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ORIGINAL RESEARCH ARTICLE

Evaluation of Antioxidant and Toxicological properties of Chicory leaves.

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ABSTRACT

Antioxidant potential of various solvent extracts of Chicory (*Cichorium intybus* L) leaf was evaluated. Among the extracts, the aqueous extract showed maximum radical scavenging activity by DPPH method (87.45%) as well as total polyphenolic content (51.69 mg/g extract). The aqueous extract was further evaluated for its *in vitro* antioxidant potency toward reducing power, superoxide scavenging activity, metal chelating property and hydroxyl radical scavenging ability. Protection against radical induced protein oxidation (BSA) and DNA damage (by pBR322 plasmid DNA nicking assay and SCGE assay) were also evaluated. The results indicates that the aqueous extract of chicory posses a marked radical scavenging properties and offered significant protection against protein oxidation and DNA damage, which could be attributed to the presence of phenolic compounds. As for the safety is concerned; the extract did not cause any toxic symptoms at acute and sub chronic toxicity levels used in the study. Moreover, it also showed to be free of any cytotoxicity towards rat's lymphocytes. Therefore, Chicory leaves can be considered as a source of natural antioxidants for pharmaceutical or dietary needs.

Key Words: Chicory leaves; Antioxidant activity; Polyhenols, Protein oxidation, DNA damage; Toxicity

INTRODUCTION

Oxidative stress plays an important causative role in initiation and progression of several diseases like cancer, cardiovascular diseases, inflammation, aging etc through excessive formation /or incomplete removal of highly reactive oxygen species (ROS). Biomolecules such as carbohydrates, proteins, DNAs, lipids undergo oxidative damage by the deleterious effect of oxygen radicals.[1-2] These destructive effects are prevented by several natural antioxidant defense mechanisms such as antioxidant enzymes (Superoxide dismutase, catalase, glutathione peroxidase) and biomolecules (glutathione, vitamins E and C). Under pathological conditions, the efficiency of the antioxidant defense mechanism is altered and over production of radicals cause imbalance among pro and antioxidants and former takes over the later leads to tissue damage.[3-4] Epidemiological studies have suggested associations between the consumption of polyphenol-rich foods or beverages and prevention of diseases. [5] There is a great deal of interest in edible plants rich in antioxidants and their health-promoting properties.

Chicory (*Cichorium intybus* L) is a member of *Asteraceae* family. It is an erect, glandular, biennial herb with a tuberous taproot and rosette of 30-70 leaves. The stem grows up to 90 cm height. It is grown as a leaf vegetable or salad green in Europe and as a fructose crop in many parts of the world. Roots are often used as a coffee substitute or supplement, particularly in India and South Africa, where more than 90% of all the coffee consumed contains Chicory.[6] The plant is being used traditionally to cure various ailments in Ayurvedic and Unani systems and found to have enormous application in food industry as well. The leaves are used for easing skin inflammations and swellings. The whole plant extracts was reported to have anti-diabetic, [7] antioxidant, [8-9] antibacterial, [10] immunotoxic, [11] antihepatotoxic, [12-13] and Cardioprotective properties. [14] Insulin, fructooligosaccharides, polyphenols such as chlorogenic acid, caffeic acid derivatives are the main active compounds present in it. [15] Though there is a regular treatment schedule for various ailments involving leaves, no detailed studies have been reported regarding antioxidant potency and its safety aspects so far.

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Therefore, the purpose of this study was to evaluate the antioxidant properties, as well as the toxicity if any, of Chicory leaves.

MATERIALS AND METHODS

Folin-Ciocalteu reagent (FCR), Na_2CO_3, Ferrozine, NBT, PMS, and NADH were purchased from Sisco Research Laboratory (SRL, Mumbai). Gallic acid, $FeCl_2$, BHT, Ferric cyanide and EDTA were procured from E-Merck, Mumbai, India. DPPH, 2-deoxy-D-ribose and HiSep^{TM} LSM 1077 media were purchased from Hi-Media. pBR322 plasmid was purchased from Genei, Bangalore. BSA, Agarose, and ethidium bromide were purchased from Sigma-Aldrich (St.Louis.MO). All other reagents were of analytical grade. For toxicity studies, kits from Agappe (Agappe Diagnostics Ltd, Kerala) were used for estimating cholesterol, creatinine, urea, SGOT, SGPT, ALP and kit from Erba (Transasia Bio-Medicals Ltd, India) was used for glucose estimation.

