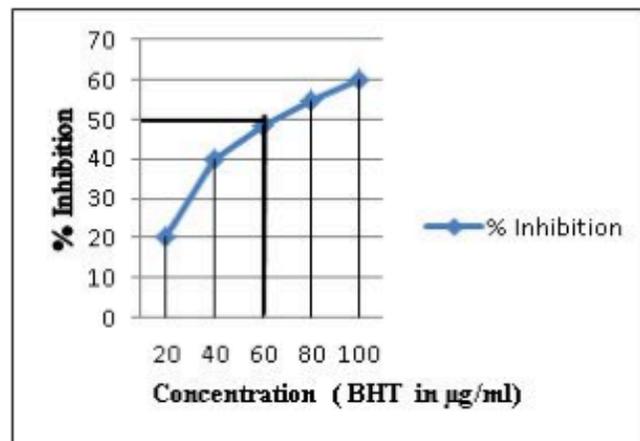
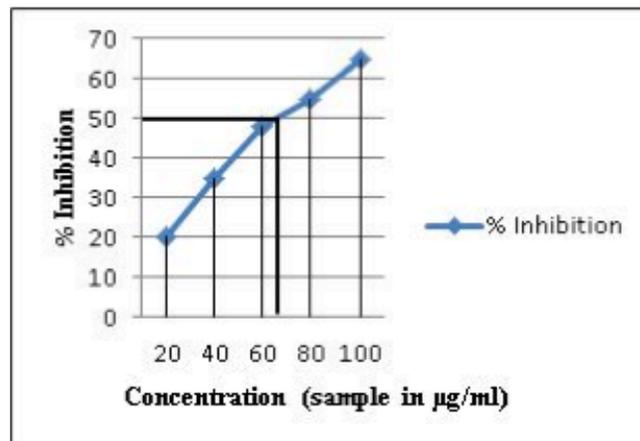


Figure 5: Effect of Serum Glucose (SG) at different time interval

Administration of standard Metformin (150mg/kg, p.o) resulted in decrease in elevated SG 112mg/dl to 103mg/dl on 7th day (**p<0.001) and 112mg/dl to 104.66mg/dl on 14th day (**p<0.01). Administration of

polyphenol rich fraction of *Cichoriumintybus* (CI) (15 mg/kg, p.o.) resulted in increase in elevated SG 111mg/dl to 124.66mg/dl on 7th day (**p<0.01) and decrease in elevated SG 111mg/dl to 108.33mg/dl on 14th day (**p<0.01).

Administration of polyphenol rich fraction of *Cichoriumintybus* (CI) (30 mg/kg, p.o.) resulted in decrease in elevated SG 122mg/dl to 109.66mg/dl on 7th day (**p<0.001) and 122mg/d to 78.66mg/dl on 14th day (**p<0.001).

