Biochemical and histological studies on *Vernonia anthelmintica*(L.) willd seed

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Abstract

It is important to recognize the biological and chemical toxicities that might take place as a consequence of using herbal combination.There fore, current study was designed to assess the biochemical, histological and toxicity of herbal plant *Vernoniaanthelmintica* seeds extract. The crude extract of *V.anthelmintica*(5mg/kg and 2mg/kg) was administered orally for three months and biomarkers for liver, kidney and cardiac function were observed along with histological changes.The results of biochemical analysis of crude extract of *V.anthelmintica* showed non-significant alteration in renal functions except change in creatinine (1.02±0.07;p<0.04; 5mg/kg) and albumin values (5.61 ±0.22,p<0.040; 5mg/kg). Liver function test showed increased in alkaline phosphatase (p<0.006) and gama GT (p<0.032). Cardiac enzymes, CPK and CK-MB were elevated whereas LDH was reduced. Lipid profile showed significant increased in values of cholesterol, triglycerides and LDL. Other blood parameters and electrolytes were produced no significant changes. The histologicalfindingsshowed moderate degree of inflammatory changes in kidney. The heart and stomach have mild inflammatory changes at 5mg/kg while 2mg/kg showed insignificant changes. These results suggested that crude extract of *V. anthelmintica* at the dose of 5 mg/kg has some toxic changes on chronic administration. It also indicated that low dose of *V.anthelmintica* needs careful monitoring if, it is used for therapeutic purpose.

Key words: *Vernonia anthelmintica*, biochemical parameters, toxicity, histology

Introduction

Pakistan hasan abundant variety of natural source of drugs/medicines. Third world countries depend on herbal medications as the individual therapeutic source available. According to 1985 estimates of World health Organization reported that common people retied on herbs for primary health care requirements (Benetely and Trimen1999 Krishnamoorthy *et al*, 1999, Joshi, 2000).

Seeds of *Vernonia anthelmintica* possess sharp pungent taste and used traditionally in different ailments (Mhaskar *et al*, 2000). It also possessed mild CNS stimulating effect (amber et al., 2016).Conventionally, herbs considered has no side effects and have been used for different health diseases of world-wide, still many people have thought herbs are safe (Khan, 2007), therefore current study has been particularly considered to evaluate the acute and chronic effects of *V. anthelmintica* seeds extract.

Material and Method

The plant of *Vernonia anthelmintica* was obtained from the herbal market of Karachi and kept in Herbarium of Faculty of Pharmacy, University of Karachi. Rabbits of either sex (1.0 to1.5 kg) were obtained from the animal house of Dow medical university of health sciences.The methanol extract was strained and evaporated further down in rotatory evaporator after evaporation of solvent it yield a dark black residue (20 gm) (Amber 2016).

Experimental design and dosing for biochemical studies

In present study rabbits were selected as experimental animals Feroz*et al*, 2011. All animals were uniformly divided into two groups. Groups received drugs in following pattern.

- Group A: Control group received saline only
- Group B, C: Crude drug (2mg/kg and 5mg/kg in 0.5 ml)

Blood sampleswere collected in gel tubes through cardiac puncture technique after completion of dosing period i. e 90 days. Various biochemical tests such as cardiac, liver, kidney function test, blood chemistry and electrolyte test were performed (Ezeonwumelu, 2011, Mehjabeen *et al*., 2015).

Microscopic Tissue Examination

Entirely important characteristics were distinguished at autopsy, liver specimen, heart and kidney specimen were preserved in 10 % buffered formation and the organs weight of were reserved. Some best slabs of these organs were preserve standard process have done through microscopic examination (Ezeonwumelu, 2011).

Statistical analysis

The method for data analysis and statistically investigated through one –way analysis ANOVA. The data were calculated by as mean ± Standard error of the mean. $P<0.05$ was occupied as level of significance. $P<0.001$was booked to be the level of extremely significance (amber, 2016).

