Anti-oxidant and Anti-hepatotoxic Activities of *Taraxacum officinale* Weber (Dandelion)

[1]Mohd Zafar, [2]Abdul Hafeez, [3]Amrita Singh, [4]Snehil Singh, [5]Bhanu P. S. Sagar

[1,3,4,5]IEC Department of Pharmacy, IEC Group of Institutions, Greater Noida, Uttar Pradesh, India
[2]School of Pharmacy, Glocal University, Saharanapur, Uttar Pradesh, India

***Abstract*- Herbal medicine is currently enjoying a revival in popularity in the west and in many parts of the world. Now a day, various herbal formulations / phyotoformulations are commercially available for the prevention and treatment of hepatotoxicity. *Taraxacum officinale* (dandelion) contains compounds with various medicinal activities. This dissertation work was an attempt to establish scientific protocols to perform pharmacological investigation / research on *Taraxacum officinale* for anti-hepatoxic effect against paracetamol. High amount of total phenolics and total flavonoid content were found in the EETOL Extract than EETOR Extract.**

In toxicity studies, mortality rates were zero percent and dandelion extracts possess wide safety margin and classified as Non-Toxic Constituent. Extracts caused slight change blood parameters whereas LFTs were not significantly altered. In anti-hepatotoxic pharmacological studies, PCM induced hepatoxicity by glutathione depletion, necrosis of hepatocytes, elevated levels LFTs with centilobular necrosis. EETOL extract produced remarkable anti-hepatotoxic activity with significant alleviation of LFTs and reddish coloration, normal anatomical architecture of the hepatocytes (reversal effects). Anti-hepatotoxic effects were comparatively lesser then standard Silymarin and pharmacodynamics includes membrane stabilization; inhibition of phopholipase A2; anti-inflammatory; anti-oxidant; attenuation of depletion of glutathione; and enhanced proteins synthesis. Further, in thiopentone sodium induced sleeping time analysis, reduction in sleeping time was recorded in dandelion EETOL extract treated animals when compared to toxic control animals.

Keywords: **Anti-oxidant, Anti-hepatotoxic, catalase, dandelion, glutathione, nitric acid, paracetamol, peroxidise, silymarin, superoxide dismutase, thiopentone sodium.**

Introduction

Pradhan *et al*., 2006, illustrated that liver is master chemist (metabolic clearing house) and most regenerative part of human body that plays astonishing vital functions in detoxification and metabolism of endogenous / exogenous toxic chemicals. Chronic liver diseases cause morbidity and mortality (hepatic coma – central coma – death) due to hepatotoxins, environmental pollutants; hepatic cancer; alcoholic and intoxicants and other drug therapy. **Radha *et al*., 2005,** revealed that drug induced liver diseases (DILI) are caused by overdose / prolonged therapy of paracetamol and idiosyncratic liver injury triggered by other drugs like anti-tubercular drug rifampicin (R-cin), Amoxicillin + Clavulanate, Flucloxacillin, Erythromycin, Ciprofloxacin. **Luper, 1998,** had reported that no significant allopathic medicines are available for the treatment of DILI.

Stickel and Schuppan, (2007) hepatotoxicity can cause mortality and morbidity (hepatic coma followed by central coma then death) and accounts for 3.5%-9.5% of all adverse drug reaction (ADRs) reports and up to 14.7% of fatal adverse reaction (FDRs). Drug induced liver injury (DILI) is caused by overdose / prolonged therapy of anti-tuberculor drugs, paracetamol, antibiotics etc. and it affect large population across the globe. (**Dienstag *et al*., 2001)**.

Sudipta *et al*., 2012 explained Drug-induced liver injury (DILI) / Drug-induced hepatotoxicity (DIH) as the most important cause of mortality and morbidity. The primary cause of liver intoxication includes alcohol (71%) psycho-pharmaceuticals & industrial exposure (11%). **Onkar *et al*., 2016** compiled hepatotoxicity caused due to lipid- peroxidation, activation of pro-inflammatory mediators, induction of nitric acid (NO) synthase, mitochondrial dysfunction and Cytochrome P450 activation. Various risk factors for hepatotoxicity like race, age (geriatrics are at high risk due to reduced hepatic blood flow; decreased clearance; variation in drug binding; drug-to-drug interactions), multiple drug therapy, gender (more common in females than in males), alcohol (due to depletion of glutathione), genetic factors (idiosyncratic reactions), AIDS (low glutathione level), Drug formulations (long-acting drugs) and fibrosis.

Mukherjee (2002), reported that paracetamol (synonym: PCM; acetaminophen; N-acetyl-p-aminophenol) intoxication / prolonged therapy produce liver toxicity. Now a days, it has become one of the most popular "over-the-counter" (OTC) drug and occupied 50 -60% of the total OTC analgesic market. PCM induced acute hepatotoxicity at the higher then 15-20g. On over dosage PCM exhaust glutathione stores and leads to necrosis of hepatocytes. Acute over-dosage may cause fatal hepatic damage, and the number of self-poisonings and suicides with PCM - especially in the United States - has grown alarmingly in recent years. **(Shah *et al*., 2011)**

Parabia *et al*., 2007, revealed that Drug-induced hepatotoxicity (DIH) / Drug-induced Liver injury (DILI) is the most common cause of acute hepatic failure and affect huge population across throughout the globe. Symptoms of liver damage include digestive disturbances such as constipation; jaundice (elevation of biochemical parameters like SGPT, SGOT, ALKP, TBIL and

Total Albumin / Liver Function Tests / LFTs); necrosis and degenerative changes in hepatocytes, ballooning and fatty changes / steotosis, depletion of glutathione in the liver, rupture of hepatic membrane, loss of proteins in blood, inflammation of hepatocytes, cirrhosis of the liver, edema; pruritus (itching), nausea and vomiting.