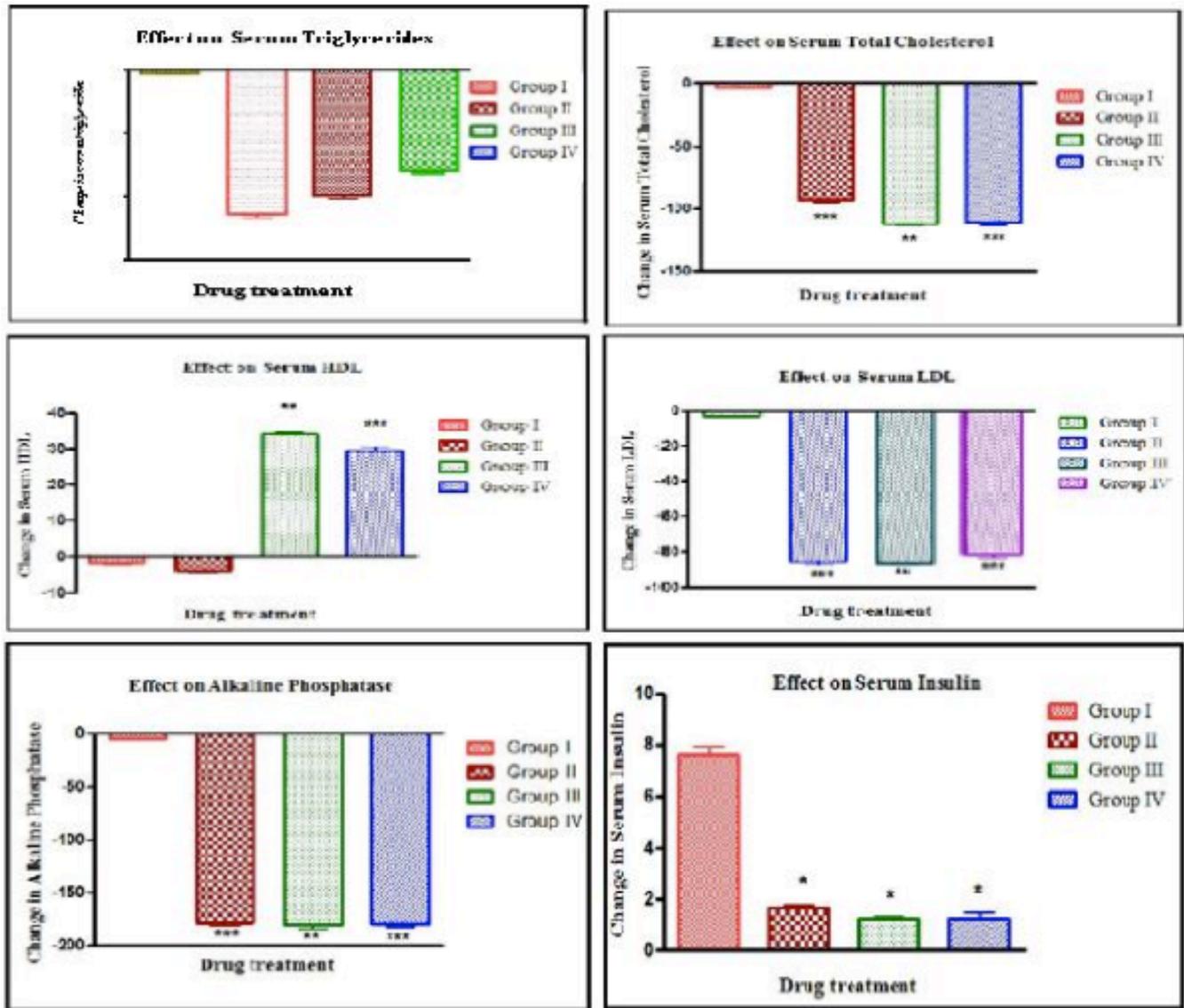


Figure 6: Effect of Polyphenol rich fraction and standard drug on various biochemical parameters

Chronic heart disease in diabetic patients is associated with increased plasma cholesterol levels, reduced HDL in non insulin dependent diabetic patients and the most common lipid abnormality i.e. raised triglyceride levels. The efficacy of conventional anti-diabetic drugs on lipid metabolism has been reported to decrease over a period of time. When treated with *Cichorium intybus*-polyphenol rich fraction (30mg/kg), there was a significant reduction in serum triglycerides (**P<0.01),

total cholesterol concentrations (**P<0.01) (Figure 6) and significant increase in serum HDL level in diabetic rats (**P<0.01) respectively as compared to untreated group. (Figure 6) 30mg/kg dose also caused significant decrease (**P<0.01) in Serum LDL and alkaline phosphatase level. (Figure 6) However, there was no significant change in serum insulin of all drug treated animals (**P<0.05). (Figure 6) While the serum triglyceride and cholesterol levels decreased significantly

in drug treated diabetic rats, the HDL cholesterol level was found to be improved. Thus the weight loss associated with drug treated diabetic rats may be attributed directly to its lipid lowering activity or indirectly to its influence on various lipid regulation systems. The comparable effect of *Cichoriumintybus* polyphenol rich fraction (30mg/kg) with Glibenclamide may suggest similar mode of action since it was able to lower blood sugar level in alloxanised rats where the pancreatic β -cells were permanently damaged indicating that the polyphenol rich fraction possesses extra pancreatic effects.

CONCLUSION

Thus it can be concluded that the polyphenol rich fraction of *Cichoriumintybus* has a promising hypoglycemic potential which might be accounted for its abundant water soluble polyphenol content which are proven to scavenge the harmful free radicals. However the confirmation can be drawn from the isolation and structural elucidation of the polyphenols from the polyphenol rich fraction and its screening in the diabetic human population to elucidate its mechanism of action.