Plant material and Extraction:

The chicory leaves (cultivar is unknown) procured from local market were used in the present study. The proximate composition of the leaves was estimated using the standard Association of Official Analytical Chemists' methods.[16] 100g dried leaf powder was used for the sequential extraction using different solvent systems with the increasing polarity namely hexane, chloroform, ethyl acetate, acetone and methanol which were used (1:10 ratio) with the aid on an orbital shaker for 12 hours. Water extract was prepared by extraction in boiling water for15 minutes. Extracts were filtered and then dried by flash evaporation/ lyophilization as per the requirements. Finally, the crude extracts were stored in a deepfreezer (-20^{O}C) until further use.

Antioxidant assays:

Total phenolic content of the extracts were determined using Folin-Ciocalteu method [17] and the results were expressed in terms of gallic acid equivalents (mg gallic acid/g extract). Antioxidant activity of the extracts, based on the DPPH radical scavenging activity, was determined as described by Braca *et al.*,[18] Metal chelating activity by Dinis *et al.*, [19] the reducing power by Oyaizu, [20] Hydroxyl radical scavenging assay by Halliwell *et al.*, [21] and superoxide anion scavenging activity by Liu *et al.*, [22] were carried out in aqueous extract.

Determination of protein oxidation:

Protein oxidation was assayed as described previously [23] with minor modifications. Oxidation of BSA (5 µg) in phosphate buffer was initiated by 20mM AAPH and inhibited by various concentrations of aqueous extracts (50-250 µg/ml). After incubation for 2 hours at 37^{o}C, 0.02% BHT was added to prevent the formation of further peroxyl radical. The samples were then analysed with normal SDS-PAGE.

DNA damage protection assay:

a) Determination of oxidative DNA strand breakage

Conversion of the supercoiled form of plasmid DNA to open circular and further linear form has been used as an index of DNA damage. DNA strand breakage assay was performed using pBR322 plasmid DNA as per the method described by Lee *et al.* [24] A mixture of 10µl of extract of different concentration (50-200µg/ml) and plasmid DNA (0.5µg) was incubated at room temperature followed by the addition of 10mM AAPH. The final volume of the mixture was made up to 20µl and incubated for 30 min at 37^{o}C. The DNA samples was electrophoresed on 1% agarose gel and band intensities were analyzed using Easy win 32 software from Herolab (Germany).

b) Single cell Gel electrophoresis (SCGE) assay

The standard alkaline SCGE, or Comet assay was performed according to the method developed by Sing *et al.* [25] Lymphocyte cells, resuspended at 1×10^6 cells/ml of phosphate buffered saline, were treated with H_2O_2 (20µM) for 30 minutes at 37^{o}C and used for SCGE assay. After electrophoresis was completed, slides were washed and stained with ethidium bromide (20µg/ml). Comets were observed under image analyzer microscope (Olympus BX51). DNA damage was analyzed for 100 randomly selected cells from each slide using Image-pro Plus software. The degree of DNA damage was expressed by tail moment value i.e. Tail Moment = (Tail length×Tail% DNA/100).

Analysis of aqueous extract by HPLC:

For the analysis of phenolics, a ternary solvent system with increasing hydrophobicity

and changing pH was used. Separation was carried out on a reverse phase C_{18} column (150×4.5mm) and the compounds were monitored with Diode array detector (JASCO HPLC system).The solvents were 50mM ammonium dihydrogen phosphate, pH 2.6 (Solvent A), 0.2 mM orthophosphoric acid, pH 1.5 (Solvent B) and 20% of solvent A in 80% acetonitrile (Solvent C). The solvent gradient elution programme was used as per the method described by Hakkinen *et al.*, [26] with minor modification. Retention times and UV-Vis spectra of the peeks were compared with those of the standards. 20µl of the sample and standards were injected into the column and the chlorogenic acid and caffeic acids were detected at 280nm.

Toxicity studies on experimental animals:

a) Acute and sub chronic toxicity

The experimental mice/rats were housed in controlled environment (temperature 25±2°C and 12 hours dark/light cycles) and fed with standard pellets diets. The study was conducted after obtaining Institutional Animal Ethical Committee approval. Acute toxicity was carried out administering orally a single dose of aqueous extract at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0g/kg body weight in six groups of mice (five males and females per group). The general behavior of mice was observed continuously for 1 hour after administration of extract and then intermittently for 4 hours, and thereafter over a period of 24 hours.[27] The mice were observed further for up to 14 days following the treatment for any sign of toxicity and mortality. Sub-chronic toxicity was performed comprising four groups of six male Wistar rats in each group (200g-250g). All the animals had free access to water and food (*ad-libitum*). Aqueous extract was dissolved in distilled water and administered orally and daily for 90 days to the groups II-IV at a dose of 100,300,500 mg/kg body weight respectively. Group I (control) received only distilled water. At the end of every 30 days, blood was collected from the retro-orbital puncture for haematological such as RBC, WBC, Platelets, haemoglobin and packed cell volume (haematology analyzer, Sysmex Kx-21, JAPAN) as well as biochemical parameters (SGPT, SGOT, Creatinine, Urea, Glucose, Alkaline phosphatase and Total cholesterol). The effect of aqueous extract on the biochemical parameters of serum was determined enzymatically using specific kits to asses the state of liver and kidney. At the end of the study, the animals were sacrificed by decapitation and a portion of liver was fixed in 10% neutral buffer formalin for histopathological examinations.

b) Cytotoxicity towards rat's lymphocytes

Lymphocytes of rats were isolated from fresh whole blood using HiSep^{TM} LSM 1077 media. Cell number and viability were determined by Trypan blue exclusion staining using a Neubauer Improved Haemocytometer before and after the treatment. Rat lymphocytes were incubated at a density of 5×10^5/ml. Cell suspensions were treated with different concentrations of aqueous extract (0.1–1.0 mg/ml) and incubated for 30 min at 37°C. Cells were then pelleted by centrifuging at 1200rpm for 3 minutes and resuspended in RPMI 1640 medium. The viable and the dead cells were scored using 0.4% trypan blue and viability % was calculated using the formulae.

% Viability = [nonstained cells/ (stained+nonstained cells)] ×100.

Statistical analysis:

All the data were expressed as mean ± standard deviation. Statistical analysis was performed using one way ANOVA followed by Dunnett's test. The *p* values less than 0.05 were considered as significantly different.

RESULT AND DISCUSSION

Proximate composition:

The leaves were found to contain 91.95% of moisture, 1.62 ±0.01% of crude protein, 0.28 ±0.15%of total fat, 1.23 ±0.02% of crude fiber, 1.55 ±0.02% of ash and 3.37 ±0.31% of carbohydrates on fresh weight basis.

Antioxidant properties:

The yield of extractable compounds determined by sequential extraction and their radical scavenging activity is presented in **Table 1.** The scavenging effect of different solvent extracts on the DPPH radical decreased in the order of water>methanol> acetone> chloroform> ethylacetate > hexane. Among these extracts, the aqueous extract showed maximum antioxidant activity followed by methanolic extract. The aqueous extract also had higher total polyphenolic content than the methanolic extract (51.69 and 39.34mg/g extract respectively, (p<0.05) which may account for its enhanced

antioxidant activity. Phenolic compounds have been studied extensively as important contributors to the antioxidant properties.[28] The hydrogen donating ability of these compounds is responsible for their effective antioxidant property and used for protecting against cellular oxidative damage.