Agarwal *et al*., 2001 found that no significant and safe hepatoprotective agent is available in modem therapeutics that stimulates liver function, offer protection to the liver from damage or help regeneration of hepatic cells.

Sampath *et al*., 2010, further, revealed that herbal drugs are more widely used than allopathic drugs as hepatoprotective because of them are inexpensive, better cultural acceptability, better compatibility, with the human body and minimal side effects. There are plants drugs such as "Milkthistle", "Bhringraja" and “tree turmeric” which can give definite protection against experimentally induced hepatotoxicity.

Sheetal *et al*., 2004, summarised Alternative System of Medicine (ASM) / Complimentary System of Medicine (CSM) which include Ayurveda (since last 5000 years) and Siddha (originated in India) Unani system (Persia; about 3,000 years), Chinese traditional System (5,000 years), Tibetan Systems (3,000 years old). **(Balunas *et al*., 2005)**
Hikino *et al*., 1984 summarised various hepatoprotective medicinal plants like *Silybum marianum, Andrographic paniculata, Wedelia calendulacea, Phyllanthus emblica, Picrorhiza kurroa, and Eclipta alba* Linn. **(Newman *et al*., 2002**)

Qiu (2007), articulated that there are reputed hepatoprotective polyherbal formulations include Amlycure DS, Liva-16, Livodin DS, Livosin, Livotone, Livotrit, Livergen, Acilvan Hepa-10, Livomap, Vimliv, Livomycin, Amylcure, Liv-52, Sanliv, etc. Hepatoprotective herbal formulations contains 33 plants which include *Berberis aristata*, *Cassia angustifolia*, *Eclipta alba*, *Picrorrhiza kurroa*, *Silybum marianum*, *Boerrhaavia diffusa*, *Andrographis panuculata*, *Ocimum sanctum Tinospora cordifolia*, *Solanum nigrum, Astercantha longifolia, Plumbago zeylanica, Circhorium intybus, Terminalia chebula, Oldenlandia corymbasa*, *Phyllanthus niruri, Solarium nigrum, Phyllanthus amarus, Taraxacum officinale, Curcuma longa, Solanum nigrum* etc. **Schuppan *et al*., 1999** reported that plants are excellent sources of phenolic antioxidants. It has been assessed that these plants have been formulated together in different doses and combinations to achieve maximum synergistic antihepatotoxic / hepatoprotective activity.

Taraxacum officinale (dandelion weed plant; Asteraceae) contains bioactive compounds like phenolic compounds, flavonoids, phenolics, coumarins, sterols, sequiterpenes, as main constituents and have shown various pharmacological activities. But, surprisingly, no significant phytochemical and pharmacological investigations are reported in literature so far. *In-vitro* anti-oxidant and *in-vivo* anti-hepatotoxic (against paracetamol) activities of leaves, aerial parts and roots of dandelion in albino rats are not reported in scientific literature published. So, it was an attempt to perform research on *Taraxacum officinale* (dandelion) with following objectives:

- Phytochemical screening and qualitative analysis by using scientific techniques.
- Estimation of TPC and TFC in dandelion extracts.
- *In-vitro* anti-oxidant activity by DPPH, NO, SOD, CAT and Peroxidase.
- *In-vivo* anti-hepatotoxic activity of dandelion extracts against PCM.
- Safety and toxicity evaluation of dandelion extracts.
- Lipid / biochemical parameters assessment with histopathological studies.

Methodologies Used

(i) Various research papers were scientifically reviewed for literature search, summarised, assessed, statistically analysed to establish research protocols.
(ii) Phytochemical analysis techniques (chromatographic methods, IR, UV etc.) were used for qualitative/quantitative analysis.
(iii) *In-vitro* antioxidant activities by DPPH free radical scavenging activity and Nitric Oxide scavenging activity.
(iv) Biochemical parameters (like SGPT, SGOT, total protein, serum albumin, serum billirubin and alkaline phosphatase etc.), histopathological analysis of liver - body weight analysis.

Harvesting and Authentication of Raw Material

Fresh entire plants of *Taraxacum officinale* Weber (dandelion) were harvested and collected from Medicinal Plant Garden of the IEC-GI campus in January 2020. Dandelion plants were washed to clean, dried under shade and finally coarsely pulverised in grinder. Dandelion parts were scientifically analysed by pharmacognostical methods for its authentication. Phytochemical screening was done to detect PPMs and SPMs and herbarium specimens were also deposited.

Figure 1: *Taraxacum officinale* plant morphological structures.

Figure 2: *Taraxacum officinale* plant morphological structures.

Preliminary Phytochemical Screening

Various chemical tests were performed to detect PPMs / SPMs like Sterols (Salkowski tests), Phenols (Ellagic acid test), Flavonoids (Pew's, Shinoda and NaOH tests), Glycosides (Keller-Kiliani; Molisch's reagent tests), Alkaloids (Iodine, Wagner's; Mayer's reagent test), Saponins (Foam test), Carbohydrates (Molisch's; Barfoed's; Seliwanoffs tests), Tannins (Ferric chloride and Lead acetate tests), Amino acids (Millon's reagent tests) and Protiens (Biuret test), tests were carried out. PPMs and SPMs like alkaloids, glycosides, tannins, saponins, anthraquinones, phenolic compounds, flavanoids, steroids, reducing sugars, and amino acids were found present in the drug samples.

The petroleum ether, chloroform, methanol and aqueous extracts Plant aerial parts, leaves and roots (dried under shade, coarsely pulverized separately) of dandelion were subjected for PPMs and SPMs using standard procedures described by **Harborne (1973); Sofowora (1993); Khandelwal (2008)**.

Preparation of EETOP, EETOL and EETOR Extract of Dandelion

Dandelion shade dried and coarsely pulverized aerial parts, leaves, and roots (1000 gm each) were Soxhlet extracted with crude ethanol (80%; Soxhletion; separately) for 16 hours, filtered, distilled, vacuum evaporated and finally lyophilized.

Estimation of Total Phenolics Contents (TPC) in EETOL, EETOP and EETOR.

The total phenolics content (TPC, possess antioxidant activity) in EETOP, EETOL and EETOR extract of dandelion were undertaken by method of **Jeong *et al.*, 2010**.

Estimation Total Flavonoid Contents (TFC) in EETOP, EETOL and EETOR

The total flavonoids content (antioxidant potential is correlated with TFC content) were determined in EETOP, EETOL and EETOR of dandelion as per method reported by **Kamtekar *et al.* 2014.**

(A) *In-vitro* Antioxidant effects of EETOP, EETOL and EETOR

In-vitro antioxidant activity of EETOL, EETOP and EETOR of dandelion by DPPH, NO, SOD, catalase, peroxidase radical scavenging method was performed.

(i) DPPH Free Radical-Scavenging Activity: DPPH radical-scavenging capacity was measured by method of **Blois (1958).**

(ii) Nitric Oxide (NO) Scavenging activity: Curcumin was used as Standard. **(Larson, 1994)**

(iii) Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP) Assay : The FRAP assay was performed as per method of **Benzie and Strain (1996).**

(iv) Reducing Power Assay : As per SOP by **Jayaprakasha *et al*., 2001**.

(B). Antioxidant Enzymes Assays in Liver Tissues

(i) Superoxide dismutase (SOD) activity **(Habbu *et al*., 2008; Chidambara *et al*. 2002);**

(ii) Peroxidase activity **(Nicholas, 1962);**

(iii) Catalase Activity **(Aebi, 1984)**

Preparation of Liver Tissue for SOD, Catalase, and Peroxidase Estimation

Hepatic tissues - excised – cleaned - homogenized (cold 1.15% KCl and10 mM phosphate buffer with EDTA (pH 7.4) - centrifuged (10,000 rpm for 10 min) - supernatant centrifuged (13,000 rpm; 60 min) - cytosolic extract – SOD / CAT / peroxidase estimated.

SOD activity (Beauchamp and Fridovich 1971; Chidambara *et al*. 2002)

SOD (Units/ mg) was estimated using Kit (Sun Pharma, India) and based upon reduction of nitrobluetetrazolium (NBT) to water insoluble blue formazan. Standard operating procedures were undertaken **(Habbu *et al*., 2008)**.

Safety and Toxicity Evaluation of EETOP, EETOL and EETOR Extracts

Toxicity studies of EETOL, EETOP, EETOR of dandelion was carried out as per OECD guidelines and IAEC Form B approval (IEC/IAEC/2022/05 dated 25-03-2022)

Physiological and behavioural changes were observed then RBC count, MCV, MCH **(Dacie and Lewis, 2001)**, Hb content **(Pla and Fritz, 1971; Crosby *et al*., 1954)**, hematocrit (Ht) **(Dacie and Lewis, 1991)**, PLT / WBC count **(Wu and Hoak, 1974)** were analysed and diagnostic kits were also used for biochemical analysis **(Trinder, 1969; Spencer, 1986; Bretaudiere *et al*., 1976)**.

Anti-hepatotoxic activity of EETOP, EETOL and EETOR of dandelion against PCM

IAEC of IEC-GI Institution as per CPCSEA guidelines approved the Form-B IEC/IAEC/2022/05 on 25-03-2022 (Registration No.–1332/PO/Re/S/10/CPCSEA).

Grouping of Animals

Table 1: Group of animals.

Group	Treatment
I	Normal Control (Vehicle)
II	Toxic Control: PCM (1gm/kg) for 03 weeks
III	PCM (1gm/kg) for 03 weeks + 200 mg/kg EETOL for 02 weeks
IV	PCM (1gm/kg) for 03 weeks + 400 mg/kg EETOL for 02 weeks
V	PCM (1gm/kg) for 03 weeks + 200 mg/kg EETOP for 02 weeks
VI	PCM (1gm/kg) for 03 weeks + 400 mg/kg EETOP for 02 weeks
VII	PCM (1gm/kg) for 03 weeks + 200 mg/kg EETOR for 02 weeks
VIII	PCM (1gm/kg) for 03 weeks + 400 mg/kg EETOR for 02 weeks
IX	PCM (1gm/kg; 03 weeks) + 140 mg/kg Silymarin (Standard) for 02 weeks

Animals were given water *ad libitum* and on eighth day liver biochemical parameters (LFTs) were estimated. The blood samples for the LFT estimations were collected from retro-orbital sinus. Animals of Group II were served as toxic control group (PCM Group) and animals were given PCM (1gm/kg) for 03 weeks. Group III to IX animals were given PCM (1g/kg) for 03 weeks to induce hepatotoxicity followed by treatment with EETOL (Group III 200 mg/kg), EETOL (Group IV; 400 mg/kg), EETOP (Group V: 200 mg/kg), EETOP (Group VI: 400 mg/kg), EETOR (Group VII: 200 mg/kg), EETOR (Group VIII: 400 mg/kg) for 02 weeks and standard drug Silymarin (140 mg/kg) for two week.(Group IX). SGPT / ALT, SGOT / AST, Abumin, Total proteins (T-Prot), alkaline phosphatase (AKLP) and Billirubin were estimated **(Gornal *et al*., 1949; Lowry *et al*., 1949; Godfried *et al*., 1935)**.

Effect of Extracts on Thiopentone sodium Induced sleeping-time and liver weight

Kulkarni, 1999 reported that barbiturates are extensively metabolized in the liver and damaged liver suffers with delay in barbiturates clearance (thiopentone sodium 40 mg/kg i.p; cause longer duration of hypnotic effect; **Gujrati *et al.*, 2007)**

Grouping of Animals

Table 2 : Groups of animals for sleeping time effect.

Group	Drug Treatment
I	Normal (Vehicle Control)
II	Toxic: PCM (1gm/kg) for 03 weeks + Thiopentone sodium (40 mg/kg i.p)
III	PCM for 3 weeks + EETOL (400 mg) + Thiopentone sodium (40 mg/kg i.p)
IV	PCM for 3 weeks + EETOP (400 mg) + Thiopentone sodium (40 mg/kg i.p)
V	PCM for 3 weeks + EETOR (400 mg) + Thiopentone sodium (40 mg/kg i.p)
VI	PCM (1gm/kg) for 03 weeks + Silymarin (Standard; 140 mg/kg p.o) + Thiopentone sodium (40 mg/kg i.p)

Group I animals were given water *ad libitum* / vehicle control. Group II (toxic control) animals were given PCM (1gm/kg) for 03 weeks. Group III to VI animals of Group II to VI were served as models for PCM induced hepatotoxicity. Animals of Group II to V were given PCM (1gm/kg) for 03 weeks to induce hepatotoxicity followed by treatment with EETOL / EETOP / EETOR (400 mg/kg) with thiopentone sodium (40 mg/kg i.p) (Group III, IV and V) and silymarin (standard drug, 140 mg/kg) with thiopentone sodium (40 mg/kg i.p) (Group VI).

Results and Discussions

The procured plant drug was authenticated as dandelion with herbarium specimen number IEC/Pharm/Herb/2020/102. Qualitative analysis of dandelion aerial parts, leaves and roots showed the presence of PPMs and SPMs like glycosides; alkaloids; amino acids; tannins; saponins; flavanoids; steroids; phenolics; reducing sugars; anthraquinones. Practical yield EETOL, EETOP and EETOR extracts were 1.86%, 1.54% and 1.12% respectively.

Different concentrations of EETOL, EETOP and EETOR extracts (200 / 400 mg in 1 % carboxymethylcellulose) were used in pharmacological studies. High concentration of TPC were found in the EETOL (34.78±1.84 mg GAE/100 gDW) and EETOP (26.28±1.62 mg GAE/100 gDW), whereas TPC concentration was low in EETOR (14.56±1.34 mg GAE/100 gDW) of dandelion.

Standard Plot was prepared and TFC in EETOP, EETOL and EETOR were analysed and calculated. It has been assessed that there is a close relationship between antioxidant activity and the amount of TFC **(Negro *et al.*, 2003; Li *et al.*, 2009)**. Comparatively high amount of TFC was found in the EETOL (9.6 mg) and EETOP (8.4 mg) than EETOR (7.2 mg) extract. Free radical-scavenging activities of the EETOL, EETOP and EETOR extracts increased with increasing concentrations (regression equations significant at $p < 0.05$; Table 4 and Figure 3). Results were means ± standard errors of mean (SEM) and data analyzed by ANOVA to determine the level of significance ($P < 0.05$).

Table 3 : TFC in EETOL, EETOP and EETOR.

Sample	Absorbance (510 nm)	TFC Concentration Eq.to Quercetin(µg/1 ml)	TFC (mg of Quercetin Eq./100mg CE)
EETOL	0.528	960	9.6
EETOP	0.452	840	8.4
EETOR	0.402	720	7.2

Table 4 : The regression curve values of DPPH.

S. No.	Concentration (µg/mL)	The cleared ratio (%)			
		EETOL	EETOP	EETOR	Ascorbic Acid
1	0	0	0	0	0
2	10	13.4	13.0	7.4	14.4
3	20	23.8	23.2	16.6	25.2
4	40	34.4	33.6	24.8	35.8
5	60	44.6	43.6	34.2	46.4
6	80	54.8	52.4	42.8	57.4
7	100	64.4	62.8	49.6	68.2

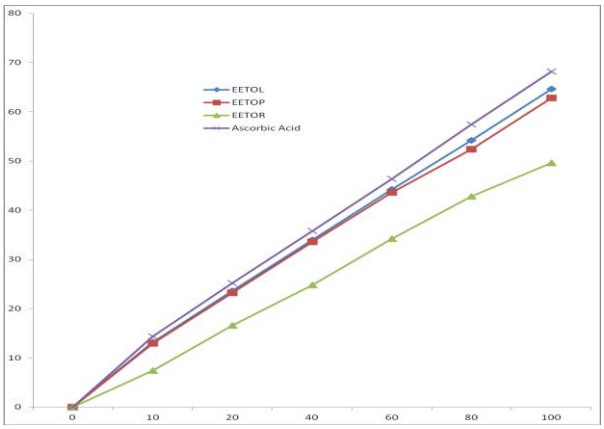

Figure 3: The regression curve of DPPH.

EETOL extracts produced highest NO scavenging activity with IC50 value 97.10 µg/ml (Table 5). Antioxidant activity by reducing power of EETOL, EETOP and EETOR assay was increased with increased dose.

Table 5 : NO scavenging activity of EETOL.

Concentration (µg/ml)	% Inhibition	IC50 µg/ml	R2
20	10.74±0.36		
40	21.40±1.11		
60	33.29±1.65	97.105	0.9947
80	40.22±1.73		
100	51.42±1.65		
Curcumin (Standard)	34.24±1.26	17.72	0.9921

Values: means ± SD (n=6). R2 : correlation of Regression.

Antioxidant activity of EETOL and EETOP extracts were significant (data were expressed as means ± S.D.) and analysis of variance was performed by the ANOVA procedures (Table 6; Figure 4-6). Dandelion extracts dose dependently elevated the reduced levels of SOD, catalase, and peroxidase activity (antioxidant activity by preventing cell membrane oxidation). Polyphenol, tannins, flavonoids and bitter principles in dandelion extracts induced antioxidant activity through significant superoxide radical scavenging activity, inhibition of lipid oxidation and by protecting glutathione in liver.

Table 6: Effect of EETOP, EETOL and EETOR on SOD, Catalase and Peroxidase.

Group	Drug	SOD (units/mg)	Catalase (units/mg)	Peroxidase (units/mg)
I	Normal (Vehicle Control)	17.24 ± 0.42	122.4 ± 5.46	0.80 ± 0.08
II	Toxic : PCM; 1gm/kg	5.28 ± 0.26	32.28 ± 2.4	0.18 ± 0.16
III	PCM (1g) + EETOL (200 mg)	11.18 ± 0.68	98.26 ± 2.2	0.56 ± 0.14
IV	PCM (1g) + EETOL (400 mg)	14.36 ± 0.42	116.6 ± 5.22	0.72 ± 0.06
V	PCM (1g) + EETOP (200)	10.26 ± 0.42	86.54 ± 3.42	0.48 ± 0.06
VI	PCM (1g) + EETOP (400 mg)	10.96 ± 0.36	98.88 ± 3.18	0.54 ± 0.06
VII	PCM (1g) + EETOR (200 mg)	6.76 ± 0.62	48.4 ± 3.26	0.28 ± 0.08
VIII	PCM (1g) + EETOR (400 mg)	7.24 ± 0.28	74.8 ± 3.8	0.34 ± 0.12
IX	PCM (1g) + Silymarin (140 mg)	15.36 ± 0.42	116.6 ± 5.22	0.72 ± 0.06

Note: means ± SEM (n = 6); ANOVA.

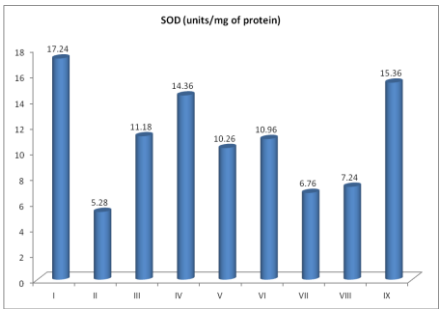

Figure 4: Effect of EETOP, EETOL, EETOR and Silymarin on SOD.

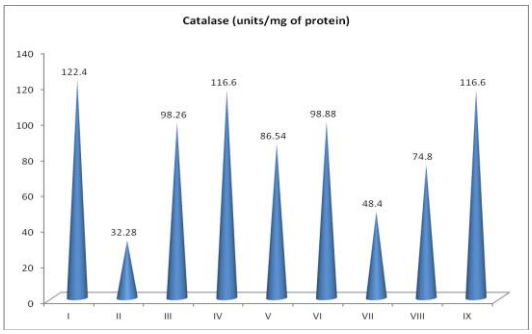

Figure 5 : Effect of EETOP, EETOL, EETOR and Silymarin on Catalase.

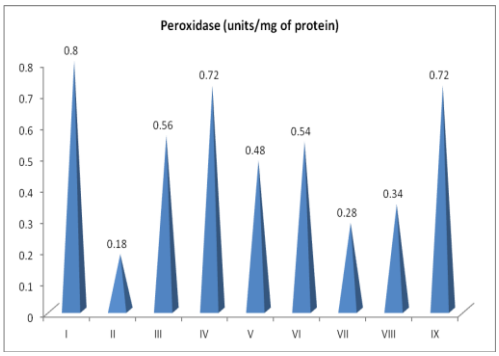

Figure 6 : Effect of EETOL, EETOP, EETOR and Silymarin on Peroxidase.

EETOL, EETOP and EETOR extracts produced potential antioxidant activity dose dependently by preventing cell membrane oxidation. Polyphenol, tannins, flavonoids and bitter principles in EETOL, EETOP and EETOR extracts induced antioxidant activity through significant superoxide radical scavenging activity, inhibition of lipid oxidation and by protecting glutathione in liver.

Table 7 : Toxicity (mortality) of EETOL, EETOP, and EETOR.

S. No.	EETOL (mg/kg.b.wt.)	EETOP (mg/kg.b.wt.)	EETOR (mg/kg.b.wt.)	Percent mortality (%)
1.	50	50	50	0
2.	100	100	100	0
3.	250	250	250	0
4.	500	500	500	0
5.	750	750	750	0
6.	1000	1000	1000	0
7.	1250	1250	1250	0
8.	1500	1500	1500	0
9.	1750	1750	1750	0
10.	2000	2000	2000	0

Table 8 : EETOL, EETOP and EETOR effects on blood parameters.

Parameter	Control Group	Groups (Dose: 400 mg/kg p.o.)		
		EETOL	EETOP	EETOR
RBC Count	8.16 ± 0.35	8.82 ± 0.87	8.92 ± 0.68	9.17 ± 1.14
Hemoglobin (Hb)	14.24 ± 092	14.72 ± 0.86	14.86 ± 0.32	14.81 ± 0.98
Hematocrit (Ht)	49.31 ± 2.45	51.63 ± 3.34	52.62 ± 3.14	51.87 ± 3.62
MCV	51.11 ± 5.04	51.04 ± 4.32	51.84 ± 4.62	52.14 ± 5.24
MCH	17.86 ± 1.22	18.12 ± 2.21	18.54 ± 2.26	18.72 ± 1.62
Platelet Counts	805.2 ± 68.72	814.4 ± 58.74	818.6 ± 62.82	828.44 ± 66.32
WBC Count	7.18 ± 1.32	7.34 ± 1.38	7.42 ± 1.28	7.56 ± 1.46
Neutrophils Count	23.52 ± 2.13	23.64 ± 3.74	23.82 ± 3.24	23.96 ± 3.46
Eosinophils Count	1.36 ± 0.54	1.42 ± 0.62	1.48 ± 0.74	1.54 ± 0.62
Basophils Count	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocyte Count	69.72 ± 6.53	67.86 ± 6.54	68.26 ± 6.78	69.24 ± 5.84

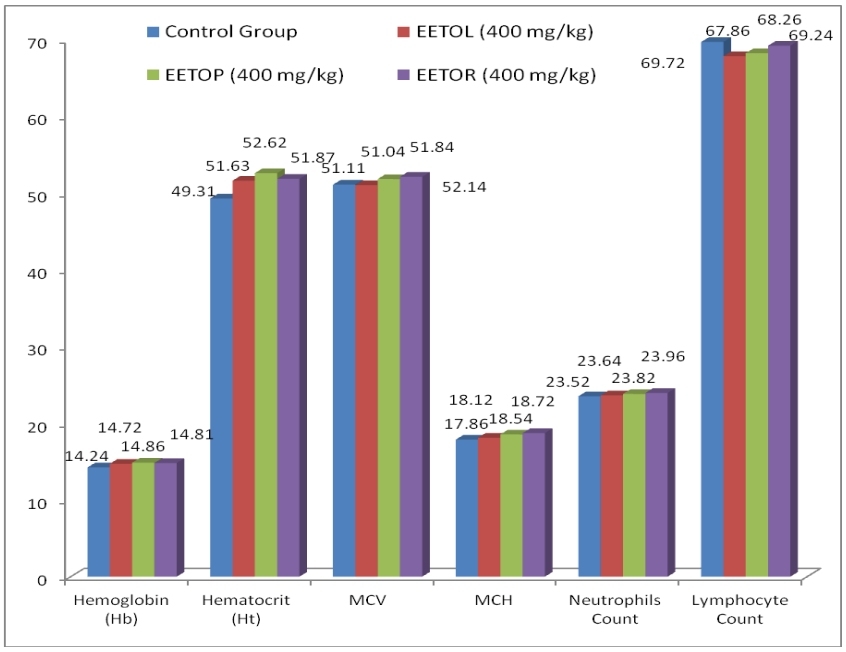

Figure : Effects of EETOL, EETOP and EETOR on blood parameters.

Table 9 : EETOL, EETOP and EETOR effects on blood chemistry and LFTs.

Parameter	Control Group	Groups (Dose: 400 mg/kg p.o.)		
		EETOL	EETOP	EETOR
Glucose	71.72 ± 6.24	63.68 ± 7.56	66.42 ± 8.44	68.28 ± 7.86
Creatinine	0.84 ± 0.12	0.86 ± 0.14	0.90 ± 0.08	0.94 ± 0.08
BUN	19.18 ± 1.62	18.86 ± 1.32	18.56 ± 1.24	18.46 ± 1.28
Tbil	0.34 ± 0.04	0.36 ± 0.06	0.38 ± 0..06	0.40 ± 0.08
SGPT	34.62 ± 4.28	34.62 ± 4.42	36.26 ± 4.18	40.26 ± 4.14
SGOT	48.6 ± 3.54	48.68 ± 2.64	50.42 ± 2.12	51.12 ± 2.52
AKLP	17.14 ± 4.42	17.34 ± 4.12	17.54 ± 4.36	18.24 ± 4.44
TC	54.86 ± 5.72	53.62 ± 5.36	55.14 ± 4.64	56.24 ± 4.76
T-Prot.	4.62 ± 0.26	4.60 ± 0.22	4.64 ± 0.48	4.68 ± 0.34
ALB	3.14 ± 0.08	3.16 ± 0.04	3.18 ± 0.04	3.20 ± 0.06

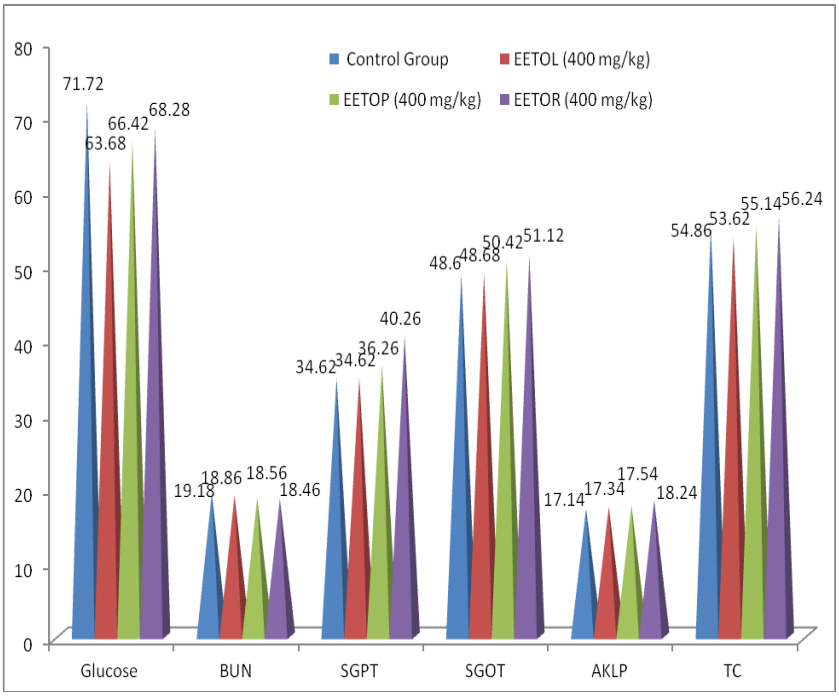

Figure : EETOP, EETOL and EETOR effect on LFTs.

In safety and toxicity evaluation studies, mortality rates were zero percent (no mortality reported even at the

dose of 2000 mg/kg. body weight). So, dandelion extracts (EETOL/EETOP/EETOR) possess wide safety margin and classified as Non-Toxic Constituent. Besides, Extracts caused slight change blood parameters and LFTs were also not significantly altered. (Table 7-9; Figure 7-8).

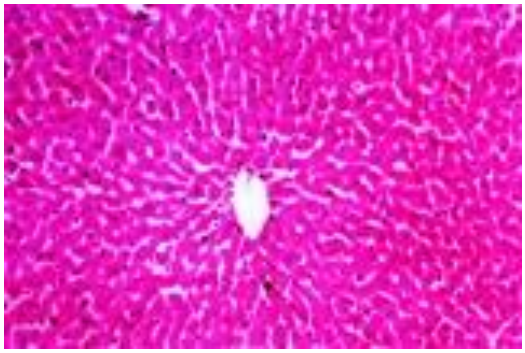

Figure 9: TS of normal liver (Group-I).

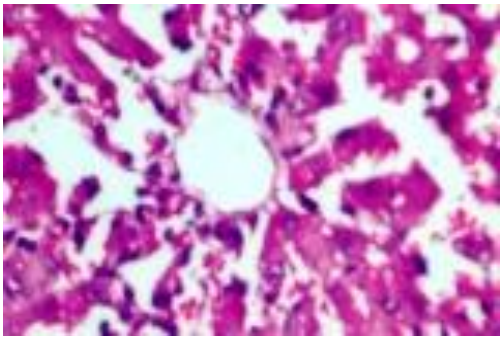

Fig.10: TS of PCM induced toxicity (Group II).

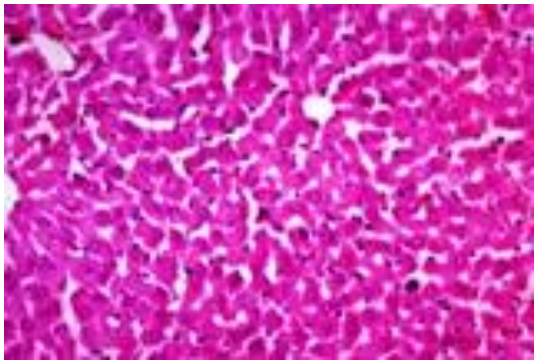

Fig. 11: TS of EETOL (400 mg) treated liver.

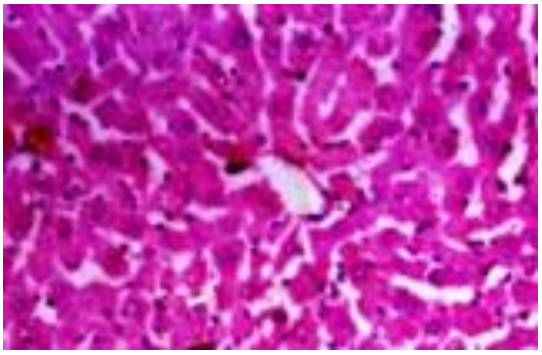

Fig. 12: TS of Standard reference drug i.e. Silymarin treated liver.

Table 10: Effects of EETOP, EETOL and EETOR on LFTs in PCM induced hepatotoxicity.

GROUP	SGPT (Units/ml)	SGOT (Units/ml)	Serum albumin	Total Protein	Serum alkaline phospha-tase	T. Bil. (mg/dl)
Group I (Normal) **Fig. 9**	34.8 ± 2.46	38.6 ± 2.28	3.58 ± 0.34	4.76 ± 0.32	18.12 ± 1.42	0.22 ± 0.14
Group II (PCM Control) **Fig. 10**	188.6 ± 4.48	198.6 ± 2.82	3.93 ± 0.42	4.96 ± 0.42	34.2 ± 2.2	0.88 ± 0.18
Group *III* (EETOL; 200 mg/kg)	136.4 ± 2.8	166.8 ± 2.34	3.64 ± 0.38	4.90 ± 0.28	27.6 ± 1.46	0.66 ± 0.18
Group *IV* (EETOL; 400 mg/kg) **Fig. 11**	116.2 ± 2.6*	126.4 ± 2.18*	3.60 ± 0.34*	4.86 ± 0.26*	25.9 ± 1.36*	0.62 ± 0.16*
Group *V* (EETOP; 200 mg/kg)	156.2 ± 2.34	168.4 ± 1.52	3.72 ± 0.28	4.90 ± 0.18	27.6 ± 0.42	0.74 ± 0.62
Group *VI* (EETOP; 400 mg/kg)	126.6 ± 2.24	138.8 ± 1.56	3.68 ± 0.28	4.82 ± 0.16	26.4 ± 0.44	0.68 ± 0.26
Group *VII* (EETOR; 200	168.8 ± 3.52	174.2 ± 2.46	3.86 ±	4.90 ±	31.4 ± 3.18	0.82 ±

Note :

mg/kg)			0.24	0.36		0.26
Group *VIII* (EETOR; 400 mg/kg)	148.2 ± 3.56	154.2 ± 2.16	3.82 ± 0.46	4.86 ± 0.32	29.2 ± 3.44	0.78 ± 0.24
Group IX (Silymarin) **Fig. 12**	78.6 ± 2.36*	87.8 ± 1.42*	3.58 ± 0.32*	4.68 ± 0.32*	22.6 ± 0.62*	0.38 ± 0.12*

Significance difference from PCM group (Group II). *P<0.05

Animals of Group I was given water *ad libitum* and on eighth blood samples were collected from retro-orbital sinus and biochemical parameters / LFTs of liver and histopathological slides were assessed (Table 10 ; Figure 9-13).

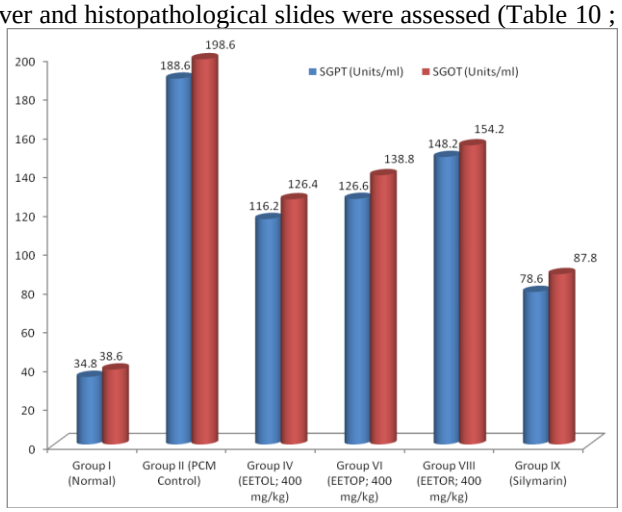

Figure 13: Effects of EETOL, EETOP, EETOR & Silymarin on SGPT & SGOT.

The administration of PCM (1gm/kg) for 03 weeks induced severe liver damage (manifested in 15-21 days of ingestion of toxic dose) which include glutathione depletion, necrosis of hepatocytes, elevated levels of plasma amino-transferases, and increased concentration of billirubin. Biopsy of the showed centilobular necrosis (which include necrosis, degeneration, and infiltration) and rise in the level of LFTs were correlated with the hepatic lesions produced. (Table 10; Figure 10)

Administration of EETOL extract (400 mg/kg) for 02 week (Group IV) produced significant alleviation of the serum enzyme activities. EETOL produced significant reversal effects which were clearly indicated by reddish coloration, normal anatomical architecture of the hepatocytes. EETOL showed a remarkable anti-hepatotoxic activity (Table 10; Figure 11) but comparatively lesser then standard reference drug i.e. Silymarin (Group IX; Table 10; Figure 12) and data represented in Table 10 was analyzed by ANOVA.

Table 11: Effect of Extracts on thiopentone induced sleeping time & liver weight.

Group	Thiopentone sod. induced sleeping time		Liver wt (g/100g bw)
	Onset (s)	Duration (min)	
I (Normal control)	204.52 ± 4.92	76.64 ± 4.92	3.94 ± 0.50
II (Toxic Control)	54.32 ± 4.24a	242.36 ± 4.72a	6.44 ± 0.30a
III (EETOL 400)	156.46 ± 4.86***	130.52 ± 6.28***	5.22 ± 0.12*
IV (EETOP 400)	132.32± 5.44	154.62 ± 6.28	5.66 ± 0.14
V (EETOR 400)	66.34 ± 4.22	214.42 ± 4.62	6.26 ± 0.18
Group VI (Silymarin)	178.18 ± 9.46***	122.64 ± 6.54***	4.82 ± 0.16*

Values are mean ± SEM of 6 animals in each group
aP < 0.001 relative to control group; ***P < 0.001 relative to Toxicant group
*P < 0.05 relative to Toxicant group

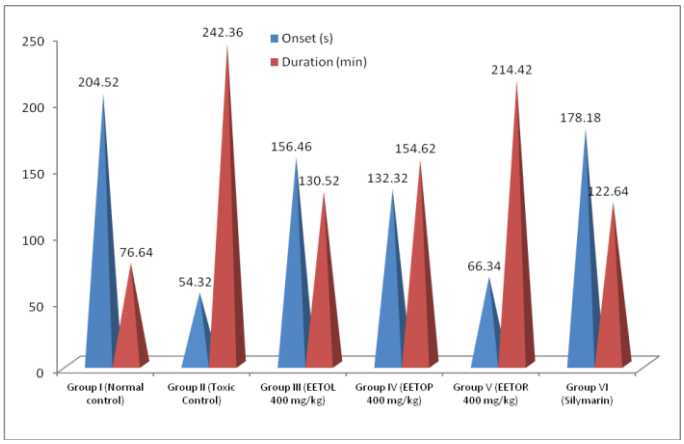

Figure 14 : Effect of Thiopentone sodium on onset and duration of sleeping time.

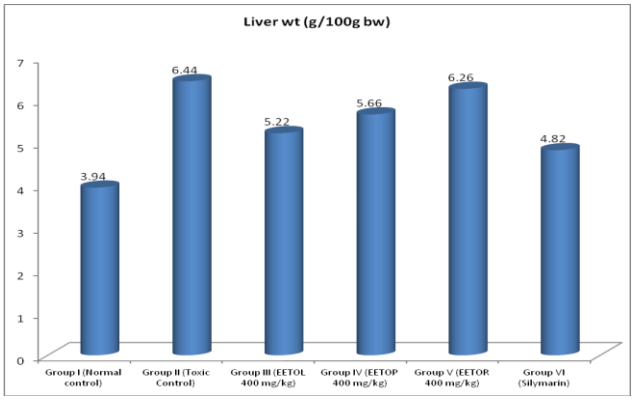

Figure 15: Effect of Thiopentone sodium on Liver Weight (g/100g bw).

Sleeping time / pattern were normal in Group-I animals. Administration of PCM (1gm/kg) for 03 weeks induced severe liver damage. Subsequently, PCM induced hepatotoxicity exhaust glutathione stores and leads to necrosis of hepatocytes and liver weight were also increased in toxic control group animals. Reduction in thiopentone sodium induced sleeping-time was recorded with EETOL when compared to PCM treated (Table 11; Figure 14-15).

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