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REFERENCES

1. Etuk, E.U. Animals models for studying diabetes mellitus. *Agric. Biol. J. N. Am.* 2010; 1(2): 130–4.
2. Beretta A. Campanha de prevencao e diagnostic do diabetes realizadapela prefeitura municipal nacidade de Araras. *Laes and Haes* . 2001; 22(131): 188–200.
3. Macedo C.S, Capelletti S.M, Mercadante M.C.S, Padovani CR, Spadella CT. Experimental model of induction of diabetes mellitus in rats. Plastic surgery, laboratory of plastic surgery, Sao Paulo –Paulista School of Medicine; 2005; 2–5.
4. Simpson, J. E, Shaw J. E. and Zimmet P. Z. The prevention of type 2 diabetes-lifestyle change or pharmacotherapy? A challenge for the 21st century. *Diab.Res. Clin. Pract.* 2003; 59: 165–80.
5. Zimmet P, Alberti KG, and Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* 2001; 4,14: 782–7.
6. Resmi CR, Fathima A. Antidiabetic effect of a herbal drug in alloxan-diabetic rat. *Indian drugs.* 2001; 38(6): 319.
7. Satyanarayan K, Mangathayary V, Sreekanth J, Kokate CK. Studies on hypoglycemic and cardiotoxic effect of roots of *Cocculus hirsutus*. *Indian Journal of Pharmaceutical Sciences.* 2001; 1763(1): 30–5.
8. Barik R, Jain S, Qwatra D, Joshi A, Tripathi G.S, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin–nicotinamide induced type II diabetes in rats. *Indian Journal of Pharmacology;* 2008; 19–22.
9. Juskiewicz J, Zdunczyk Z, Zary-Sikorska E, Krol B, Milala J, Jurgonski A. Effect of the Dietary polyphenolic fraction of Chicory root, peel, seed and leaf extracts on Caecal fermentation and blood parameters in rats fed diets containing prebiotic fructans. *Br J Nutr, Mar.* 2011; 105(5): 710–20.
10. Milala J, Grzelak K, Krol B, Juskiewicz J, Zdunczyk Z. Composition and Properties of Chicory extracts rich in Fructans and Polyphenols. *Pol. J. Food Nutr.* 2009; Sci. Vol. 59, No. 1: 35–43.
11. Carazzone C, Mascherpa D, Gazzani G, and Papetti A. "Identification of phenolic constituents in red chicory salads *Cichoriumintybus* by high-performance liquid chromatography with diode array detection and electrospray ionisation tandem mass spectrometry. *Food Chemistry.* 2013; Vol. 138: 1062–71.
12. Judzentiene A and Budiene J. "Volatile constituents from aerial parts and roots of *Cichoriumintybus* L. (chicory) grown in Lithuania. *Chemija.* 2008; Vol. 19: 25–8.
13. Mukherjee P.K. *Quality Control of Herbal Drugs.* 1st edition; 2002; 133, 176, 177, 189, 193, 380, 384, 492). 12. Kokate.
14. Wagner H, Bladt S. *Plant Drug Analysis; Thin Layer Chromatography,* Springer. 2nd edition; 1996; 196–7, 353.
15. Harborne J.B. *Phytochemical Methods.* Chapman and Hall, London; 1998; 60.
16. Olayinka A, Aiyegoro Anthony I, Okoh. *Phytochemical Screening and Polyphenolic Antioxidant Activity of Aqueous Crude Leaf Extract of Helichrysum pedunculatum.* *Int. J.Mol. Sci;* 2009.
17. Feng Xu, Linling Li, Huang X, Cheng H, Wang Y, Cheng S. Antioxidant and antibacterial properties of the leaves and stems of *Premna microphylla*. *Journal of Medicinal Plants Research.* 2010; Vol. 4, Issue-23: 2544–50.
18. Džilani A, Toudert N, Džilani S. Evaluation of the Hypoglycemic Effect and Antioxidant Activity of Methanol Extract of *Ampelodesma mauritanica* Roots. *Life Sciences and Medicine Research;* 2011; Vol. 31.
19. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC.* 2008.
20. Barik R, Jain S, Qwatra D, Joshi A, Tripathi G.S, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin–nicotinamide induced type II diabetes in rats. *Indian Journal of Pharmacology.* 2008; 40: 19–22.
21. Chude MA, Orisakwe OE, Afonne OJ, Gamaniel KS, Obi E, Vongtall OH. Hypoglycemic effect of the aqueous extract of *Boerhariadiffusa* leaves. *Indian Journal of Pharmacology.* 2001; 33: 215–6.
22. Jain S, Bhatia G, Barika R, Kumar P, Jain A, Dixit V.K. Antidiabetic activity of *Paspalum scrobiculatum* Linn. in alloxan induced diabetic rats. *Journal of Ethno pharmacology.* 2010; 127: 325–8.
23. Lenzen S, Panten U. Alloxan: history and mechanism of action. *Diabetologia.* 1988; 31: 337–42.