Aqueous extract with high antioxidant activity was further evaluated for its metal chelating property, superoxide scavenging activity, hydroxyl radical scavenging ability and reducing power in *in vitro* systems and their corresponding IC_{50} values are presented in **Fig 1**. The IC_{50} value for DPPH radical scavenging activity was 118 ± 1.26µg and compared with ascorbic acid (IC_{50}, 7.43±0.23µg) and BHA (IC_{50}, 9.77 ± 0.44µg) standards. The observed antioxidant activity of the extracts may be due to the neutralization of DPPH free radical either by transfer of an electron or hydrogen atom. [29] Metal chelating activity of antioxidants plays a significant role in reducing the concentration of transition metals responsible for catalyzing the lipid peroxidation.[30] The chelating property of the aqueous extract was analysed against Fe^{2+}. Ferrozine can quantitatively form complex with Fe^{2+}. In the presence of other chelating agent the complex formation is inhibited and the absorbance of ferrozine- Fe^{2+} complex decreased linearly in the presence of extract in a dose dependant manner with IC_{50} value of 466±8.8µg. The results were compared with EDTA, a standard metal chelator which showed 50% inhibition of metal chelation at 10.57µg. The chelating power of the extract may be attributed to polyphenolic content which is in conformity with the observation made in the literature.[31-32]

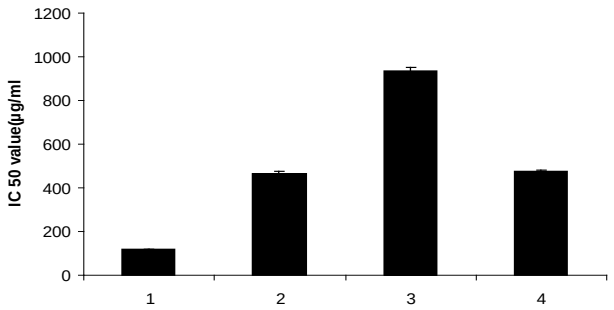


Fig 1: Radical scavenging activities of aqueous extract of chicory leaves (1-DPPH radical scavenging activity, 2-metal chelation property, 3-hydroxyl radical scavenging activity), 4-superoxide radical scavenging activity. Results are expressed as means ± SD of triplicate measurements.

Hydroxyl radical is the most reactive radical known in chemistry. It can abstract hydrogen atoms from biological molecules such as thiols leading to the formation of sulfur radicals, some of which can damage biological molecules also.[33] These hydroxyl radicals are produced by Fenton-type reaction in which metal iron reduces the hydrogen peroxide and reducing agent such as ascorbic acid, accelerate the formation of OH radicals by reducing Fe^{3+} ions to Fe^{2+}. [34] Inhibitory effect of extract on deoxyribose degradation was examined and the IC_{50} value for hydroxyl radical was at 0.936±17.6mg. It is evident from the present study that the aqueous extract acted as an effective OH radical scavenger in competition with 2-deoxy-D-rbose.The extract potentially inhibited the metal ion dependant generation of OH as well as capturing the released radicals. The superoxide radical scavenging property of the aqueous extract in PMS/NADH-NBT system was determined. Superoxide ion generated from dissolved oxygen by PMS/NADH coupling reaction reduces the NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The IC_{50} value of aqueous extract was 477±5.9µg/ml.

The reducing properties are generally associated with the presence of reductones. They exert antioxidant action breaking the free radical chain by donating hydrogen atom.[35] Reducing ability of the aqueous extract was studied by Fe^{2+}-Fe^{3+} transformation. The reducing power of the aqueous extract was increased with the increasing concentration from 0.1 to 0.8mg/ml in reaction mixture. Based on this observation it may be said that aqueous extract is capable of terminating free radical initiated chain reaction by neutralizing free radicals.

Inhibition of Protein oxidation:

Accumulation of macromolecular oxidative damage including protein has been reported as a fundamental cause in many pathological conditions. AAPH is a water soluble initiator, which decomposes at physiological temperature producing alkyl peroxyl radicals with oxygen to initiate the protein oxidation.[36] **Fig. 2** shows that two hours after the incubation, the BSA was completely degraded by 20 mM AAPH in positive control as studied by SDS-PAGE electrophoresis (lane 2). Pretreatment of

BSA with aqueous extract (lane 3-7) showed protective effect significantly by restoring the band intensity to 31.5, 35.7, 47.8, 61.4 % of negative control at the concentration of 50, 100, 150, 200, 250 µg/ml respectively in a dose dependent manner. Quercetin, a standard antioxidant, completely prevented the oxidative degradation of BSA at 10µg/ml concentration. The inhibitory effect of extracts might operate by scavenging the peroxyl radical generated in the reaction mixture

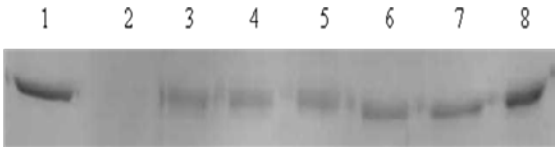


Fig 2. The inhibitory of aqueous extract of Chicory on 20 mM AAPH induced BSA oxidative fragmentation. Lane 1, BSA; lane 2, BSA + AAPH; lane 3, BSA + AAPH+50µg/ml extract; lane 4, BSA + AAPH+100µg/ml extract; lane 5, BSA + AAPH+150µg/ml extract; lane 6, BSA + AAPH+200µg/ml extract; lane 7, BSA + AAPH+250µg/ml extract; lane 8, BSA + AAPH+10µg/ml Quercetin.

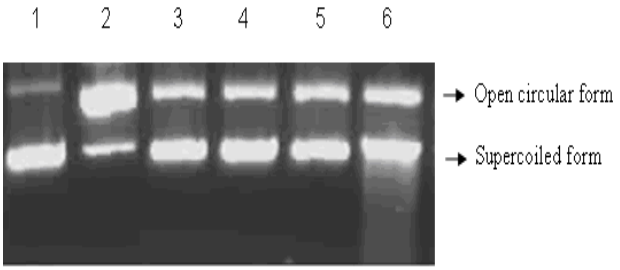


Fig 3: The inhibitory effect of aqueous extract on 10 mM AAPH induced pBR322 plasmid DNA strand breakage. Lane 1, DNA; lane 2, DNA + AAPH (positive control); lane3, DNA + AAPH + 200 µg/ml of extract; lane 4, DNA + AAPH + 100 µg/ml of extract; lane 5, DNA + AAPH + 50 µg/ml of extract; lane 6, DNA + AAPH + 8µg/ml Quercetin.

DNA damage protecting properties:

DNA protection properties of the plant extracts is important since ROS mediated DNA damage causes major problems such as aging, cancer, degenerative disorders etc and dietary antioxidants have been reported to exert a protective effect against such diseases by inhibiting DNA damage.[37] Therefore, DNA damage protecting activity of aqueous extract was evaluated using prokaryotic and eukaryotic DNA in the present study.

a) Plasmid DNA nicking assay

Free radical scavenging effect of aqueous extract on plasmid DNA (pBR322) damage protection was studied along with quercetin as standard. Exposure of plasmid DNA to AAPH caused introduction of strand breakage which let to extensive conversion of super coiled (Sc) form to open circular form. Fig 3 clearly shows that in the presence of aqueous extract (lane 3, 4 and 5) there is a significant reduction in the formation of nick in the plasmid DNA. The band intensity of the Sc DNA was decreased by 62.85 % in positive control (with AAPH) compared to negative control (without AAPH). In the presence of aqueous extract at 200, 100, 50µg/ml concentration the band intensity of the Sc DNA were comparable to that of negative control. The extent of protection at 200,100 and 50µg/ml was 53.9, 46.3 and 45.1% respectively. Quercetin at 8µg/ml concentration offered 50.76 % protection against nick formation. It is inferred from these results that aqueous extract effectively protected the peroxyl radical induced DNA strand breakage significantly (p<0.05). The protection offered at 200µg/ml concentration was close to that of 8µg/ml standard quercetin.

b) Single cell Gel electrophoresis (SCGE) assay

SCGE is one of the methods used in genetic toxicology for detection and quantification of DNA lesion at single cell level.[38] This method allows biomonitoring of human exposure to variety of genotoxic agents. Polyphenols from six Mediterranean plants extracts (*Crepis vesicaria* L, *Origanum heracleoticum*, *Scandix australis* L, *Amaranthus* sp, *Scolymus hispanicus* L, *Thymus piperella* L) and other plant species have been reported to be responsible for the scavenging activity of ROS, inhibiting DNA damage in lymphocytes and cell lines of Caco-2 cells by H_2O_2. [39-40] In the present study, 20µm of H_2O_2 was used to produce extensive DNA damage in lymphocyte cells. The % of tail momentum for positive control (cells +20µm H_2O_2) and sample treated groups at the concentration 0.5mg and 1.0mg/ml extract were 23.4±2.1, 17.4±1.3 and 12.93±1.4 respectively (**Fig.4**). The inhibition of DNA damage was concentration dependant and at 0.5mg and 1mg/ml concentration it provided 25.64% and 44.74% DNA damage protection compared to the control group (p<0.05). The hydrogen peroxide scavenging property of the extract may be contributing for the observed DNA protection and could be due to polyphenol content.

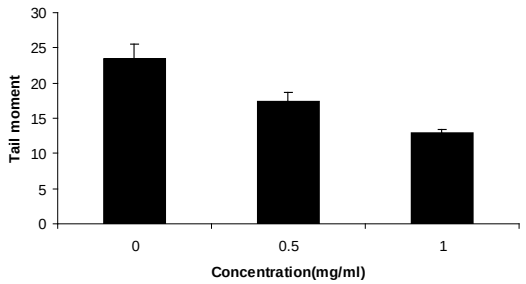
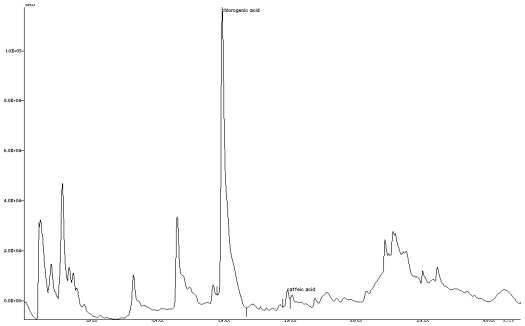


Fig 4: Effect of aqueous extract on H_2O_2 induced DNA damage in rat blood lymphocytes. Inhibition of DNA damage is shown as percentage. Results are expressed as means ± SD of triplicate measurements. Extract concentrations at 0.5 and 1mg/ml is significant at $p<0.05$ compared to without extract.

HPLC Analysis of aqueous extract:

Phenolic acids have been proved to have antioxidant activity, attributed to scavenging reactive oxygen species.[41] HPLC chromatogram of major phenolic acid in the extract is shown in **Fig 5**. Based on the retention time chlorogenic acid and caffeic acid were identified at 29.83 and 40.16 min respectively. In the present study chlorogenic acid was detected



as one of the major polyphenol in the extract and this result is similar to that of the earlier report by Kocsis *et al*. [15] These phenolics are well known to have physiological properties such as antioxidant; antibacterial effects etc. Chlorogenic acid along with other polyphenols may be primarily responsible for the observed *in vitro* antioxidant activity of aqueous extract.

Fig 5: HPLC chromatogram of aqueous extract of Chicory leaves; Retention time for chlorogenic acid - 29.83 min and caffeic acid - 40.16 min.

Toxicity studies:

In case of acute toxicity, no sign of toxicity (hypoactivity, diarrhea, salivation, and anorexia) or death was observed in mice up to the dose of 3 g/kg body weight. The sub-chronic toxicity study in orally administered aqueous extract, at the dose of 100,300 and 500 mg/kg body weight for the period of 3 months showed that all the haematological parameters, such as red blood cell count, platelet count, white blood cell count, packed cell volume and percentage of haemoglobin were within the limits throughout the treatment period (data not shown). The change in body weight has been used as an indicator of adverse effect of plant extracts, drugs and chemicals. [42] No change in body weights observed in the present study also indicate no adverse effect of the extract. The serum biochemical profile of the treated and control rats are presented in table No.2. Administration of the extract did not cause any significant changes in serum biochemical parameters such as SGPT, SGOT, creatinine, urea and alkaline phosphatase in control and treated groups through out the study period (30, 60, 90 days**)** suggesting nontoxic effect of extract. However, blood glucose level was significantly decreased ($p<0.05$) after 30 days in groups IV and 60 days in group III compared to control group I. The hypoglycemic effect was attenuated at the end of the treatment period (90 days). No significant change in the levels of serum tranaminases (SGPT and SGOT), urea and creatinine, which are biomarkers for liver and kidney functions confirms normal function of the organs. This was further confirmed by histopathological examination of liver tissues which also showed normal architecture of liver in treated to that of control group (**Fig 6a-d**). Hence it can be stated from the study that chicory leaves is not toxic.

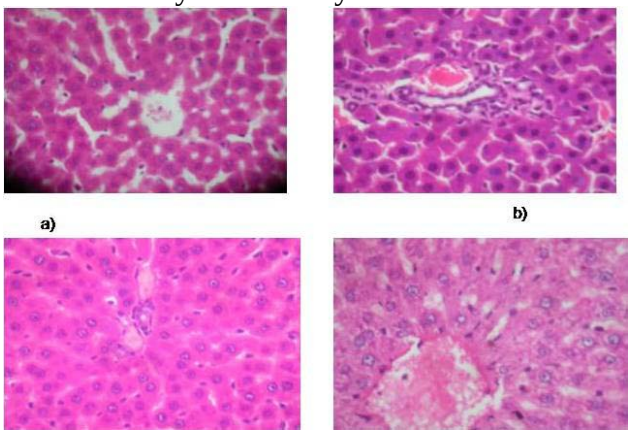


Fig 6: Representative Section of liver tissue shows well maintained architecture with normal hepatocytes (HE 100X). a) Control rats, b) Rats treated with 100mg aqueous extract/kg body weight, c) Rats treated with 300mg aqueous extract /kg body weight d) Rats treated with 500 mg aqueous extract /kg body weight. No obvious pathology noted.

Cytotoxicity:

In relation to cytotoxic study, the results clearly showed that the aqueous extract did not cause any toxicity ($p<0.05$) up to the concentration of 1.0 mg/ml in rat lymphocytes. The high % of viability indicated high live cells and overall the

viability was above 95% at all the concentrations used (**Table.3**).

Table1: Antioxidant activity and total phenolic content of sequential extract of *C. intybus* leaves. The values are mean ± SD (n=3)

Solvents	Extract yield (%)	% of DPPH radical scavenging activity	Total phenolic content (mg/g extract)
Hexane	3.20	1.66 ± 0.18	-
Chloroform	3.35	4.73 ± 0.21	-
Ethyl	0.88	2.09 ± 0.10	-
acetate	1.04	7.62 ± 0.11	-
Acetone	11.14	72.76 ± 0.38	39.34 ± 0.48
Methanol	11.56	87.45 ±1.10	51.69 ± 0.35
Water			

Table 3: Cytotoxicity study for aqueous extract of Chicory on rat lymphocytes. The values are mean ± SD (n=3).

Concentration (mg/ml)	% of Cell viability
0	98.2±1.2
0.1	98.8±0.5
0.2	98.1±1.4
0.4	97.9±1.3
0.8	98.4±0.7
1.0	97.8±1.1

CONCLUSION

The results obtained from the present study revealed that the aqueous extract of Chicory leaf was effective in scavenging the free radicals generated through various *in vitro* systems. It also significantly protected the oxidation of biomolecules such as protein and DNA.

Table 2: Serum biochemical analysis of rats administered with aqueous extract of Chicory leaves.*significantly different vs control (*P*<0.05).

Biochemical parameters	Period of treatment (days)			
	0 days	30 days	60 days	90 days
Group I(control)				
SGPT (U/l)	37.5±3.80	39.2 ±2.74	36.5 ±1.64	38.80±1.27
SGOT (U/l)	69.20±0.59	68.93 ± 2.24	71.52 ± 3.50	70.98±4.22
Creatinine (mg/dl)	0.53±0.06	0.52±0.02	0.54±0.05	0.51±0.02
Urea (mg/dl)	35.2±1.70	37.5±1.93	38.7±0.95	36.90±1.70
Glucose(mg/dl)	100.36 ±3.88	102.60 ±2.54	103.77 ±4.25	99.67 ±4.27
Alkaline phosphatase (U/L)	255.42 ±3.59	260.42 ±5.23	262.42 ±3.25	266.42 ±4.47
Total cholesterol (mg/dl)	63.81±1.66	64.81±2.01	68.81±3.55	65.81±4.63
Group II(100mg/kg)				
SGPT (U/l)	41.70±6.18	40.9 ±5.47	39.0 ±3.80	41.0 ±3.29
SGOT (U/l)	67.78 ± 2.17	70.13 ± 3.07	68.80 ± 2.55	71.23± 3.40
Creatinine (mg/dl)	0.48±0.07	0.50±0.04	0.51±0.04	0.49±0.02
Urea (mg/dl)	37.6±1.18	39.4±1.85	36.6±1.56	38.7±1.08
Glucose(mg/dl)	98.43 ±4.18	99.55 ±4.32	97.65 ±3.57	101.35 ±4.46
Alkaline phosphatase (U/L)	261.02 ± 2.17	258.02 ± 4.66	259.02 ± 3.74	263.02 ± 3.71
Total cholesterol (mg/dl)	61.97±4.71	65.97±3.27	66.97±4.19	62.97±4.24
GroupIII(300mg/kg)				
SGPT (U/l)	40.3 ±4.02	42.8 ±3.21	40.1 ±2.53	39.1 ±4.30
SGOT (U/l)	67.15 ± 4.46	69.52 ± 2.60	70.20 ± 2.94	69.85 ± 2.40
Creatinine (mg/dl)	0.54±0.09	0.53±0.05	0.50±0.08	0.50±0.05
Urea (mg/dl)	40.2±0.94	36.3±1.07	40.8±1.72	41.5±0.94
Glucose(mg/dl)	101.85 ±4.02	100.34 ±3.20	89.17 ±3.21*	101.22 ±3.22
Alkaline phosphatase (U/L)	256.46 ± 4.46	265.46 ± 3.74	260.46 ± 5.62	259.46 ± 2.89
Total cholesterol (mg/dl)	63.5±1.98	66.5±4.04	61.5±3.86	67.5±4.28
Group IV(500mg/kg)				
SGPT (U/l)	39.0 ±5.63	40.5 ±5.11	41.9 ±2.36	40.8 ±2.23
SGOT (U/l)	68.32 ± 4.45	67.23 ± 1.47	70.27 ± 1.48	72.23 ± 5.46
Creatinine (mg/dl)	0.50±0.07	0.56±0.03	0.52±0.09	0.55±0.07
Urea (mg/dl)	39.5±1.00	40.1±1.50	38.9±1.03	42.8±1.07
Glucose(mg/dl)	103.64 ±2.63	87.11 ±4.27*	95.92 ±4.24	98.37 ±4.28
Alkaline phosphatase (U/L)	257.94 ± 4.45	259.94 ± 5.37	263.94 ± 4.52	260.94 ± 4.67
Total cholesterol (mg/dl)	62.72±4.57	61.72±3.24	63.72±3.83	62.72±4.82

The scavenging activity of the extract could be attributed to the presence of phenolics compounds. At the oral dose tested, aqueous extract did not cause any toxicity in mice and rat and also showed no cytotoxicity towards the rat's lymphocytes. Therefore, the consumption Chicory leaves, as a dietary source of antioxidants could provide health benefits in preventing cellular oxidative damages.

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