"CHEMICAL AND BIOLOGICAL SCREENING OF *ARTEMISIA PALLENS (ASTERECEAE FAMILY)"*

A MINOR RESEARCH PROJECT PROPOSAL FOR FINANCIAL ASSISTANCE

COMPLETION REPORT
JUNE 2014

SUBMITTED TO

UNIVERSITY GRANTS COMMISSION
NEW DELHI

BY

Mrs.Varsha Sanjeev Honmore
M. Sc. (Organic Chemistry)

Abasaheb Garware arts and science college Pune 411004 INDIA.
NAAC ACCREDITED A GRADE
(AFFILIATED TO UNIVERSITY OF PUNE)

UNIVERSITY GRANTS COMMISSION
COMPLETION REPORT
Reference: UGC (WRO) sanction letter no. F.47-1749/10(WRO) dt.12.5.2011

CONTENTS

ANNEXURE-I
Actual submitted proposal

PROJECT TITLE

"CHEMICAL AND BIOLOGICAL SCREENING OF*ARTEMISIA PALLENS (ASTERECEAE FAMILY)"*

INTODUCTION

Artemisia pallens wall is a shrub endemic to south India generally found in Karnataka, Tamilnadu, Andhrapradesh and in Maharashtra. Leaves are very small, bluish green with yellow flowers and inconspicuous.

The chemical components of *Artemisia pallens* were found to be Cis-davanone,isodavanone and nondavanone.The oil contains linalool,dehydro-1-linalool,terpinen-4-ol and four devanafurans. These davanafurans were found to be responsiblefor the characteristic odour of the oil.The minor constituents of the oil are camphene,P-cymene,sabinene,3-terpinene,1,8-cineol,borneol,isoborneol,geraniol,eugenol,methylleugenol,methyllisoeugenol,eugenol, aceto-eugenol,γ-cadinene and farnesol(1).

The chemical composition of the oil Artemesia Pallens has been investigated by the number of research groups(2). Recently, Lamparsky et al(3),tried to summarize the role of minor components in importing the delicate and exquisite aroma of the oil.It has been found that devanone,the major component of the oil,is odorless when purified rigorously. Sipma G et al(4) isolated and characterized devanone I,a sesquiterpene ketone,Nageli et al(5) isolated artemone II from the essential oil of Artemesia Pallens,which was also synthesized Thomas et al (6) reported the isolation structure elucidation and synthesized of davana ether III,an odoriferous compound. The photosensitized oxidation of devanone I to get hemiacetal IV and an allyl alcohol V was reported(6).Epoxidation of davanone I yielded a stereo-isomeric mixture ofdenone ether III(7). Thomas et al(8) reported the isolation and synthesis of nordavanone VI,aterpenoid,a sesquiterpenoid VII (9) and four furan type stereo isomers of devana.(10)

Lamparsky D et al (3) reported same analytical results of devana oil with respect to biogenetically possible structural features.Akhila et al (11) reported a novel biosynthesis of irregular sesquiterpene artemone II.Chandra A et al (12) isolated a dihydrofuranoterpenoid VIII-XIII from the essential oil.Catalan et al (13) reported sesquiterpene ketone from the extract of aerial parts of devana,these compounds included mainly 3,4 epoxy derivative of isodavanone XIV-XVI.Misra et al (14) reported 34 fragment components of devana oil.Rojatkar el al (15) reported a germacranolide XVII from aerial parts. Further chemical investigations by Pujar et al (16) have reported one more germacranolide XVIII from aerial of Artemesia Pallens.

ORIGIN OF RESEARCH PROBLEM:
Isolation and characterization of biologically active molecule.

INTERDISCIPLINARY RELEVANCE:
Collaboration with Botany, Microbiology and Pharmacology departments.

INDIAN STATUS :
Artemisia Pallens was cultivated in South India for its fragrant leaves and flowers, which are used in floral decorations and religious offerings. Two distinct morphological types have been isolated in which the flowering is in different seasons (17). The leaves and flowers yield an essential oil. The oil has delicate aroma and is used in high-grade perfumes. Davana oil is obtained by steam distillation of the dried plant in the yield of 0.22-0.58 %.It is much prized in India for its delicate fragrance and is chiefly used in floral compositions. The high cost of the oil permits its use only in expensive perfumes compositions. Artemisia Pallens are under commercial cultivation for Oil production, however, it is not attained wide popularity, perhaps because of its high price.

INTERNATIONAL STATUS:
Nevertheless, the USA,Europe and Japan have shown increasing interest in the oil, mainly for use in the flavoring of cakes,pastries,Tobacco and also some costly beverages(18). "Effects of **Artemisia pallens** Wall on Blood Glucose Levels in sesquiterpene lactones from Artemisia judaica was studied in US (Yr 2002).

SIGNIFICANCE OF STUDY :

An ethanomedical search conducted by Tropical Botanic Garden and research Institute ,Palode,Trivendrum(India),revealed that this plant is used as folk medicine for the treatment of diabetes mellitus. For the first time the anti-hyper glycaimice effect of *artemesia pallens* suggested that the active principles from this plant are effective for the treatment of diabetes.The extract of *Artemesia Pallans* led to significance blood glucose lowering effect in glucose-fedhyperglycmic and alloxan- induced diabetic rats.This effect of the extract was dose dependant and significant at 100-mg/kg levels in glucose-fed rats and fasted normal- rats ,the extract cause the moderate hypoglycemic effect at a higher dose(1000 mg/kg).The water extract of this plant was inactive (19).We are planning to isolate and characterize the bioactive molecule from *Artemisia pallens*.

OBJECTIVES :

1. Isolation of sesquiterpene lactones and biologically active compounds from *Artemisia Pallens*.
2. Feedback obtained from the reported activity of the Artemisia pallens from the other countries will be useful in deciding the details of bio-evaluation.
3. The compounds found active will be isolated in more quantities, their simple derivative will be prepared and the bio activity of all the compounds will be determined. This will finally lead to the structure activity relationship.

4.The specific objectives are as follows:

- Collection of Artemisia Pallen plant material and shade dry.
- Fine grinding of plant material.
- Extraction and fractionation of the extract.
- Evaluation of anti-diabetic and pharamalogical activities of the extract and the fractions.
- Purification of active fractions and isolation of pure compounds.
- Structure elucidation of the isolated pure compounds by using spectroscopic methods like UV,IR,1HNMR,13CNMR,1D and 2D NMR and LC-mass.
- Semi synthetic studies will be carried out on the isolated molecules.
- Molecules obtained from the above plant will be tested for the biological activity.

METHODOLOGY :

5. Collection of Artemisia Pallen plant material and shade dry.
6. Fine grinding of plant material.
7. Preparation of extract of above plant with different solvents of increasing polarity.
8. Separation and purification of the compounds from extracts by different chromatographic techniques.eg. Column and preparative chromatography.
9. Structure determination of the bioactive molecules using modern spectroscopic methods and chemical conversions.
10. Biological activity of the extract and isolated compounds will be studied in collaboration.
11. The bioassay of the compounds and their derivatives will be carried out to determine their pharmacological activities.

YEAR WISE PLAN OF WORK :

First Year :

- Literature survey of the plant species.
- Collection of plant material.
- Authentication of plant.
- Extraction of plant material using different solvents.
- Column chromatographic seperation of *Artemisia Pallens*.
- Isolation and purification of the molecules from the column fractions of *Artemisia Pallens*.
- Submission of progress report.

Second Year :

- Structure determination of the isolated compounds by using modern spectral methods.
- Chemical transformation work will be carried out on the important isolated molecules.
- Biological activity of the important molecules will be studied.
- Submission of Final Report.

(a) Amount sanctioned

	Item	Expenditure
i)	Field Work and Travel	10,000.00
ii)	Chemicals and glassware	50,000.00
iii)	Contingency	10,000.00
iv)	Equipments	30,000.00
iv)	Books and Journals	20,000.00
v)	Special needs	25,000.00
	Total	1,45,000.00

10. Whether teacher has received support for the research for the research project from the UGC under Major,Minor,scheme support for the research or from any agency?If so,please indicate: No.