RESULTS

Biochemical Tests

Haematological Parameters

Effect of *V. anthelmintica* on haematological parameters were presented in table 1.At the dose of 5 mg/kg of *V. anthelmintica* crude extract results showed significant increased in PT test: 8.50 ± 0.29, RBC count (5.50 ± 0.25 $x10^6/mm^3$)and Platelet counts (601.25 x 10^9± 5.5) total leucocyte count (8.98 x 10 ± 0.89) and Haematocrit (35.67 ± 1.17).While significant decreased in MCV (64.35 ± 1.16) MCHC (29.98 ± 1.27) and MCH (19.02 ± 0.07) values at 2mg/kg dose.

Table 1: Evaluation of haematological parameters of *V. anthelmintica* at dose of 2mg and 5mg/kg

Parameters	Control group	CE ± SEM		*p* value	
		2mg/kg	5mg/kg	2mg	5mg
Hb	10.4 ± 0.23	07.87 ±0.41	10.78 ± 0.61	$p<0.380$	$p<0.590$
RBC	4.9 ± 0.45	3.90 ± 0.65	5.50 ± 0.25	$p<0.127$	$p<0.334$
Hematocrit	33.2 ± 1.30	31.35 ±0.98	35.67 ± 1.17	$p<0.121$	$p<0.284$
MCV	67.3 ± 0.78	60.24±1.09	64.35 ± 1.16	$p<0.026$*	$p<0.094$
MCH	21.2 ± 0.98	17.11±0.03	19.02 ± 0.07	$p<0.032$*	$p<0.071$
MCHC	31.3 ± 1.01	26.78 ±0.98	29.98 ± 1.27	$p<2.943$	$p<0.345$
Total leucocyte count (WBC)	$7.2x10^9$ ± 0.11	6.9 ±0.19	8.910 ± 0.89	$p<0.0512$	$p<0.097$
Platelet Count	$357\times10^9\pm$ 3.87	590.112 x $10^9\pm4.2$	601.25 x $10^9\pm$ 5.5	$p<0.1130$	$p<0.211$
HbA1C	4.14 ± 0.09	3.71 ±0.07	4.14 ± 0.09	$p<0.6210$	$p<1.002$
PT Test	14 ± 0.25	12.12 ±0.23	8.50 ± 0.29	$p<0.120$	$p<0.0132$*
INR-International Normalise ratio	0.44 ± 0.01	0.34 ±98	0.64 ±0.02	$p<0.0892$	$p<0.0132$*
APTT	35±2.11	57±1.89	72±1.90	$p<0.012$*	$p<0.034$*
Blood Glucose Random	90.75 ± 0.85	87 ±1.43	90 ± 2.86	$p<0.131$	$p<0.810$

Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Renal Parameters

Results showed increasedlevel of urea and creatinine.Whereas other parameters such as total protein, uric acid albumin and globulin were decreased (Table 2). All these changes were found non significant at p<0.05.

Table 2: Renal Parameters of *V. anthelmintica* at dose of 2mg/kg and 5mg/kg

Renal parameters	Control ± SD	CE ± SEM 2mg/kg	CE ± SEM 5mg/kg	Level of Significance 2mg/kg	Level of Significance 5mg/kg
Urea	31.25 ± 0.85	38±3.21	42 ± 5.12	$p<0.0553$	$p<0.0801$
Creatinine	0.84 ± 0.01	0.79±0.01	1.02 ± 0.07	$p<0.0645$	$p<0.0503$
Uric Acid	0.04 ± 0.01	0.034±0.091	0.03 ± 0.01	$p<0.0845$	$p<0.1302$
Total protein	9.69 ± 0.03	8.41±0.44	8.61 ± 0.88	$p<0.1923$	$p<0.2610$
Albumin	6.19 ± 0.04	3.91±0.11	5.61 ± 0.22	$p<0.0226$	$p<0.0400$
Globulin	3.5 ± 0.04	3.41±0.32	3.00 ± 0.93	$p<0.3097$	$p<0.6103$
A/G Ratio	1.77 ± 0.01	4.09±3.9	7.96 ± 6.54	$p<0.19$	$p<0.38$

Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Hepatic parameters

Table 3 represented the effect of *V. anthelmintica*on liver enzymes. The dose of 5 mg/kg results showed significant increased level of alkaline phosphatase(89.00 ± 12.75; control: 36 ± 0.41) and also significant increased in gamma GT enzyme (20.50 ±10.87) as compared to control animals (13 ± 0.41).

Table 3: Hepatic parameters of *V. anthelmintica* at dose of 2mg/kg and 5mg/kg

Liver function Test	Control Male ± SEM	CE ± SEM 2mg/kg	CE ± SEM 5mg/kg	Level of Significance 2mg/kg	Level of Significace 5mg/kg
Total bilirubin	0.79 ± 0.01	0.712±0.98	0.80 ± 0.01	$p<0.398$	$p<0.543$
Direct Bilirubin	0.05 ± 0.01	0.04±0.08	0.04 ± 0.01	$p<0.293$	$p<0.320$
SGPT	55 ± 0.41	51.43±07.9	46.25 ±10.55	$p<0.156$	$p<0.441$
Alkaline Phosphatase	36 ± 0.41	76.01±09.9	89.00 ±12.75	$p<0.012$*	$p<0.006$*
Gamma GT	13 ± 0.41	16.68± 0.31	20.50± 0.21	$p<0.012$*	$p<0.032$*
SGOT	94 ± 0.48	51.3±11.09	55.50 ± 12.45	$p<0.0081$*	$p<0.01904$

Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Cardiac Parameters

Result showed significant increased in the level of CPK and CK-MB at 2 and 5 mg/kg dose as compared to control. On the other hand LDH levelwas slightly decreased (Table 4).

Table 4: Effect of *V. anthelmintica* on cardiac parameters at dose of 2mg/kg and 5mg/kg

Cardiac enzyme	Control Male ± SEM	CE ± SEM 2mg/kg	CE ± SEM 5mg/kg	Level of Significance 2mg/kg	Level of Significance 5mg/kg
LDH	385 ± 0.5	290.98±51.56	283.50± 58.23	$p<0.110$	$p<0.130$
CPK	803± 0.41	1135±221.11	1511.75± 239.02	$p<0.019$*	$p<0.030$*
CK-MB	1168±0.41	1341±425.01	1523 ± 468.06	$p<0.2980$	$p<0.480$

Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Lipid Profile

Table 5 showed significant increased in the level of all parameters of lipid profile such as cholesterol HDL ratio, cholesterol, triglyceride, HDL, LDL and VLDL Cholesterol at both doses of crude extract of *V. anthelmintica*.

Table 5: Lipid profile of *V. anthelmintica* at dose of 2mg/kg and 5mg/kg

Lipid profile	Control Male ± SD	CE ± SEM 2mg/kg	CE ± SEM 5mg/kg	Level of significance 2mg/kg	Level of significance 5mg/kg
Cholesterol HDL Ratio	3.18 ± 0.05	07.98±3.91	10.45 ± 5.17	$p<0.170$	$p<0.210$
Cholesterol	19 ± 1.58	70.91±16.21	74.50 ± 19.41	$p<0.020$*	$p<0.031$*
Triglycerides	110 ± 2.16	190.67±75.90	194.75 ± 80.82	$p<0.170$	$p<0.336$
HDL	6 ± 0.41	6.45±1.98	9.50 ± 2.22	$p<0.9503$	$p<0.175$
LDL	12 ± 0.41	32.98±10.98	37.75 ± 13.63	$p<0.100$	$p<0.1103$
VLDL	22 ± 0.65	34.94±14.9	38.75 ± 16.05	$p<0.110$	$p<0.3502$

Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Electrolytes

Table 6 revealed the comparison of crude extract of *V. anthelmintica*at 2mg/kg and 5mg/kg with control group. Animals of this group did not show significant variation in the levels of either sodium, potassium, chloride or bicarbonate and calcium.

Table 6: Electrolytes test of *V. anthelmintica* at dose of 2mg/kg and 5mg/kg

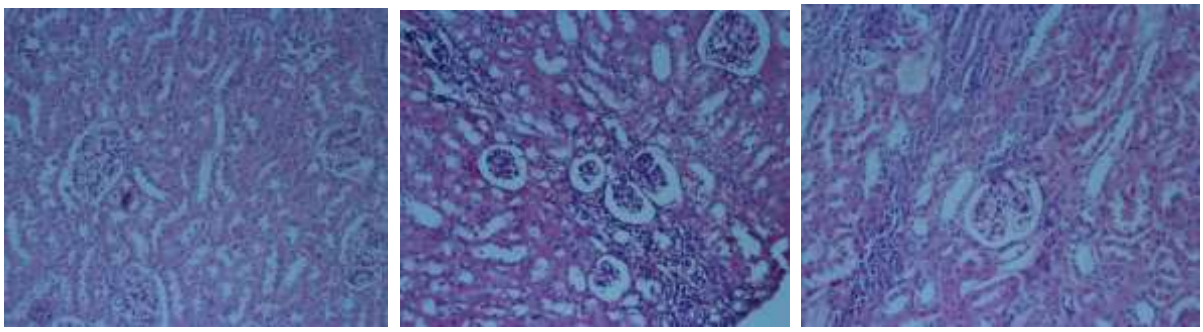
Electrolyte	Control Male ± SD	CE ± SEM 2mg/kg	CE ± SEM 5mg/kg	Level of significance 2mg/kg	Level of significance 5mg/kg
Sodium	145.75 ± 0.63	137.41±0.45	141.50 ± 0.65	$p<0.099$	$p<0.369$
Potassium	4.28 ± 0.07	3.12±0.19	4.70 ± 0.29	$p<0.109$	$p<0.203$
Chloride	103 ± 0.41	97.10±0.12	101.75 ± 0.25	$p<0.216$	$p<0.815$
Bicarbonate	26 ± 0.41	20.11±0.21	25 ± 0.41	$p<0.9018$	$p<0.1342$
Calcium serum	14.23 ± 0.01	10.76±0.121	13.74 ± 0.43	$p<0.1982$	$p<0.3152$

Values showed at less than $p<0.05$. $p<0.001$Values $p<0.05$=significant*,$p<0.001$ =highly significant**,CE: crude extract

Microscopic tissue examination

Renal tissue examination

Gross examinations of renal tissue did not reveal any microscopic changes in control group. Microscopic examination of renal tissue of at 5mg/kg and 2 mg/kg doses (orally) showed mild to moderate inflammation together with focal mild tubular atrophy (Fig 1a-1c).



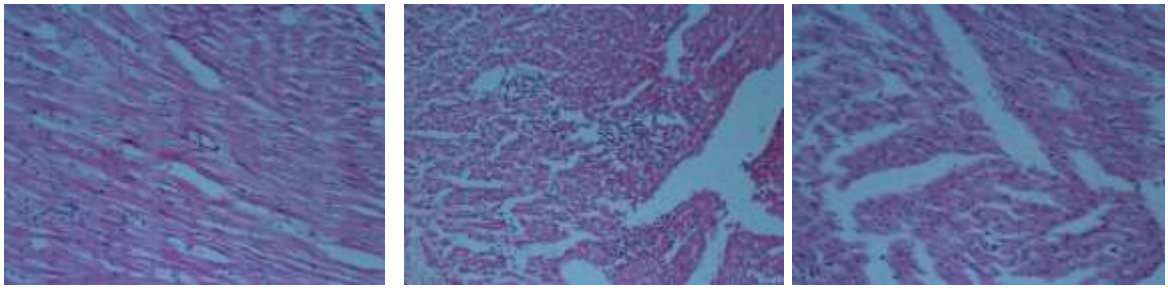
a) **b)** **c)**

Figure 1a: 20X Photomicrograph showed control kidney
Figure 1b: 20X Photomicrograph of kidney treated animal showed moderate inflammation together with focal mild tubular atrophy at the dose of 5mg/kg

Figure 1c: 20X Photomicrograph of kidney treated animal showed mild inflammation together with focal mild tubular atropy at 2mg/kg

Cardiac tissue examination

Gross examination of cardiac tissue did not reveal any microscopic changes in control group. Microscopic examination of cardiac tissue of control animals and treated animal with 2 mg/kg dose of *V. anthelmintica*did not reveal any significant changes in cardiac tissue. On the other hand animals kept on 5mg/kg dose of *V. anthelmintica* according showedmild patchy inflammation (Fig 2a-2c).

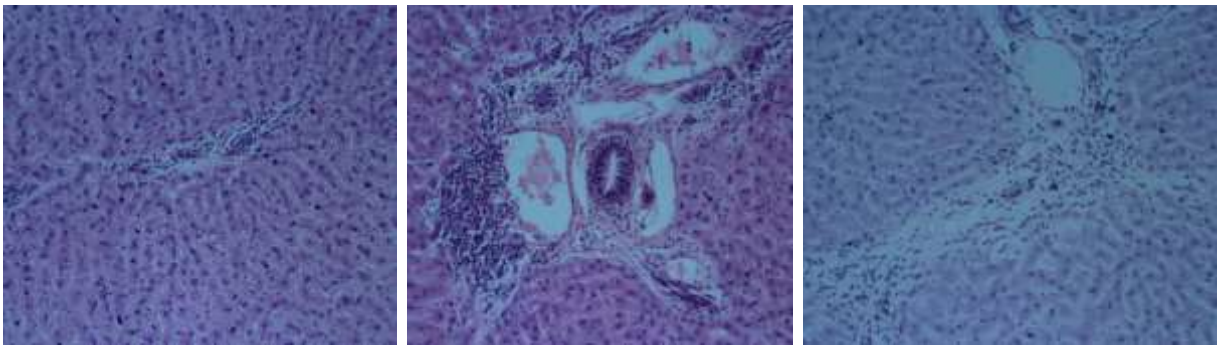


a) b) c)

Figure 2a: 20X Photomicrograph showed control heart
Figure 2b: 20X Photomicrograph of heart showed mild patchy inflammation at 5mg/kg
Figure 2c: 20X Photomicrograph of heart showed no remarkable change at 2mg/kg

Hepatic tissue examination

Microscopic examination of hepatic tissue of control animals did not reveal any significant changes in hepatic tissue.*V. anthelmintica*crude extract (5mg/kg) showed periportal moderate inflammation with periportal fibrosis Moderate to severe lobular and periportal inflammation, abscess and necrosis was also observed at 5mg/kg dose. Figure 3 reveal mild periportal inflammation together with periportal fibrosis (Fig 3a-3c).

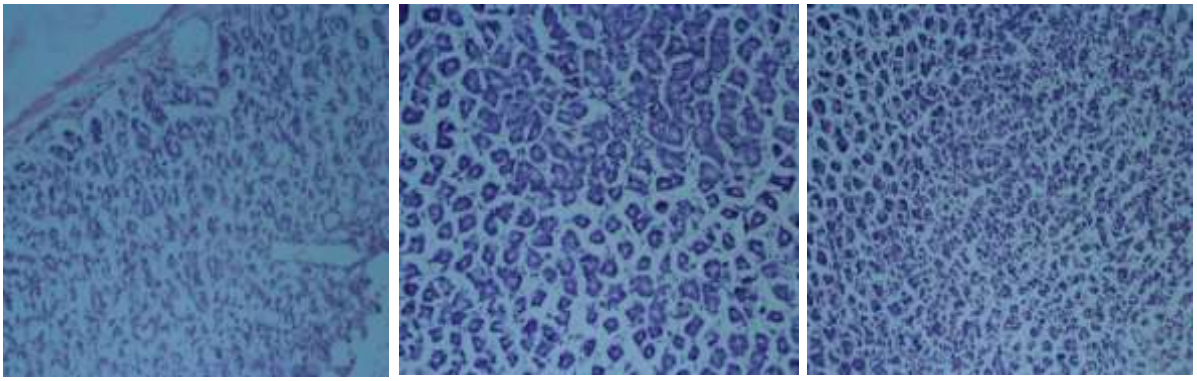


a) b) c)

Figure 3a: 20 X. Photomicrograph of control liver
Figure 3b: 20X. Histopathology of liver showed periportal moderate inflammation together with periportal fibrosis at the dose of 5mg/kg
Figure 3c: 20X. Photomicrograph of liver showed mild periportal inflammation together with periportal fibrosis at the dose of 2mg/kg

Gastric Tissue Examination

Microscopic examination of gastric tissue of control groupand 2mg/kg dose of *V. anthelmintica*did not reveal any significant changes in gastric tissue. On the other hand animals kept on 5mg/kg dose of *V. Anthelmintica* showed mild to moderate inflammation together with lymphoid aggregates.Mild inflammation was also observed (Fig 4a-4c).



a) b) c)

Figure 4a: 20 X. Photomicrograph of control stomach
Figure 4b: 20 X. Photomicrograph of stomach showed mild inflammation at the dose of 5mg/kg
Figure 4c: 20 X. Photomicrograph of stomach showed no change at the dose of 2mg/kg

Discussion

Vernonia anthelmintica seeds traditionally used in leukoderma (Zhou 2012), literature reported its various important pharmacological actions (Rajani 2014), it also has central nervous system depression effect (amber, 2016). It contained elemanolide dimers, vernodalidimers C, D, and E, and four known elemanolides (Ablajan 2015), oxygenated stigmastane-type steroids, vernoanthelcin and stigmastane-type steroidal glycosides (Rajani, 2014). Its seeds oil contained epoxy acid (Gunstone, 1954). Present study carried on crude methanol seed extract at 2 mg/kg and 5mg/kg.

Results of biochemical parameters showed changes in the level of total, direct bilirubin and ALT at 2mg/kg and increased in the level of alkaline phosphatase in at 5mg/kg dose. Elevation in serum alkaline phosphatase initiate primarily from liver as well as from bone (Renner and Dallenbach, 1992). In this study microscopic examination of hepatic tissue showed periportal moderate inflammation together with periportal fibrosis. Mild hepatic damage may be one of the reason of elevation of alkaline phosphatase and gamma GT.

Similarly elevated cardiac enzymes (CPK,CK-MB)were observed with chronic use of crude extract of *Vernoniaanthelmintica* and insignificant decreased level of LDH and decreased level of SGOT were found at 2 mg/kg and 5mg/kg. Cardiac enzymes are proteins in nature that escape out from injured myocardial cells resulting in elevated levels in blood. Increase in the levels of AST was initially used to evaluate cardiac injury, however now a days CK is thought to be more specific for myocardial injury. The decreased level of LDH SGOT revealed chronic inflammation of liver which correlated with the histological observations. CPK (an enzyme needed in creatin-creatinine pathway in muscle cells and brain tissue) has found with increased concentration in heart and skeletal muscle and decreased concentration in brain tissue. Increased level of CPK-MB (> 5%) showed the damage of myocardial cells (Richard and Denise, 2011). However significant change in CK, along with histological slide might be indicating mild cardiac damage at 5mg/kg. Results of histopathology showed insignificant changes at 2mg/kg.

The human kidneys description for less than 1% of body weight, yet collect approximately20% of the cardiac output (Loh and Cohen,2009).Renal function tests such as urine analysis and glomerular filtration rate (GFR) have enthusiastic approach in assessment the extent and rigorousness of renal damage. The GFR is possibly the only significant pointer for the measurement of renal function (Mouton and Holder, 2006) for which Creatinine level in blood has used to assess the GFR (Watson *et al*, 2002; Tschupper*et al*, 2007).

The impact of the extract, *V. anthelmintica* on renal parameters i.e. total protein, Urea and Creatinine levels revealed significant elevation in urea, creatinine and A/G ratio. The increased level of urea was reported might be due to dysfunction of kidney (Etenget al,; 2009). Increased level of creatinine suggested that there was decreased creatinine clearance because of declined glomerular filtration rate. Filtration becomes slow due to renal damage or it may be due to muscular dystrophy or dehydration. Greater the creatin phosphate is broken down in muscle, greater is creatinine formed and it should be released from the body at fairly constant rate (Bazari,*et al,*; 2007). Total protein including albumin was significantly decreased in this study it might be due to both renal and hepatic dysfunction along with renal and hepatic abnormal histopathological findings. However, serum globulin and A/G ratio was insignificantly changed.

The effect of crude extract *V. anthelmintica* on electrolytes i.e. sodium, chloride, bicarbonate, calcium, potassium showed decreased in sodium and chloride while insignificant increased in potassium. Sodium is an important electrolyte in body fluid and it has regulated by kidney and hormone (vasopressin and aldosterone).Sodium has absorbed in renal tubules and co transport with chloride while potassium is counter transport. When sodium concentration changed, ultimately potassium and chloride concentration become altered.Hyponatremia is common condition usually seen in cardiac, liver and kidney disease. Hyponatremia results from the inability of the kidney to eliminate waste water load. Kidney control water balance in body via anti diuretic hormone performances on the V_2 receptors situated at the basolateral aspect of the collecting duct cells and leads to rises water absorption. Hyponatremia occur if ADH stimulated,it has seen in obstinate and abnormal ADH secretion (Sahay *et al,* 2014). Hyponatremia in cerebral salt wasting is moderately unstated, but the best data suggested the possibility of increased B-type natriuretic peptide levels while aldosterone repressed, both contributing to the natriuresis. Hypopituitarism and primary adrenal insufficiency (Addison's disease) are rare but often unexploited causes of hyponatremia. In primary adrenal insufficiency, aldosterone deficiency subsidise to hyponatremia, with hyperkalemia. Heart failure and liver failure lead to a low effective arterial blood volume. As a response to the condensed baroreceptor activity, the renin angiotensin system is stimulated first, whereas the vasopressin axis is activated after a better decrease in arterial filling (Hoorn, 2008).In this study significant hyponatremia and hypochloremia lengthways with insignificant hypocalcemiaat 5 mg/kg may suggested renal function has impaired, this was also supported by high serum creatinine and renal histopathology findings.

The effect of *V. anthelmintica* on lipid profile such as cholesterol, triglyceride, HDL, LDL and VLDL showed animals received 2mg/kg and 5mg/kg dose exhibitedinsignificant increased in triglyceride, HDL, LDL, VLDL. On the other hand cholesterol has significantly increased which may be due to decreased metabolic function of liver. Elevated levels of cholesterol and triglycerides plainly prelude to atherosclerosis and other cardiovascular disorders (Nielsen, 1996). Irregular lipoproteins level is also responsible factor of increased cholesterol level. These random levels may be due to liver dysfunction or other factors (Durrington, 2003, Feroz*et al*, 2011).

Influence of the extract on haematological parameters such as haemoglobin, RBC, WBC, platelet, haematocrit, MCV, MCH, MCHC, Hb A1C PT, APTT revealedthe*p* values of these result of Hb, R.B.C, Platelets counts,HbA1C has no significant range, that indicated*V. anthelmintica* has nontoxic effects on bloodparametres. On the other hand result showed increased in platelets. Schaefer has reported inflammatory disorder and iron deficiency anaemia as one of the cause of increased platelets counts (Schaefer,2003). However PT, and INR revealed highly significant in*P* values at 5mg/kg. This might be due to poor liver function test that has correlated with histological slides of liver.At 2mg/kg all values were insignificant as compare to control group.

Histological findings suggested that at 5 mg/kg seed extract of *V. anthelmintica* has some potential to injured the liver heart and kidney tissues. These effects may be due to the presence of bitter component vernodlin, vernodalol, andvernolicacid (Iqbal 2006). Similarly one of the

research (Alam 2010) suggested it has larvicidal toxicity. Therefore, it is again possible that toxicity might be appear on long term usage of *V. anthelmintica* extract in this animal study.

Conclusion

On the basis of above findings, it can be concluded that chronic use of *Vernoniaanthelmintica* possessed alteration in biochemical and histological parameters, and this drugs possess mild toxic effect on histological findings in animal studies.Although 2 mg/kg dose showed insignificant changes but one should be very careful if uses for long time therapy.

References

Bazari H. Approach to the patient with renal disease. In: Gold man L, Ausiello D, eds. Cecil Medicine. 23 ed. Philadelphia, Pa: Saunders: Elsevier; 2007: chapter 115.

Bentely, R, Trimen, H. Medicinal Plants: 1999, Asiatic Publishing House, New Delhi, India.

Durrington PN. Seminar: dyslipidaemia. *The Lancet* 2003; 362; 717–731.

Eteng MU, Ibekwe HA, Abolaji AO, Okoi AL, Onwuka FC and Osuchukwu FC. Effect of *Rauwolfia vomitoria* Afzel (Apocynaceae) extract on serum amino transferase and alkaline phosphatase activities and selected indices of liver and kidney functions. *Africa. J. Biotech*, 2009; 8(18): 4604-4607.

Feroz Z, Khan RA,andAfroz S. Cumulative toxicities on lipid profile and glucose following administration of anti-epileptic, anti-hypertensive, anti-diabetic and anti-arrythemic drugs. *Pakistan journal of Pharmaceutical sciences*, 2011; 24 (1): 47-51.

Hoorn E.J. Zietse R Hyponatremia Revisited: Translating Physiology to Practice *Nephron Physiol.*, 2008; 108: 46.

Joshi SG *Medicinal Plants*. Oxford and IBH Publishing Co. Pvt. Ltd 2000., New Delhi, India..

Khan RA, Sadruddin, Mirza T.Evalution of safety profile of Herbal tea.*J Basic Appl Sci*., 2007; 3(1): 49-53.

Krishnamoorthy V, Madalageri, MB. Bishop Weed (*Trachyspermumammi*): an essential crop for north Karnatka. *Journal of Medicinal and Aromatic Plant Sciences*1999; 21 (4): 996-998.

Loh AH and Cohen AH. Drug-induced kidney disease-pathology and current concepts. Annals academy of medicine: 2009; 38(3):240-250.

Mhaskar KS, Blatter E, Caius JF.*Kirtikar and Basu's illustrated, Indian Medicinal Plants.*Their usage in Ayurvedic and Unani medicines.Volume 6.Third revised and enlarged edition.Sri Satguru Publications. A division of Indian Books Center, Delhi, India: 2000; 1832-1834.

Mouton R and Holder K. Laboratory tests of renal function. Anesthesia and intense care medicine, 2006; 7: 240-243.

Renner, E.L. and Dällenbach, A., 1992. Increased liver enzymes: what should be done?. *Therapeutische Umschau. Revue Therapeutique*, *49*(5), pp.281-286.

Sahay Mand Sahay R. Hyponatremia: A practical approach Indian *J EndocrinolMetab*. 2014; 18 (6): 760-771.

Schaefer A.I; Thrombocytosis and Essential Williams Hematology, 6th edition, Beutler E,*ET AL,* New York; McGraw-Hill Book Company, 2003: 1541-1547.

Tscuppert Y, BuclinT, Rothuizen LE, Decostered LA, Galleyrand J, Gaud C and Biollaz J. Effect of dronedarone on renal function in healthy subjects. *British J. of clinical pharmacol.,* 2007; 64 (6): 785-791.

Watson AD, Lefeebvre H.P, Concordet D, Laroute V, Ferre JP, ConchouFandToutain PL. Plasma exogenous creatinine clearance test in dogs; Comparison of other methods and proposed limited sampling strategy. *J. Vet. Int. Med*., : 2002; 16(1): 22-33.

Amber, Mehjabeen, Mahyrookh, Noorjahan, Farah Owais, Rehman, Mansoor Ahmad. Standardization and neuropharmacological effects of *Vernonia anthelmintica* (Linn.)Crude extract andFractions. *Acta Biologica Indica* 2016, 5(1):38-45

Ezeonwumelu J O C, JuliusA K, Muhoho CN, Ajayi AM, Oyewale A A, Tanayen J K, Balogun SO, Ibrahim A, Adzu B, AdiukwuC P, OloroJ, KiplagatDM, GojiA D T, OkoruwaAG., OnchweriAN and ReddyPMK. Biochemical and Histological Studies of Aqueous Extract of Bidenspilosa Leaves from Ugandan Rift Valley in Rats. *British Journal of Pharmacology and Toxicology* 2011; 2(6): 302-309.

Mehjabeen, