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BIOCHEMICAL, HEMATOLOGICAL AND HISTOLOGICAL CHANGES IN RESPONSE TO GRADED DOSE OF EXTRACT OF EQUISETUM ARVENSE IN ADULT FEMALE WISTAR RATS

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ABSTRACT: *Equisetum arvense*, commonly known as field horsetail, is a perennial herb belonging to the genus Equisetum. Traditionally, *Equisetum arvense* is used as diuretic; anticoagulant, skin antiseptic, in kidney and liver related problems and gastric ulcers. *E. arvense* has also been suggested for the treatment of bone related disorders such as osteoporosis but the data is very scarce on its dose range and its influence on basic biochemical parameters. The present study was carried out to study its effect in female wistar rats at three levels of dose: L (30 mg/ kg of body weight), M (60 mg/ kg of body weight) and H (120 mg/ kg of body weight) for a period of 30 days. We report here complete blood count, LFT, KFT, blood glucose, total protein, and albumin and lipid profile. No toxic effects were observed in reference to clinical signs, body weight and organ weight. HBG, HCT, MCV, PLT, glucose and serum cholesterol, HDL and LDL level at H dose was found significantly different to Control group. Histology of liver tissue of all the three groups showed normal cellular architecture with prominent central vein compared. Histological examination of femur bone showed decreased trabecular width suggesting the toxic effect of extract at H dose. Thus from the study it can be concluded that *E. arvense* produces toxic effect in a dose dependant manner and that M dose of *E. arvense* (60 mg/ kg body weight) can be used as a therapeutic dose for further studies.

INTRODUCTION: In developing countries like India, where herbs form a part of lifestyle, herbal medicine is one of the trusted systems of medicine. Traditional medicinal plants have attained a significant importance in the treatment of diseases and also in maintenance of optimal health for humans and animals [1, 2]. They are used as they are cost effective and safe to use with minimum or no side effects. As these plants are usually taken in crude form, its accurate dosage and validation of safety is necessary [3, 4]. The data from these studies helps in selection of the dose for humans and also in predicting toxicity [5].

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For age related diseases like osteoporosis, long term treatment with synthetic drugs results in various side effects such as hypercalcemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding, hot flashes, dyspepsia and gastrointestinal ulcers [6]. For this reason many medicinal plants such as *Cissus quadrangularis, Cimicifuga racemosa, Trifolium pratense, Bambusa arundinacea, Cimicifuga foetida, Griffonia simplicifolia* etc. have been reported in the literature for the treatment of osteoporosis [6, 7].

Equisetum arvense, a perennial herb belonging to order Equisetales, has been used as a folk medicine to treat diuresis; to stop bleeding, as a skin antiseptic, to treat kidney and liver related problems and gastric ulcers [8, 9]. Its role in the treatment of bone related diseases has been

implicated but not yet proven. There are different controversies over the use of *E. arvense* and its toxicity [10, 11, 12].

Till date no data is available on its effect on basic biochemical parameters in female albino wistar rats. The choice of female wistar rats was in view of the future work involving the post menopausal osteoporosis. The selection of dose was based on data available in literature [13]. The objective of the present study was to study effect of extract of *E. arvense* on biochemical, haematological and histological parameters in female albino wistar rats at graded doses (30, 60 and 120mg/ kg of body weight) and whether 120 mg/ kg of body weight of extract causes any adverse effects on various parameters in female albino wistar rats.

MATERIALS AND METHODS:

The collection of plant material:

The whole plant of *Equisetum arvense* was collected from Vainganga River at Bhandara District of Maharashtra, India and was identified and authenticated by University Department of Botany, RTM Nagpur University, Nagpur by depositing a voucher specimen (No: R/ 9698). The aerial part of the plant was washed with distilled water and was shade-dried, powdered and stored in air-tight containers.

Preparation of extract:

20g of dried powder was extracted with 95% ethanol using a Soxhlet apparatus. The extract was concentrated in vacuum, dried and weighed to determine its yield (14.17 %). The extract was used in graded doses: Low (L), Moderate (M) and High (H) (30 mg, 60 mg and 120 mg/ kg of body weight respectively) [13].

Animals:

3 months old female albino wistar rats weighing (160-200gms) (n=20) were obtained from National Institute of Nutrition (NIN), Hyderabad and were housed in a temperature-controlled room under 12-hr-light/-dark period in polypropylene cages (5 rats/ cage). They had access to food and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (IAEC) constituted and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The female rats were divided into 4 groups:

Control- receiving saline water

L dose- receiving 30mg/ kg of body weight of *Equisetum arvense* extract

M dose- receiving 60 mg/ kg of body weight of *Equisetum arvense* extract and

H dose- receiving 120 mg/ kg of body weight of *Equisetum arvense* extract

The extract was given orally for 30 days. Weight of individual rat was monitored weekly and diet and water intake of each cage was monitored daily during the entire treatment period.

At the end of the treatment, blood samples were withdrawn from orbital plexus into heparinised tubes for haematological studies and into non heparinised centrifuge tubes for biochemical parameters. The animals were sacrificed by CO_2 asphyxiation after blood collection. The internal organs were excised, cleaned off extra fat and weighed. Blood samples were centrifuged at 2500 rpm for 10 minutes and the serum was used for the analyses. The biochemical parameters analysed were total cholesterol (Liquizyme Cholesterol), HDL (Liquizyme Cholesterol), triglycerides (Liquizyme Triglycerides), hepatic biochemical parameters such as SGOT and SGPT, serum ALP (Merck Alkaline phosphatase kit), albumin (Rodkey; 1965), total proteins (Folin Lowry method), total Bilirubin and direct Bilirubin, kidney biochemical parameters such as serum creatinine (Modified Jaffe's method), serum urea (Accurex kit) and blood glucose by glucometer.

Complete blood count was immediately analysed by using a haematological analyser (Model No. ABX Micros 60). The parameters measured were haemoglobin (HGB), haematocrit (HCT), red blood cells (RBC), platelets (PLT), total leucocytes count (TLC), platelet crit (PCT), Differential leucocytes count (DLC- Lymphocytes, Monocytes and Granulocytes), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MHC), mean haematocrit (MCHC), mean platelet volume (MPV), red blood cell distribution width (RDW) and platelet distribution width (PDW). Kidneys, liver, heart, brain, spleen, femur bone and lungs were preserved in 10% neutral buffered formalin

solution. The tissues (liver and femur bone) were embedded in paraffin then sectioned, stained with haematoxylin and eosins and were examined microscopically.

Statistical analysis:
AnalyseIt software (Version 2.26) for Windows excel was used for statistical analysis.

RESULTS:

Effect of extract of *E. arvense* on the general behaviour: All the animals belonging to L dose, M dose and H dose showed normal general behaviour as well as skin and fur when compared to control group.

Effect of extract of *E. arvense* on the body weight and organ weight: The increase in body weight of rats receiving H dose was comparable to control group and H dose group showed negative gain (10%) which was significant when compared to control. Some trend was observed in case of organ weight where rats from group receiving H dose showed non-significant variation when compared to control.

Effect of extract of *E. arvense* on the biochemical parameters: Both L and M dose of *E. arvense* did not significantly alter biochemical parameters **(Table 1)** but H dose showed significantly higher blood glucose, cholesterol, HDL and LDL.

Effect of extract of *E. arvense* on the haematological parameters:
Hematological parameters (**Table 2**) of the rats in L and M group did not show significant deviation from control group. At H dose HGB, HCT and MCV was significantly low and PLT significantly high as compared to control group. This group also demonstrated significantly lower MCV when compared to L and M group. The rats in H group also had non-significantly raised TLC, RDW and PDW.

The differential white blood cell count values of all the rats in various groups were examined and shown (**Table 3**). Although there were no significant changes in lymphocytes, monocytes and granulocytes H dose resulted in high lymphocytes and low granulocytes count in H dose group.

TABLE 1: EFFECT ON BIOCHEMICAL PARAMETERS OF RATS RECEIVING VARIOUS DOSES OF EXTRACT OF *E. ARVENSE*

Parameter	Control	L dose	M dose	H dose
Glucose (mg/dl)	63.3 ± 3.84	65.00 ± 2.27	71.5 ± 3.71	77.3 ± 2.10*
Lipid Profile:				
Total Cholesterol (mg/dl)	92.03 ± 22.39	78.92 ± 8.58	74.39 ± 14.51	36.03 ± 3.24*
Triglycerides (mg/dl)	108.08 ± 6.33	108.76 ± 3.13	108.42 ± 16.65	93.30± 9.11
HDL (mg/dl)	11.01 ± 1.59	10.65 ± 0.99	10.80 ± 2.01	25.28 ± 4.51*
LDL (mg/dl)	91.52 ± 22.36	78.43 ± 8.06	73.84 ± 14.45	34.69 ± 3.19*
LFT:				
Total Bilirubin (mg/dl)	0.89 ± 0.21	0.85 ± 0.31	0.75 ± 0.33	0.79 ± 0.26
Direct Bilirubin (mg/dl)	0.08 ± 0.02	0.27 ± 0.24	0.14 ± 0.04	0.42 ± 0.18
Serum ALP (U/L)	320.58 ± 41.36	331.60 ± 38.48	338.94 ± 52.39	376.52 ± 14.75
AST (U/l)	175.30 ± 11.24	199.70 ± 12.55	229.69 ± 20.40	233.07± 30.36
ALT (U/l)	44.40 ± 5.86	51.53 ± 2.98	60.68 ± 9.46	32.21 ± 2.47
Total protein (g/dl)	6.00 ± 0.40	5.25 ± 0.36	5.50 ± 0.25	5.83± 0.15
Albumin (g/dl)	3.47 ± 0.12	2.90 ± 0.12	2.92 ± 0.02	3.64 ± 0.74
KFT:				
Urea (mg/dl)	12.36 ± 1.03	13.33 ± 1.07	14.51 ± 2.2	14.12 ± 3.30
Creatinine (mg/dl)	0.25 ± 0.014	0.26 ± 0.007	0.26 ± 0.017	0.26 ± 0.002

Values are expressed as mean ± S.E., n = 5, * value indicates significance over control

TABLE 2: EFFECT OF EXTRACT OF *E. ARVENSE* ON HAEMATOLOGICAL PARAMETERS IN RATS

Parameter	Control	L dose	M dose	H dose
HGB (g/dl)	13.80 ± 0.50	12.68 ± 0.35	12.60 ± 0.24	11.80 ± 0.40*
HCT (%)	36.53 ± 1.6	33.80 ± 0.75	33.60 ± 1.79	30.30 ± 0.94*
PLT (10^3/mm^3)	625.5 ± 88.78	710.3 ± 17.42	754.5 ± 32.83	936.0 ± 102.21*
PCT (%)	0.45 ± 0.07	0.52 ± 0.01	0.52 ± 0.03	0.55 ± 0.00

TLC (10^3/mm^3)	6.00 ± 0.71	6.05 ± 0.69	6.30 ± 0.80	6.70 ± 0.40
RBC (10^6/mm^3)	6.44 ± 0.31	6.05 ± 0.15	5.83 ± 0.27	5.56 ± 0.20
MCV (µm^3)	56.5 ± 0.29	57.0 ± 0.41	57.8 ± 0.63	54.3 ± 0.85*
MCH (pg)	21.50 ± 0.54	20.95 ± 1.08	21.73 ± 1.00	21.10 ± 0.59
MCHC (g/dl)	37.95 ± 0.96	37.00 ± 0.55	37.68 ± 1.78	38.40 ± 0.90
MPV (µm^3)	7.13 ± 0.17	7.00 ± 0.30	6.88 ± 0.17	6.53 ± 0.07
RDW (%)	14.70 ± 0.21	14.43 ± 0.46	14.88 ± 0.50	14.70 ± 0.21
PDW (%)	13.90 ± 0.15	13.28 ± 0.36	13.68 ± 0.29	13.90 ± 0.37

Values are expressed as mean ± S.E., n = 5, * value indicates significance over control

TABLE 3: EFFECT OF EXTRACT OF *E. ARVENSE* ON DIFFERENTIAL LEUCOCYTES COUNTS

Parameter	Control	L dose	M dose	H dose
Lymphocytes (10^3/mm^3)	1.45 ± 0.16	1.50 ± 0.23	1.50 ± 0.21	1.80 ± 0.20
Monocytes (10^3/mm^3)	0.08 ± 0.03	0.18 ± 0.03	0.20 ± 0.06	0.10 ± 0.00
Granulocytes(10^3/mm^3)	0.48 ± 0.08	0.40 ± 0.07	0.40 ± 0.11	0.33 ± 0.07

Values are expressed as mean ± S.E., n = 5

Effect of extract of *E. arvense* on liver and femur bone histology:

The effect of extract of *E. arvense* on liver and femur bone histology is represented in **Fig. 1** and **2**. No significant damage in the liver tissue was observed in all the three groups: L, M and H dose treated groups as compared to control group.

Histological observations of liver tissue of all three groups showed normal cellular architecture with prominent central vein. In some areas in H dose treated rats hepatocellular necrosis was observed. Chords of Hepatocytes are well preserved in all the groups. Cytoplasm is seen vacuolated in H dose treated rat group.

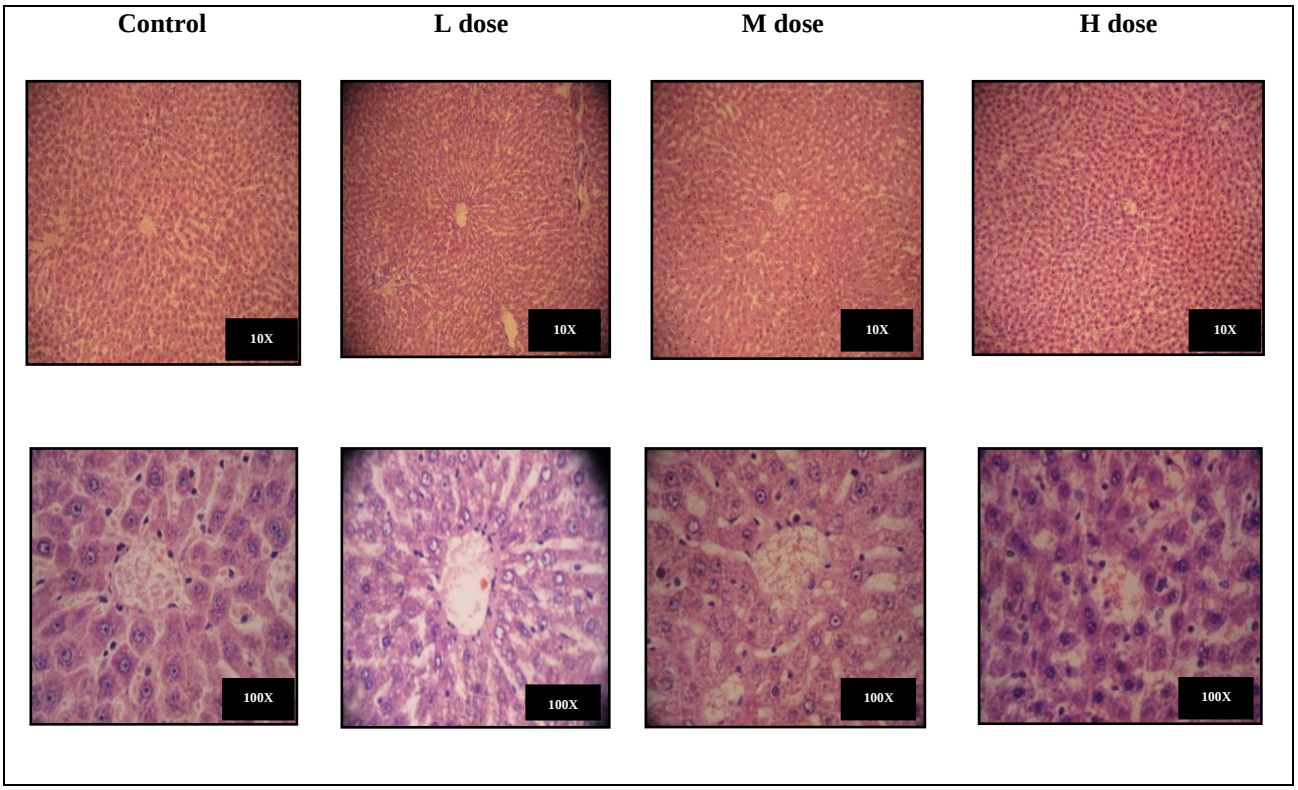

FIG.1: EFFECT OF EXTRACT OF E. ARVENSE ON LIVER HISTOLOGY

The width of trabecular network (**Fig.2**) in H dose is decreased. The normal architecture of trabecular network in H group was found to be disrupted when compared to control, L and M dose treated groups.

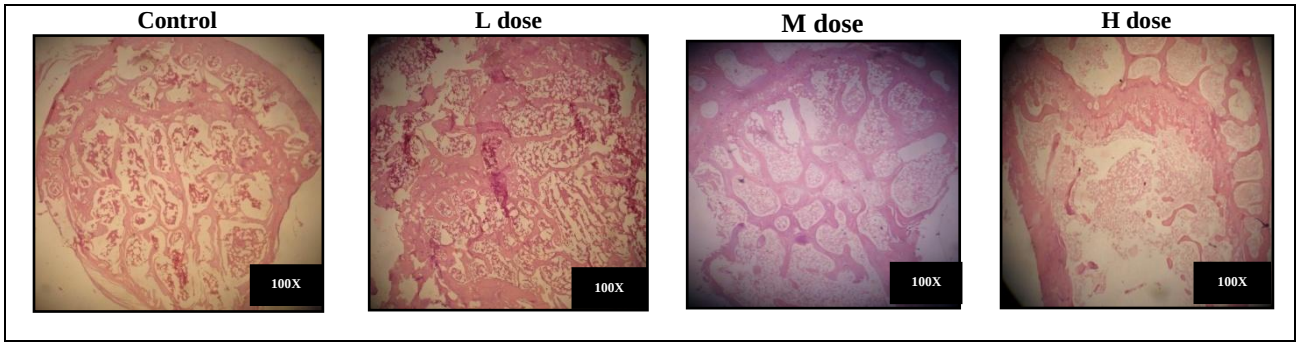

FIG. 2: EFFECT OF EXTRACT OF *E. ARVENSE* ON FEMUR BONE HISTOLOGY

DISCUSSION: For many years medicinal plants have been used to treat various diseases. The initial step for screening of medicinal plants for its pharmacological properties involves assessment and evaluation of its toxic properties [5, 14]. *E. arvense* is a perennial herb which has been used traditionally for the treatment of various ailments. In an acute toxicity study in male wistar rats graded dose (30 mg, 50mg and 100mg/ kg) of *E. arvense* did not produce any toxicity [15]. Oh et al. demonstrated that aerial parts of *E. arvense* contain hepatoprotective and antioxidant activities [11]. But Semprini et al. reported that the oral administration of *E. arvense* in wistar rats produced hepatic damage in rats [10]. But no clinical data is available reporting its range of dose to be given and its effects in female albino wistar rats.

Hence, the present study was carried out to study the effect of extract of *E. arvense* on biochemical, haematological and histological parameters in female albino wistar rats when given in graded dose. The lack of mortality or toxicity at L and M doses suggests that extract of *E. arvense* is practically non toxic at this dose. Negative weight gain of H dose treated animals at the end of treatment indicates its toxic effect at the dose, but no mortality was observed.

Haematological parameters evaluation indicates the extent of damaging effects of foreign compounds such as plant extract on blood constituents of the animals. It also provides information about the status of bone marrow activity and haemolysis. In regards to haematological analysis most of the values were normal in comparison with the control group. MCH, MCHC and MCV relates to individual red blood cells while HB, RBC and PCV are associated with the total population of red blood cells [16, 17]. The significant difference in HGB, HCT, PLT and MCV in H dose treated animals indicates the unlikelihood of the extract to induce anemia. MCV is an indication of the size of RBCs. The decreased HGB and MCV in H dose treated group are associated with less hemoglobin production resulting in smaller sized RBC_s [18, 19]. The insignificant change in TLC in rats receiving H dose was probably due to foreign bodies or stress associated with toxicity due to high dose.

Furthermore blood chemical analysis was performed to evaluate any toxic effect on blood glucose, lipid profile and on liver and kidney function tests. As ALT is specific to liver, a non-significant decrease in serum ALT in H dose treated group probably suggests that the extract does not possess hepatotoxic effect. The significant increase in blood glucose level in H dose treated group probably suggests its toxic effects on pancreas. The decrease in cholesterol level contributes to high serum HDL in animals as major part of cholesterol is transported in the form of HDL-Cholesterol.

In the last part of the study, histopathological examination was performed on liver and femur bone. Histological sectioning of liver showed mild spotty hepatocellular necrosis in H dose treated group. As changes in femur bone is the major site for manifestation of osteoporosis and as *E. arvense* is suggested for the treatment of osteoporosis, histology of femur bone was performed. Histopathological examination of femur bone revealed decrease in trabecular width in H dose treated group. This result suggests toxic effect of extract of *E. arvense* on femur bone at H dose (120 mg/ kg body weight).

CONCLUSION: The objective of the present work was to study effect of extract of *E. arvense* in female albino wistar rats when given in graded doses (30 mg, 60 mg and 120 mg/ kg of body weight), to determine the range of therapeutic dose to be used for the further studies and whether H dose of *E. arvense* (120 mg/ kg of body weight) produces any adverse effects in these rats. Thus from the present study it can be concluded that *E. arvense* produces toxic effect in a dose dependant manner in female albino wistar rats and that M dose of *E. arvense* (60 mg/ kg body weight) can be used as a therapeutic dose for further studies.

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