(i) Name of the agency from which the assistance was approved.- No

(ii) Sanction letter No.and date under which the assistance was approved: No.
(iii) Amount approved and utilized :
(iv) Title of the project for which assistance was approved : NIL

(v) In case the project was completed, whether the work on the project has been published. No

(vi) If the candidate was working for the doctoral degree, whether the thesis was submitted and accepted by the university for the award of degree. NA

(vii) If the project has not been completed, please state the reasons.-NIL

11. Any other information, which the investigator may like to give in support of this proposal, which may be helpful in evaluating. NIL

ANNEXURE-II
WORK DONE:

YEAR WISE PLAN OF WORK:

First Year:

- Literature survey of the plant species.
- Collection of plant material.
- Authentication of plant.
- Extraction of plant material using different solvents.
- Submission of progress report.

Second Year:

- Crude methanol extract showed promising hepatoprotective activity.
- In conclusion, oral administration of *Artemisia pallens* promoted renal and hepatic antioxidant enzyme activity to protect against paracetamol induced hepatotoxicity as well as renotoxicity.
- Results of present investigation suggest that *Artemisia pallens* may find immense therapeutic potential in clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress. However, further study is in progress for elucidation of actual mechanism of action of *Artemisia pallens* at molecular level.

Introduction: Acetaminophen (APAP) i.e. paracetamol, has been the preferred medication for treatment of pain especially in children suggesting that it is safer and perhaps more efficacious than aspirin (Ahmad *et al*., 2012). APAP exert its analgesic potential via formation of its conjugated metabolite i.e. *p*-aminophenol with arachidonic acid by fatty acid amide hydrolase to form archidonic acid metabolite which exerts its effect through cannabinoid receptors (Anderson *et al*., 1999). APAP does not produce any gastrointestinal complication or affects blood coagulation or impair renal functions within the recommended doses. However, in the cancer patients who receive chemotherapy anticancer drug are usually concomitantly administered with higher doses of APAP to relieve pain (Nassar *et al*., 2009). Therefore, APAP toxicity at higher doses is usually very common and is often associated with hepatic (Nelson, 1990) as well as renal damage (Ghosh & Sil, 2007) in clinical settings.

Moreover, APAP induced toxicity is a well established animal model to screen various hepatoprotective agents (Kandhare *et al*., 2011b). It has been reported that administration APAP results in hepatic and renal damage in humans as well as in experimental animals, although nephrotoxicity is less common than hepatotoxicity (Larson *et al*., 2005). The hepatic and renal damage by administration of APAP has complex events. Primarily APAP is converted into water-soluble metabolites in the liver via glucuronidation as well as sulfuration reactions and the metabolites are excreted via kidney. The microsomal CYP-450 enzyme system (Cytochrome P450 (CYP) 2E1 and CYP 3A4) plays a vital role in the metabolism of APAP. During the biotransformation of APAP, a highly reactive intermediate N-acetyl-*p*-benzoquinoneimine (NAPQI) is produced (Mitchell *et al*., 1973). NAPQI reacts with intracellular glutathione (GSH)

and causes depletion of GSH. Moreover, NAPQI also bind to cellular proteins and initiate lipid peroxidation, leading to renal (Hart *et al*., 1994) and hepatic injury (Mitchell *et al*., 1977). In a recent study involvement of various mechanisms beside GSH depletion by NAPQI in hepatic and renal tissue by APAP has been reported. Formation of reactive oxygen species (ROS), nitrogen species and macrophages have been identified to be involved in the development of APAP toxicity (Michael *et al*., 1999).

Pharmacotherapy for the management of APAP induced toxicity is multivariate. Aminoguanidine, zinc sulfate, lobenzarit, N-acetylcysteine are treatment options for APAP induced toxicity (Michael *et al*., 1999; Prescott, 1979). However, these synthetic moieties are associated with severe undesirable adverse effects which limit their use. Now a days, orthotopic liver transplantation (OLT) is a therapeutic option for liver failure caused by APAP (Starzl *et al*., 1987). OLT also depends upon defined prognostic factors for the effective management of APAP toxicity. Therefore, search for novel agents such as plant derived products and their bioactive compound has been carried out management of APAP toxicity.

In Ayurveda, the Indian system of medicine a number of medicinal plants has been recommended as treatment option for liver and kidney diseases (Subramoniam & Pushpangadan, 1999). At present, for the treatment of liver cirrhosis and alcoholic liver diseases extensively used plant derived phytoconstituent is silymarin which is a polyphenolic flavanoid isolated from the fruits and seeds of *Silybum marianum* (L.) Gaertn. (Asteraceae) which acts via its antioxidant potential by inhibiting lipid peroxidation (Valenzuela & Garrido, 1993). Hence in the present investigation silymarin was used as a standard hepatoprotective drug (positive control).

Artemisia pallens Walls ex D.C. (Davana) (Asteraceae) is an aromatic medicinal herb native in the southern part of India, especially in the states of Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra. Number of researchers has reported chemical composition of oil obtained from *A. pallens* plant (Sipma & Van der Wal, 1968; Rojatkar *et al*., 1996; Pujar *et al*., 2000). The oil of *A. pallens* is used as a flavoring agent for cakes, pastries, tobacco, and in some costly beverages. Reported pharmacological properties of the plant are anthelmintic, tonic, antipyretic, antidiabetic, antifungal, antibacterial, antimicrobial, antioxidant, analgesic and anti-inflammatory activity (Ashok & Upadhaya, 2010; Ruikar *et al*., 2011). However, effectiveness of *Artemisia pallens* in APAP induced toxicity has not been yet reported. The objective of the present investigation was to evaluate protective effect of methanolic extract of *Artemisia pallens* in APAP induced toxicity by studying various biochemical and molecular parameters along with histopathological aberration in kidney and liver of rats.

Material and method

Animals: Adult male Wistar rats (180-220 g) were obtained from the National Institute of Biosciences, Pune (India). They were housed in cages in animal house maintained at 24 ± 1^{o}C, with relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro Industries Ltd., Sangli, India) and filtered water throughout the experimental period. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of

Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. Animals were transferred to testing laboratory 1 h before the experiment for adaptation purpose.

Chemicals: 1,1´,3,3´-Tetraethoxypropane, crystalline beef liver catalase, reduced glutathione (GSH), 5,5´-dithiobis (2-nitrobenzoic acid) were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthalamine diamine HCl and phosphoric acid were obtained from LobaChemi Pvt. Ltd., Mumbai, India. APAP and silymarin were obtained as a gift sample from Symed Pharmaceutical Pvt. Ltd.

Preparation of extract: Arial parts of *Artemisia pallens* plant were collected from Jejuri, Maharashtra state, India in the month of December, 2011. The plant was authenticated by Dr. Mrs. Upadhey, Department of Botany, Agharkar Research Institute, Pune, India. The voucher specimen of plant material was maintained under the reference number WP-091. The methanolic extract was prepared by macerating air dried and course powdered plant material (500 g) with methanol (5 L) for 24 h at room temperature. The extract was filtered and the filtrate was concentrated *in vacuo* at 40^0 C. This process was repeated three times. The yield of methanolic extract was 52.50 g. The aqueous solution of methanolic extract of *Artemisia pallens* in DMSO was prepared for the pharmacological evaluation.

Induction of APAP induced toxicity and drug treatment schedule

The selection of dose for APAP was based on the studies carried out previously (Ahmad *et al*., 2012). Silymarin was administered to rats orally at a dose of 25 mg/kg for 14 days (Hegde & Joshi, 2010). APAP (1% aqueous solution of gum acacia) was administered to rats orally at a dose of 700 mg/kg (Ahmad *et al*., 2012). Fasted rats were randomly divided into 6 groups of 6 rats as follows:

Group I: Normal control: Rats were administered single daily dose of DMSO (10 mg/kg), p.o. for 14 days.

Group II: APAP: Rats that served as the model control was administered single daily dose of DMSO (10 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

Group III: Silymarin (25): Rats were administered single daily dose of Silymarin (25 mg/kg, p.o.) in distilled water, 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

Group IV: APME (100): Rats were administered single daily dose of APME (100 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

Group V: APME (200): Rats were administered single daily dose of APME (200 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

Group VI: APME (400): Rats were administered single daily dose of APME (400 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

After 14 day treatment, rats were fasted overnight and after 24 h, were sequentially anesthetized with anesthetic ether for about 30-40 s. The blood was withdrawn by retro orbital puncture. Each blood sample was collected into separate vials for determination of serum parameters. After blood collection the animals were sacrificed by cervical dislocation and then liver as well as kidneys were removed. The specimens were divided into two portions; one portion was used for biochemical estimation and other portion was processed for histopathological examination.

Serum biochemistry: The serum was separated by centrifugation using Eppendorf Cryocentrifuge (model No. 5810, Germany), maintained at 4 ^{o}C and run at speed of 7000 rpm for 15 min. The levels of serum albumin, ALP (Alkaline Phosphatase), BUN (Blood Urea Nitrogen), cholesterol, creatinin, direct Billurubin, HDL (High-density lipoprotein), LDH (Lactate dehydrogenase), LDL (Low-density lipoprotein), AST (Aspartate transaminase), ALT (Alanine transaminase), triglyceride, total Billurubin and uric acid were measured by spectrophotometer (UV-Visible spectrophotometer, Jasco V-530, Japan) using commercially available reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India).

The biochemical estimations

Preparation of tissue homogenate: For liver and kidney homogenization, tissue segments were mixed with 0.1 M phosphate buffer and homogenized on ice for 60 sec at 10000 r.p.m. in a homogenizer (Remi Equipment Pvt. Ltd., Remi Motors Ltd., Mumbai, India). Supernatant of tissue homogenates was employed to estimate superoxide dismutase (SOD), reduced GSH, lipid peroxidation (malondialdehyde content) and nitric oxide (NO content).

Determination of total protein, SOD, GSH, MDA and NO

The level of total protein, SOD, GSH, MDA and NO in liver and kidney homogenate were determined according to earlier reported methods (Gosavi *et al*., 2012; Kandhare *et al*., 2011a; Kandhare *et al*., 2013a, b, c).

Histopathological examination: Liver and kidney tissues were stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70, 90 and 100%) for 2 h respectively. The infiltration and impregnation was carried out by treating with paraffin wax twice, each time for one h. Tissue specimens were cut into sections of 3-5µm thickness and were stained with hematoxylin and eosin (H&E). The specimen was mounted on slide by use of Distrene Pthalate Xylene (DPX) as mounting medium. Sections were examined under a light microscope for inspection of the histopathology features of specimen and infiltration of cells. The various changes in histological features were graded as Grade 0 (not present or very slight); Grade 1 (mild); Grade 2 (moderate); and Grade 3 (severe) as described earlier (Yamasaki *et al*., 2001).

Statistical analysis: Data were expressed as mean ± standard error mean (SEM). Data analysis was performed using software (v 5.0, Graph Pad, San Diego, CA, USA). Data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA), and Dunnett's test was applied for post hoc analysis. A value of $P < 0.05$ was considered to be statistically significant.

Results

Effect of treatment of APME on APAP induced alteration in body weight and relative organ weight in rats.

There was significant ($P < 0.001$) decrease in body weight of APAP rats as compared to normal rats. Whereas relative organ weights of liver and spleen were significantly ($P < 0.001$) increased in APAP rats as compared with normal rats. Treatment with APME (200 and 400 mg/kg, p.o.) did not show any significant change in body weight as compared to APAP rats but, relative liver weight as well as relative spleen weight were significantly ($P < 0.001$) decreased as compared to APAP rats. Administration of silymarin (25 mg/kg, p.o.) for 14 days significantly ($P < 0.01$) increased body weight as compared to APAP rats whereas relative liver weight and spleen weight were significantly ($P < 0.001$) decreased as compared to APAP rats (Table 1).

Table 1. Effect of treatment of APME on body weight, liver weight and spleen weight in paracetamol induced toxicity in rats.

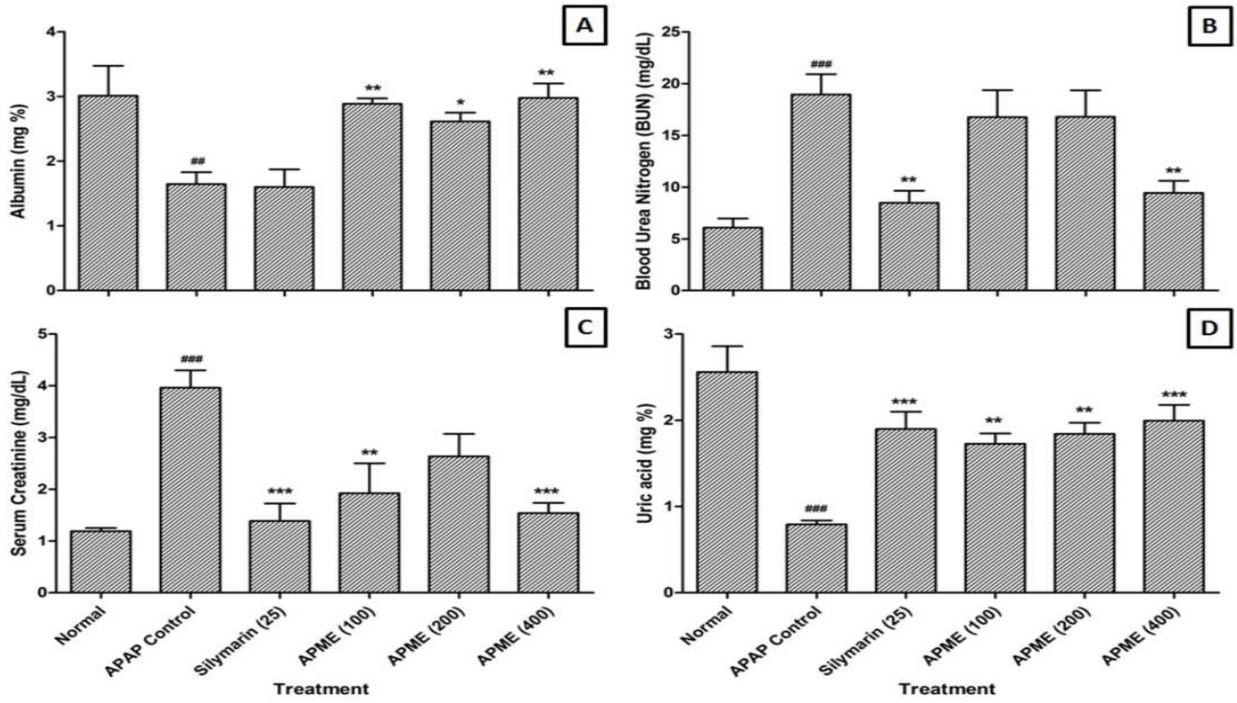
Treatment	Body weight (BW) (gm)	Liver weight (LW) (gm)	LW/BW ratio	Spleen weight (SL) (gm)	SL/BW ratio
Normal	257.16 ± 5.00	5.12 ± 0.26	0.019 ± 0.001	0.36 ± 0.02	0.001 ± 0.00008
APAP	224.66 ± 6.03$^{###}$	8.86 ± 0.26$^{###}$	0.03 ± 0.001$^{###}$	0.92 ± 0.03$^{###}$	0.004 ± 0.0001$^{###}$
Silymarin (25)	253.50 ± 7.29**	6.52 ± 0.55***	0.025 ± 0.002***	0.40 ± 0.05***	0.001 ± 0.0002***
APME (100)	246.00 ± 2.87	7.68 ± 0.39	0.03 ± 0.001*	0.73 ± 0.04**	0.002 ± 0.0001***
APME (200)	243.00 ± 4.95	6.70 ± 0.32***	0.027 ± 0.001***	0.59 ± 0.03***	0.002 ± 0.0001***
APME (400)	244.33 ± 2.23	5.92 ± 0.27***	0.024 ± 0.001***	0.45 ± 0.04***	0.002 ± 0.0001***

Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as compared to APAP group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal group.

Effect of treatment of APME on APAP induced alteration in serum albumin, blood urea nitrogen, serum creatinine and serum uric acid in rats

The level of serum albumin and serum uric acid were significantly ($P < 0.01$ and $P < 0.001$) decreased in APAP rats as compared to normal rats whereas blood urea nitrogen and serum creatinine level were significantly ($P < 0.001$) increased in the APAP rats as compared to normal rats. Chronic administration of APME (100, 200 and 400 mg/kg, p.o.) for 14 days significantly increased serum albumin ($P < 0.01$, $P < 0.05$, $P < 0.01$, reps.) and serum uric acid ($P < 0.01$, $P < 0.01$ and $P < 0.001$, reps.) level whereas the level of blood urea nitrogen and serum creatinine were significantly ($P < 0.01$ and $P < 0.001$) decreased by treatment of APME (400 mg/kg, p.o.) as compared to APAP rats. When compared with APAP rats, the serum uric acid levels were significantly ($P < 0.001$) increased in silymarin (25 mg/kg, p.o.) treated rats whereas the levels of blood urea nitrogen and serum creatinine were significantly ($P < 0.01$ and $P < 0.001$) decreased. However, silymarin (25 mg/kg, p.o.) failed to produce any significant change in serum albumin level as compared to serum albumin level of APAP rats (Fig. 1).

Fig. 1 Effect of treatment of APME on albumin (A), blood urea nitrogen (B), serum creatinine (C) and uric acid (D) in paracetamol induced toxicity in rats.



Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as compared to APAP group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal group.

Effect of treatment of APME on APAP induced alteration in alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT in rats

Chronic administration of APAP caused significant ($P < 0.001$) increase in level of alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT in APAP rats as compared to normal rats. When compared with APAP rats, there was significant ($P < 0.01$ and $P < 0.001$) and dose dependant decrease in the level of total bilirubin, direct bilirubin after chronic treatment of APME (200 and 400 mg/kg, p.o). However, administration of APME (200 and 400 mg/kg, p.o.) significantly ($P < 0.001$) decreased elevated levels of AST and ALT as compared to APAP rats. Administration of APME (100, 200 and 400 mg/kg, p.o.) failed to produce any significant reduction in elevated level of alkaline phosphatase as compared to APAP rats. Silymarin (25 mg/kg, p.o.) administration for 14 days significantly reduced these elevated levels of alkaline phosphatase ($P < 0.05$), total bilirubin ($P < 0.001$), direct bilirubin ($P < 0.05$), AST ($P < 0.001$) and ALT ($P < 0.001$) when compared with APAP rats (Table 2).

Table 2. Effect of treatment of APME on alkaline phosphatase, total bilirubin, direct bilirubin, SGOT and SGPT in paracetamol induced toxicity in rats.

Treatment	Alkaline Phosphatase (IU/I)	Total Bilirubin (mg %)	Direct Bilirubin (mg %)	AST (IU/I)	ALT (IU/I)
Normal	39.28 ± 3.03	0.094 ± 0.02	0.18 ± 0.04	125.8 ± 7.85	21.87 ± 1.87
APAP	217.9 ± 15.83$^{###}$	0.29 ± 0.02$^{###}$	0.54 ± 0.08$^{###}$	293.5 ± 29.15$^{###}$	142.0± 10.24$^{###}$
Silymarin (25)	126.2 ± 8.92*	0.12 ± 0.02***	0.30 ± 0.02*	146.4 ± 19.81***	33.04 ± 7.70***
APME (100)	199.7 ± 25.15	0.21 ± 0.02	0.33 ± 0.02*	248.3 ± 15.74	116.6 ± 12.98
APME (200)	211.6 ± 34.03	0.17 ± 0.02*	0.29 ± 0.02**	316.8 ± 27.90	137.5 ± 17.93
APME (400)	171.3 ± 15.29	0.12 ± 0.01***	0.17 ± 0.04***	133.3 ± 4.90***	39.06 ± 12.15***

Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as

compared to APAP group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal group.

Effect of treatment of APME on APAP induced alteration in serum cholesterol, HDL, LDL, LDH, triglyceride and VLDL in rats

There were significant ($P < 0.001$) increase in level of serum cholesterol, LDL, LDH, triglyceride and VLDL in APAP rats as compared to normal rats. Chronic administration of APAP for 14 days significantly ($P < 0.001$) decreased level of HDL in APAP rats as compared to normal rats. The level of serum HDL was significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$, reps.) and dose dependently increased by the chronic treatment with APME (100, 200 and 400 mg/kg, p.o.) when compared with APAP rats. Moreover, administration of APME (200 and 400 mg/kg, p.o.) for 14 days significantly and dose dependently prevented APAP caused increase in level of LDH ($P < 0.01$, $P < 0.001$ and $P < 0.001$, resp.), triglyceride ($P < 0.01$ and $P < 0.001$, resp.) and VLDL ($P < 0.01$ and $P < 0.001$, resp.) as compared to APAP rats. The increased levels of serum cholesterol and LDL after APAP administration were significantly ($P < 0.01$) decrease by chronic administration of APME (400 mg/kg, p.o., 14 days) as compared to APAP rats. Silymarin (25 mg/kg, p.o.) significantly ($P < 0.001$) decreased the elevated levels of serum cholesterol, LDL, triglyceride and VLDL as compared to APAP rats. However, administration of silymarin (25 mg/kg, p.o.) did not produce any significant change in the level of HDL and LDH as compared to APAP rats (Table 3).

Table 3. Effect of treatment of APME on cholesterol, HDL, LDL, LDH, triglyceride and VLDL in paracetamol induced toxicity in rats.

Treatment	Cholesterol (mg %)	HDL (mg %)	LDL (mg %)	LDH (mg %)	Triglyceride (mg %)	VLDL
Normal	14.32 ± 1.32	65.32 ± 4.74	1.12 ± 0.31	203.5 ± 48.62	63.12 ± 4.02	12.62 ± 0.80
APAP	48.85 ± 5.40$^{\#\#\#}$	21.54 ±2.81$^{\#\#\#}$	5.63 ±0.70$^{\#\#\#}$	2989.00 ± 362.2$^{\#\#\#}$	159.5 ± 4.49$^{\#\#\#}$	31.90±0.89$^{\#\#\#}$
Silymarin (25)	27.24 ± 2.94***	40.67 ± 5.42	1.64±0.60***	2470.00 ± 192.1	106.5 ± 7.11***	21.29±1.42***
APME (100)	44.28 ± 1.85	47.03 ± 3.77*	4.76 ± 0.48	1829.00±199.7**	133.3 ± 9.30	26.66 ± 1.86
APME (200)	43.15 ± 3.45	57.16 ±8.04**	3.83 ± 0.65	1149.00±252.7***	123.2 ± 7.44*	24.55 ± 1.48*
APME (400)	31.68 ± 1.81**	59.12±6.82***	2.44 ± 0.43**	662.6 ± 127.9***	114.2 ± 12.24**	22.84 ± 2.44**

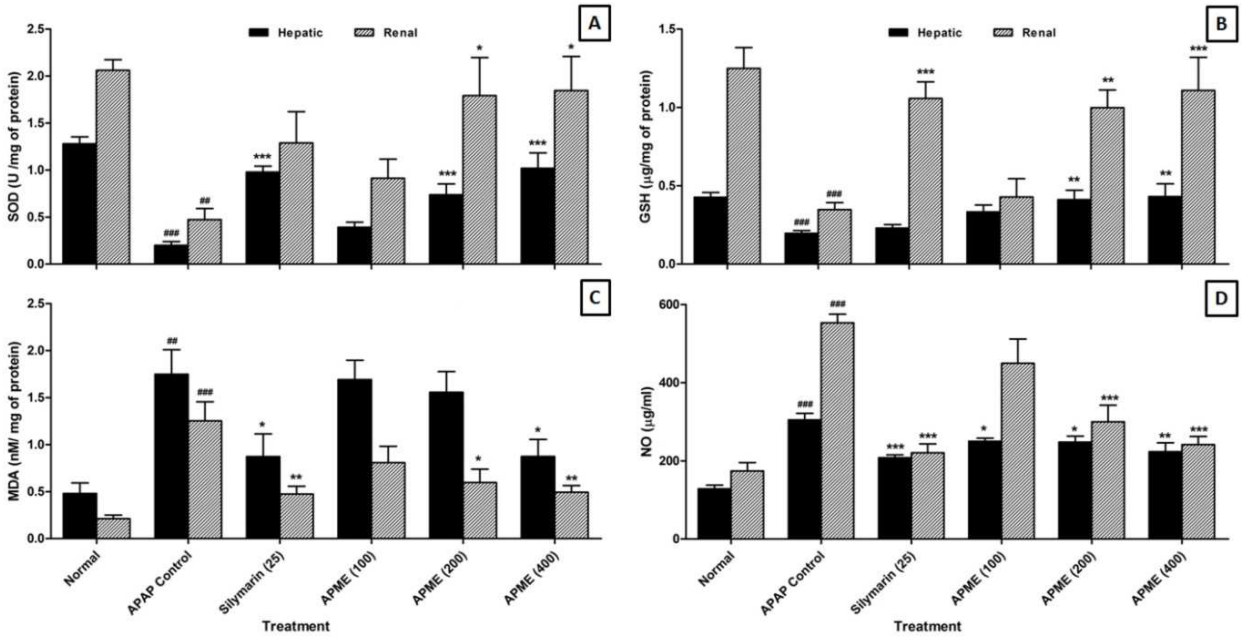
Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as

compared to APAP group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal group. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, LDH: Lactate dehydrogenase, VLDL: Very low-density lipoprotein

Effect of treatment of APME on APAP induced alteration in hepatic as well as renal SOD and GSH in rats

Chronic administration of APAP for 14 days resulted in significant ($P < 0.001$) decrease in level of SOD and GSH in hepatic as well as renal tissue in APAP rats as compared to normal rats. Treatment with APME (200 and 400 mg/kg, p.o.) showed significant elevation in level of hepatic SOD ($P < 0.001$) and renal SOD ($P < 0.05$) as well as hepatic GSH ($P < 0.01$) and renal GSH ($P < 0.001$) as compared to APAP rats. However, administration APME (100 mg/kg, p.o.) for 14 days did not show any significant increase in level of SOD and GSH in both liver as well as kidney when compared with APAP rats. Silymarin (25 mg/kg, p.o.) showed significant ($P < 0.001$) increase in level of hepatic SOD and renal GSH as compared to APAP rats but, it failed to produce any significant increase in the level of hepatic GSH and renal SOD as compared to APAP rats (Fig. 2A and 2B).

Fig. 2 Effect of treatment of APME on hepatic and renal SOD (A), GSH (B), MDA (C) and NO (D) in paracetamol induced toxicity in rats.



Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to APAP group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal group.

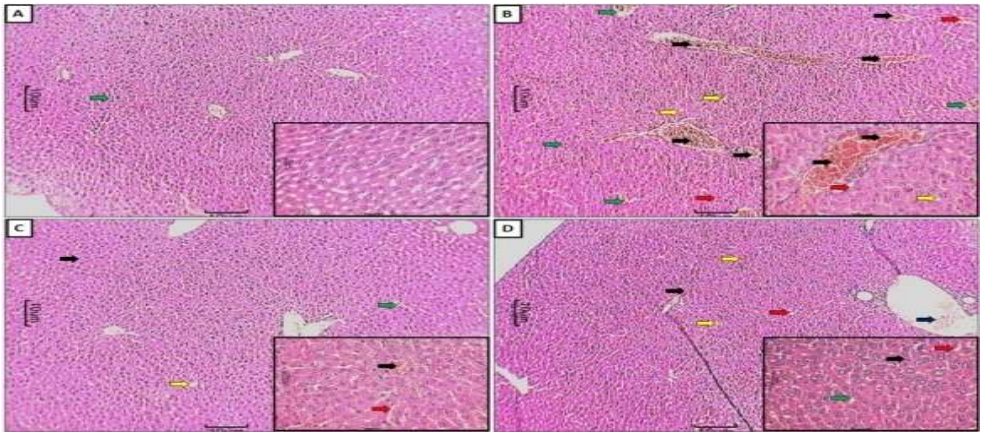
Effect of treatment of APME on APAP induced alteration in hepatic as well as renal MDA and NO in rats

There were significant increase in the levels of hepatic MDA ($P < 0.01$) and NO ($P < 0.001$) as well as renal MDA ($P < 0.001$) and NO ($P < 0.001$) in APAP rats as compared to normal rats. Treatment with APME (400 mg/kg, p.o.) showed significant decrease in hepatic as well as renal MDA ($P < 0.05$) and NO ($P < 0.01$) whereas levels of hepatic NO ($P < 0.05$) and renal MDA ($P < 0.05$) as well as renal NO ($P < 0.001$) were significantly decreased in APME (200 mg/kg, p.o.) treated rats as compared to APAP rats. However, APME (100 mg/kg, p.o.) showed significant ($P < 0.05$) attenuation in hepatic NO as compared to APAP rats. Treatment with APME (200 mg/kg, p.o.) did not show any significant decrease in the level of hepatic MDA as compared to APAP rats. When compared with APAP rats, silymarin (25 mg/kg, p.o.) treated rats showed significant decrease in level of hepatic MDA ($P < 0.05$) and NO ($P < 0.001$) as well as renal MDA ($P < 0.01$) and NO ($P < 0.001$) (Fig. 2C and 2D).

Effect of treatment of APME on APAP induced pathological alteration in rat liver and kidney

In the histopathological studies, normal control animals showed normal central vein in liver parenchymal cells and without any signs of inflammation as well as necrosis in cells (Fig. 3A). However there was evidence of congestion (grade 1) and vacuolization (grade 1). The histopathological examination of liver of APAP administered rats showed inflammatory cells (grade 4) and shrunken hepatocytes with chromatin condensation. Administration of APAP caused hepatocellular injury reflected by presence of diffuse cytoplasmic vacuolation (grade 3), centrilobular necrosis (grade 4), vascular congestion (grade 3) and nuclear pyknosis (grade 3) of the hepatocytes. Macrovesicular fatty changes (grade 2) were also evident in liver histological observation of APAP rats (Fig. 3B). However, silymarin (25 mg/kg) showed moderate histopathological changes in liver marked by sinusoidal congestion (grade 2), cytoplasmic vacuolation (grade 1) and presence of only few inflammatory cells (grade 1) along with few fatty globules (grade 1) (Fig. 3C). In the APME (400 mg/kg) treated rats, the histology of liver showed mild degree of vacuolization (grade 1), necrosis (grade 1) and congestion (grade 1) around central vein. Moderate inflammatory cells (grade 2) were present in APME (400 mg/kg) treated rats (Fig. 3D) (Table 4).

Fig.3Effectoftreatmentof APME on paracetamol induced pathological alteration in rat liver



Photomicrograph of sections of liver of normal (A), APAP (B), Silymarin (25 mg/kg) treated (C) and APME (400 mg/kg) treated (D) rats. Inflammatory infiltration (black arrow), oedema (yellow arrow), congestion (green arrow) and necrosis (red arrow). H&E staining at 40 X and 100 X (inset).

Table 4. Effect of treatment of APME on paracetamol induced pathological alteration in rat liver

Treatment	Vesicular Fat	Inflammatory infiltration	Plasma cells	Congestion	Edema	Vaculization	Pyknosis	Necrosis
Normal	0	0	0	+	0	+	0	0
APAP	++	++++	++++	+++	+++	+++	+++	++++
Silymarin (25)	+	+	+	++	++	+	0	+
APME (100)	+++	+++	+++	++	+++	+++	++	+++
APME (200)	+	+++	++	+	+	++	++	+++
APME (400)	0	++	0	+	0	+	+	+

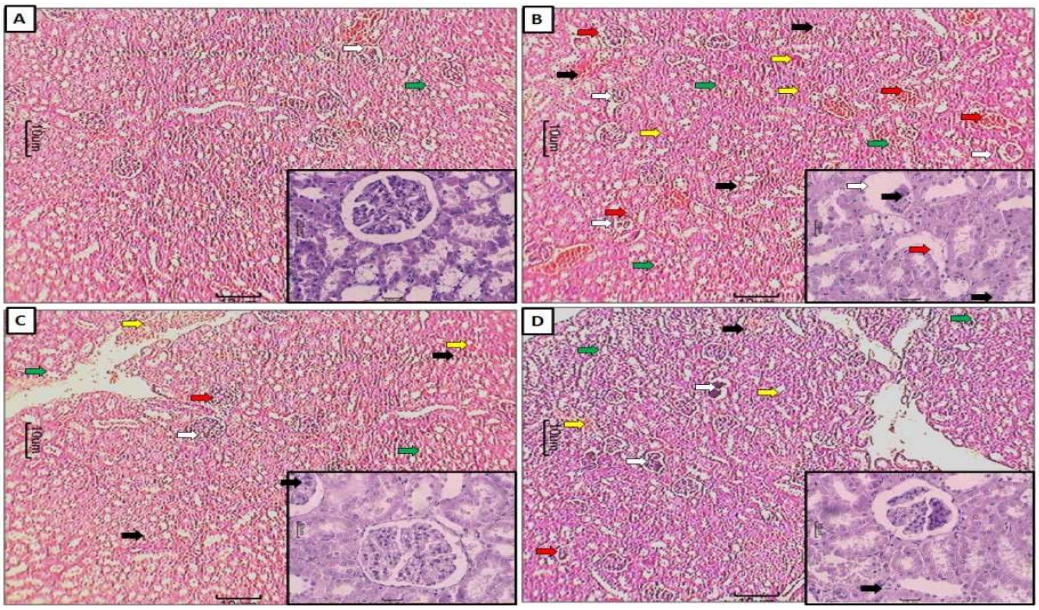
Note: 0: no abnormality detected +: damage/ active changes up to less than 25 %

++: damage/ active changes up to less than 50 % +++: damage/ active changes up to less 75 %

++++: damage/ active changes up to more than 75 %

Figure 4A showed normal architecture of kidney. Chronic administration of APAP caused renal damage evident by glomerular structural disruption (grade 4) and partial endothelial rupture in capsule. APAP animals showed presence of intraluminal cell debris, karyorrhexis and pyknosis (grade 2) indicating cell death (Fig. 4B). Treatment with silymarin (25 mg/kg) as well as APME (400 mg/kg) showed moderate number of inflammatory (grade 2) and apoptotic cells (Fig. 4C). APME (400 mg/kg) treated rats showed mild architectural damage with few intraluminal cell debris (grade 1) and infiltration of inflammatory cells (grade 1) as compared to APAP (Fig. 4D) (Table 5).

Effect of treatment of APME on paracetamol induced pathological alteration in rat kidney



Photomicrograph of sections of kidney of normal (A), APAP (B), Silymarin (25 mg/kg) treated (C) and APME (400 mg/kg) treated (D) rats. Glomerular hypertrophy (white arrow), inflammatory infiltration (black arrow), oedema (yellow arrow), congestion (green arrow) and necrosis (red arrow). H&E staining at 40 X and 100 X (inset).

Table 5. Effect of treatment of APME on paracetamol induced pathological alteration in rat kidney

Treatment	Glomerular hypertrophy	Inflammatory infiltration	Congestion	Edema	Pyknosis	Necrosis
Normal	0	0	+	0	0	0
APAP	++++	+++	++++	++++	++	+++
Silymarin (25)	+	++	+	0	+	0
APME (100)	++++	+++	+++	++++	++	++
APME (200)	+++	++	++	++	+	++
APME (400)	++	+	+	0	0	+

Note: 0: no abnormality detected +: damage/ active changes up to less than 25 %
++: damage/ active changes up to less than 50 % +++: damage/ active changes up to less 75 %
++++: damage/ active changes up to more than 75 %

Discussion

In recent time, APAP induced toxicity has significant clinical problem and generated a lot of heated debate (Watkins & Seeff, 2006). Hence, understanding of pathophysiological processes behind APAP induced toxicity played decisive role in development of new therapeutic strategies.

Administration of APAP caused significant saturation of glucuronidation and sulfuration conjugation pathway that results in glutathione depletion and formation of reactive metabolites. An elevated levels of reactive metabolites caused mitochondrial dysfunction and generation of oxidative stress which leads to hepatotoxicity as well as nephrotoxicity (Ahmad *et al*., 2012).

Various endogenous biomarkers such ALT and AST serves as gold standard for hepatotoxicity. ALT is considered as the better index of liver injury than AST, since ALT played vital role in conversion of alanine to pyruvate and glutamate which is released in a similar manner and that represents almost 90% of total enzymes present in the body. Hence, ALT is more specific parameter of hepatotoxicity (Drotman & Lawhorn, 1978; Visnagri *et al*., 2012, 2013b). In the present investigation, elevated levels of ALT and AST in serum are the consequence of APAP induced liver dysfunction and denotes the damage to the hepatic cells. This elevated levels of ALT and AST is accompanied by hepatocellular fatty deposition and centrilobular necrosis which was corroborated with the histopathological findings of liver tissue of APAP rats. Administration of methanolic extract of *Artemisia pallens* showed significant reduction in elevated levels of serum ALT and AST and may restore the functional integrity of hepatic cells thus revealing hepatoprotective nature of APME against APAP hepatotoxicity. APME treatment also reduced the hepatocellular fatty deposition as well as necrosis revealed by the histopathological examination of APME treated liver tissue.

In APAP induced hepatotoxicity, bile canaliculi cell lining caused release of ALP in response to cholestasis as well as increased biliary pressure (Gaw *et al*., 2008). On the other hand, serum bilirubin (total as well as direct) is conventional indicator of hepatic diseases. During the process of glucuronidation, bilirubin is uptaken by liver parenchyma cells from the blood and conjugates with glucoronic acid in presence of enzyme glucuronyl-transferase and further this conjugated product is excreted into bile. Damage to hepatic parenchymal cells caused increased level of total bilirubin and direct bilirubin in serum (Chakrapani & Satyanarayana, 2013). Administration of APME decreased the level of total bilirubin and direct bilirubin level suggesting its role in promotion of glucuronidation.

Lactate dehydrogenase (LDH) is mainly present in cell cytoplasm and it is extruded into the serum during cell injury or necrosis (Shiva Kumar *et al*., 2014). Serum LDH is a sensitive intracellular enzyme which is most useful in the diagnosis of hepatic damage (Kim *et al*., 2000). APAP caused significant increase in the level of serum LDH which was restored to the normal value after APME treatment.

It has been well documented that oxidative stress also played important role in induction of APAP induced toxicity which may result in damage of some susceptible amino acids of proteins (Kandhare *et al*., 2013a). Decreased level of serum albumin is most common in liver diseases which is followed by an elevated level of β and γ globulins via production of IgG and

IgM (Kaplan *et al*., 2003). Spleen is a vital organ of the immune system and its relative weight has been found to increase with increased production of IgG and IgM. It has been previously reported that APAP induced toxicity is associated with enlargement of the spleen (Spellberg, 1954). Hypoalbuminia was observed after acetaminophen administration but the trend turns towards normal after APME treatment which in turn also decreased splenic enlargement.

Kidneys remove metabolic wastes such as urea, uric acid, creatinine and ions. It has been reported that BUN, serum creatinine and serum uric acid are the most important and reliable hallmark of renal dysfunction (Praveen *et al*., 2008; Visnagri *et al*., 2013a). In the present investigation, administration of APAP resulted in significant increase in BUN and serum creatinine on the other hand serum uric acid level was significantly decreased after APAP administration. The similar results with APAP administration were reported earlier (Ahmad *et al*., 2012). APME treatment maintained these biochemical variables closer to those in normal rats which suggest that *Artemisia pallens* plays a role, either directly or indirectly, in providing protection against APAP induced renal toxicity or delay its development. The results of present investigation are in accordance with the findings of previous researcher who showed that substantial reduction of BUN and serum creatinine level by administration of *Artemisia campestris* leaf extract in early diabetes induced nephropathy (Sefi *et al*., 2012).

It has been reported that APAP caused significant alteration in lipoprotein and cholesterol metabolism (Kobashigawa & Kasiske, 1997). Clinically it has been proven that high cholesterol levels and hyperlipidemia is associated with decreased oxygen release leading to vascular complications. The level of erythrocyte membrane peroxidation was significantly elevated in APAP toxicity that leads to haemolytic changes. With marked increase in cholesterol to phospholipids ratio, the micro viscosity of cell membrane was significantly elevated leading to cellular rigidity (McConnell & Hubbell, 1971). Alteration in cell membrane structure is reflected by increase in cholesterol level which leads to impaired fluidity, permeability, activity of associated enzymes and transport system. With increasing the availability of free fatty acids, the tryglycerides levels were significantly increased in APAP induced toxicity that leads to decreased hepatic release of lipoprotein and increased esterification of free fatty acids. In the present study these fatty changes were observed in the histological examination of liver tissue of APAP rats which showed the presence of few fatty globules with vesicular fat (grade 2) (Fig. 3B). Treatment with APME showed the significant reduction in the cholesterol and triglyceride. Histopathological evaluation of liver tissue from APME treated rats (Fig.3D) also supports these findings.

Liver plays major role in protein metabolism and also in synthesis of serum protein. Hypoproteinemia is a key feature of liver damage and decreased serum protein level occurrs due to defect in protein biosynthesis by disruptions and disassociation of polyribosome from endoplasmic reticulum after APAP toxicity (Dubey *et al*., 1994). The serum albumin and globulin are the main components of total protein and mainly synthesized by the liver (Chakrapani & Satyanarayana, 2013). In APAP rats, the level of protein was decreased due to cirrhosis that in turn decreased the level of serum albumin. Treatment with APME significantly restored the altered level of total protein in the liver as well as kidney.

During the metabolism of APAP via glucuronidation and sulfuration reactions by the microsomal CYP-450 enzyme system in liver, a highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) is produced (Mitchell *et al*., 1973). NAPQI has high affinity for the glutathione (GSH) which causes depletion of cellular GSH leading to renal and hepatic damage (Mitchell *et al*., 1973). Generation of reactive oxygen species (ROS) played pivotal role in depletion of intracellular GSH and cell damage in APAP induced toxicity (Manov *et al*., 2002). Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidants (Kandhare *et al*., 2012c, d). Glutathione redox cycle played important role in detoxification of free radical species such as hydrogen peroxide, superoxide and alkoxy radicals to maintaining cell metabolism and integrity (Prakash *et al*., 2001). It has been reported that SOD is one of the most important enzymes in the enzymatic antioxidant defense system (Curtis *et al*., 1972; Patil *et al*., 2012a) and it reflects as most sensitive enzymatic index in tissue toxicity caused by ROS and oxidative stress. It has central role in detoxification of ROS via scavenging superoxide anion to form hydrogen peroxide (Patil *et al*., 2012b, c). Therefore, reduction in the activity of SOD indicates the toxic effects of ROS produced by APAP. Consistent with previous studies (Olaleye & Rocha, 2008; Visarius *et al*., 1996) administration of APAP in present investigation caused significant depletion in the SOD and GSH level in renal as well as hepatic tissue. Results of present investigation suggested that APME treatment significantly restored the declined level of intracellular SOD and GSH that plays an essential role in detoxification as well as prevention of APAP-induced liver and kidney toxicity.

An elevated level of ROS caused depletion of protective antioxidant moieties (SOD and GSH) resulting in widespread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital bio-membrane (Pesh-Imam & Recknagel, 1977; Patil *et al*., 2012d; Raygude *et al*., 2012a). It has been reported that lipoperoxidation is responsible for destruction of cell membrane via rearrangement of the double bond in the unsaturated fatty acids in the lipid membrane (Kandhare *et al*., 2012a; b). In the APAP induced toxicity lipid peroxidation has been postulated to initiate the destructive process of liver and kidney (Baskin & Salem, 1997). In the present investigation, elevated level of MDA was observed in APAP rats as compared to normal rats which suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. Treatment with APME showed significant reduction in elevated level of MDA via its antioxidant potential to prevent formation of excessive free radicals.

APAP induced increased oxidative stress caused induction of vicious cycle which releases various pro-inflammatory mediators such as nitric oxide (NO) via inducible nitric oxide synthase resulting in cellular dysfunction (Ahmad *et al*., 2012; Kamble *et al*., 2013; Patil *et al*., 2011; Raygude *et al*., 2012b). Results of present investigation are in agreement with the finding of previous study where administration of APAP significantly increased NO level in liver as well as kidney (Ahmad *et al*., 2012; Michael *et al*., 1999). It has been documented that administration of substance with NO scavenging or NOS (nitric oxide synthase) inhibitory potential prevented APAP-induced toxicity via inhibition of hepatic microvascular constriction to improve hemodynamic (Ishida *et al*., 2002). Reduction of elevated levels of NO by APME administration showed substantial protection bestowed to rats against APAP hepatotoxicity and nephrotoxicity.

The QSAR analysis carried out on series of sesquiterpene lactones with respect to hepatoprotective activity by previous researcher showed predictive ability (r^2 test) is lies within 0.942-0.969 (Paukku *et al*., 2009). It has been reported that *Artemisia pallens* possess sesquiterpene lactones in their chemical composition (Puranik & Deshpande, 2010; Suresh *et al*., 2011). The presence of sesquiterpene lactones moieties in *Artemisia pallens* may contribute to its hepatoprotective potential.

In conclusion, oral administration of methanolic extract of *Artemisia pallens* promoted renal and hepatic antioxidant enzyme activity to protect against APAP induced hepatotoxicity as well as nephro-toxicity. Results of present investigation suggest that *Artemisia pallens* may find immense therapeutic potential in clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress. However, further study is in progress for elucidation of actual mechanism of action of *Artemisia pallens* at molecular level.

Acknowledgements

I acknowledge our principal DR.S.G.Gupta and Dr. S. S. Kadam, Vice chancellor, Bharati Vidyapeeth University and Dr. K. R. Mahadik, Principal, Poona College of Pharmacy for keen interest and providing the necessary facilities to carry out the study.I also thankful to University Grants Commission for financial assiatance for my Ph.D. work.

References

Ahmad ST, Arjumand W, Nafees S, Seth A, Ali N, Rashid S, Sultana S (2012). Hesperidin alleviates acetaminophen induced toxicity in Wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicol lett,* 208, 149-61.

Anderson B, Holford N, Armishaw J, Aicken R (1999). Predicting concentrations in children presenting with acetaminophen overdose. *J pedia,* 135, 290-95.

Ashok P, Upadhaya K (2010). Analgesic and Anti-inflammatory properties of *Artemisia pallens* Wall Ex. DC. *Pharma Res,* 3, 249-56.

Baskin SI, Salem H (1997). Oxidants, antioxidants, and free radicals. CRC Press, Taylor & Francis, Bristol, PA pp. 193-202.

Chakrapani U, Satyanarayana U (2013). Biochemistry 4th Edition. Elsevier India.

Curtis S, Moritz M, Snodgrass P (1972). Serum enzymes derived from liver cell fractions. I. The response to carbon tetrachloride intoxication in rats. *Gastroenterol,* 62, 84-92.

Drotman R, Lawhorn G (1978). Serum enzymes as indicators of chemically induced liver damage. *Drug Chem Toxicol,* 1, 163-71.

Dubey G, Agrawal A, Dixit S (1994). Effect of Liv. 52 on different bio-chemical parameters in alcoholic cirrhosis. *Antiseptic,* 91, 205-8.

Gaw A, Murphy M, Cowan R, O'reilly D, Stewart M, Shepherd J (2008). Clinical biochemistry: An illustrated colour text. Elsevier Health Sciences.

Ghosh A, Sil PC (2007). Anti-oxidative effect of a protein from *Cajanus indicus* L. against acetaminophen-induced hepato-nephro toxicity. *J biochem molec biol,* 40, 1039-49.

Gosavi TP, Kandhare AD, Ghosh P, Bodhankar SL (2012). Anticonvulsant activity of *Argentum metallicum,* a homeopathic preparation. *Der Pharmacia Lettre,* 4, 626-37.

Hart SE, Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD (1994). Acetaminophen nephrotoxicity in CD-1 mice: I. Evidence of a role for *in situ* activation in selective covalent binding and toxicity. *Toxicol appl pharmacol,* 126, 267-75.

Hegde K, Joshi AB (2010). Hepatoprotective and antioxidant effect of *Carissa spinarum* root extract against CCl_4 and paracetamol-induced hepatic damage in rats. *Bangladesh J Pharmacol,* 5, 73-76.

Ishida Y, Kondo T, Ohshima T, Fujiwara H, Iwakura Y, Mukaida N (2002). A pivotal involvement of IFN-γ in the pathogenesis of acetaminophen-induced acute liver injury. *FASEB J,* 16, 1227-36.

Kamble H, Kandhare AD, Bodhankar S, Mohan V, Thakurdesai P (2013). Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. *Biomed Aging Pathol*, 3, 145-51.

Kandhare A, Raygude K, Ghosh P, Bodhankar S (2011a). The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. *Inter J Green Pharm*, 5, 236-43.

Kandhare AD, Bodhankar SL, Singh V, Mohan V, Thakurdesai PA (2013a). Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. *Biomed Aging Pathol*, 3, 23-30.

Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL (2013b). Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol induced neuropathic pain. *Fund Clin Pharmacol*, 27, 603-22.

Kandhare AD, Ghosh P, Ghule AE, Zambare GN, Bodhankar SL (2013c). Protective effect of *Phyllanthus amarus* by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. *Apollo Med*, 10, 87-97.

Kandhare AD, Kumar VS, Adil M, Rajmane AR, Ghosh P, Bodhankar SL (2012a). Investigation of gastro protective activity of *Xanthium strumarium* L. by modulation of cellular and biochemical marker. *Orient Pharm Exper Med*, 12, 287-99.

Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL (2012b). Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. *Fitoterapia*, 83, 650-9.

Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL (2012c). Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. *Neurosci Lett*, 511, 18-22.

Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Gosavi TP, Badole SL, Bodhankar SL (2012d). Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. *Asian Pac J Trop Biomed*, 5, 337-44.

Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL (2011b). Patentability of animal models: India and the globe. *Int J Pharm Biol Arc*, 2, 1024-32.

Kaplan LA, Pesce AJ, Kazmierczak SC (2003). Clinical chemistry: theory, analysis, and correlation. Mosby London.

Kim K-A, Lee W, Kim J, Seo M-S, Lim Y, Lee K-H, Chae G, Lee S-H, Chung Y (2000). Mechanism of refractory ceramic fiber-and rock wool-induced cytotoxicity in alveolar macrophages. *Inter Arc Occup Envir Health*, 74, 9-15.

Kobashigawa JA, Kasiske BL (1997). Hyperlipidemia in solid organ transplantation. *Transplantation,* 63, 331-38.

Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO (2005). Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study. *Hepatol,* 42, 1364-72.

Manov I, Hirsh M, Iancu TC (2002). Acetaminophen hepatotoxicity and mechanisms of its protection by N-acetylcysteine: A study of Hep3B cells. *Exper Toxicol Pathol,* 53, 489-500.

Mcconnell HM, Hubbell WL (1971). Molecular motion in spin-labeled phospholipids and membranes. *J Amer Chem Soc,* 93, 314-26.

Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA (1999). Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. *Hepatol,* 30, 186-95.

Mitchell J, Jollow D, Potter W, Gillette J, Brodie B (1973). Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther,* 187, 211-17.

Mitchell JR, Mcmurtry RJ, Statham CN, Nelson SD (1977). Molecular basis for several drug-induced nephropathies. *Amer J Med,* 62, 518-26.

Nassar I, Pasupati T, Judson JP, Segarra I (2009). Reduced exposure of imatinib after coadministration with acetaminophen in mice. *Ind J Pharmacol,* 41, 167-72.

Nelson SD 1990. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Semin Liver Dis* 10(4), 267-78.

Olaleye MT, Rocha B (2008). Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. *Exper Toxicol Pathol,* 59, 319-27.

Patil M, Kandhare A, Bhise S (2011). Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus* L. fruit formulation on wound healing in Wistar rats. *Chronicles of Young Scientists,* 2, 207-13.

Patil M, Kandhare A, Bhise S (2012a). Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. *Int J Pharm Pharm Sci,* 4, 337-43.

Patil MVK, Kandhare AD, Bhise SD (2012b). Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanol extract in Freund's complete adjuvant induced arthritis. *Biomed Aging Pathol,* 2, 6-15.

Patil MVK, Kandhare AD, Bhise SD (2012c). Effect of aqueous extract of *Cucumis sativus* Linn. fruit in ulcerative colitis in laboratory animals. *Asian Pac J Trop Biomed,* 2, S962-S69.

Patil MVK, Kandhare AD, Ghosh P, Bhise SD (2012d). Determination of role of GABA and nitric oxide in anticonvulsant activity of *Fragaria vesca* L. ethanol extract in chemically induced epilepsy in laboratory animals. *Orie Pharm Exper Med*, 12, 255-64.

Paukku Y, Rasulev B, Syrov V, Khushbaktova Z, Leszczynski J (2009). Structure-hepatoprotective activity relationship study of sesquiterpene lactones: A QSAR analysis. *Inter J Quantum Chem*, 109, 17-27.

Pesh-Imam M, Recknagel RO (1977). Lipid peroxidation and the concept of antioxygenic potential: Vitamin E changes in acute experimental CCl_4, $BrCCl_3$, and ethanol-induced liver injury. *Toxicol App Pharmacol*, 42, 463-75.

Prakash J, Gupta S, Kochupillai V, Singh N, Gupta Y, Joshi S (2001). Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumours in Swiss albino mice. *Phytotherapy Res*, 15, 240-44.

Praveen S, Kumar KD, Venkatesan D, Sathendra ER (2008). Effect of the ethanol extract of *Indigofera barberi* (L) in acute acetaminophen-induced nephrotoxic rats. *Adv Biotech*, 7, 28-31.

Prescott L (1979). The third Lilly Prize Lecture. University of London, January, 1979. The nephrotoxicity and hepatotoxicity of antipyretic analgesics. *British J Clin Pharmacol*, 7, 453-62.

Pujar PP, Sawaikar DD, Rojatkar SR, Nagasampagi BA (2000). A new germacranolide from *Artemisia pallens*. *Fitoterapia*, 71, 590-92.

Puranik V, Deshpande N (2010). GC-MS study and isolation of a sesquiterpene lactone from *Artemisia pallens*. *Orie J Chem*, 26, 143-46.

Raygude KS, Kandhare AD, Ghosh P, Bodhankar SL (2012a). Anticonvulsant effect of fisetin by modulation of endogenous biomarkers. *Biomed Prev Nut*, 2, 215-22.

Raygude KS, Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL (2012b). Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. *Inflammopharmacol*, 20, 331-41.

Rojatkar S, Pawar S, Pujar P, Sawaikar D, Gurunath S, Sathe V, Nagasampagi B (1996). A germacranolide from *Artemisia pallens*. *Phytochemistry*, 41, 1105-06.

Ruikar AD, Khatiwora E, Ghayal N, Misar A, Mujumdar A, Puranik V, Deshpande N (2011). Studies on aerial parts of *Artemisia pallens* wall for phenol, flavonoid and evaluation of antioxidant activity. *J Pharm Bioall Sci*, 3, 3025.

Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N (2012). *Artemisia campestris* leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. *Pathol Res Practice*, 208, 157-62.

Shiva Kumar V, Rajmane AR, Mohammad A, Kandhare AD, Ghosh P, Bodhankar SL (2014). Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. *J Biomed Res,* 28(2), 132-45.

Sipma G, Van Der Wal B (1968). The structure of davanone a new sesquiterpene from davana:(*Artemisia pallens*, Wall.). *Recueil des Travaux Chimiques des Pays-Bas,* 87, 715-20.

Spellberg MA (1954). Diseases of the liver. *Amer J Med Sci*, 228, 243-48.

Starzl TE, Esquivel C, Gordon R, Todo S (1987). Pediatric liver transplantation. *Transplantation proceedings,* 19(4), 3230-35.

Subramoniam A, Pushpangadan P (1999). Development of phytomedicines for liver disease. *Ind J Pharmacol,* 31, 166-175.

Suresh J, Singh A, Vasavi A, Ihsanullah M, Mary S (2011). Phytochemical and pharmacological properties of *Artemisia pallens*. *Inter J,* 2, 3081-90.

Valenzuela A, Garrido A (1993). Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin. *Biol Res,* 27, 105-12.

Visarius TM, Putt DA, Schare JM, Pegouske DM, Lash LH (1996). Pathways of glutathione metabolism and transport in isolated proximal tubular cells from rat kidney. *Biochem Pharmacol,* 52, 259-72.

Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL (2013a). Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. *Pharm Biol* DOI: 10.3109/13880209.2013.870584.

Visnagri A, Kandhare AD, Ghosh P, Bodhankar SL (2013b). Endothelin receptor blocker bosentan inhibits hypertensive cardiac fibrosis in pressure overload-induced cardiac hypertrophy in rats. *Cardiovasc Endocrinol,* 2, 85-97.

Visnagri A, Kandhare AD, Shiva Kumar V, Rajmane AR, Mohammad A, Ghosh P, Ghule AE, Bodhankar SL (2012). Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. *Biomed Aging Pathol,* 2, 157-72.

Watkins PB, Seeff LB (2006). Drug-induced liver injury: Summary of a single topic clinical research conference. *Hepatol,* 43, 618-31.

Yamasaki ML, Sasaki K, Mizutani N, Nabe T, Sakura Y, Matsumoto T, Ashida Y, Kohno S (2001). Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. *Inflamm Res,* 50, 474-82.

ANNEXURE-I
OUTCOME OF PROJECT

1. Principal Investigator has registered for Ph.D. under the guidance of Dr. A.D.Natu at Post Graduate and Research Center, Department of Chemistry, MES Abasaheb Garware College, Pune.

2. Presented poster in national conference and communicated one paper in international journal.

2.1. National Conference organized by Poona College of Pharmacy, Bharati Vidyapeeth , Pune 411038. India,entitled “Artemisia pallens alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers”.

2.2. Communicated paper in Pharmaceutical Biology.

Varsha S.Honmore, Amit D.Kandhare, Anand A. Zanwar, Supada R. Rojatkar, Subhash L.Bodhankar, Arun Natu. 2014, *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers.


hipatoprotective poster.pdf


pharmaceutical biology.pdf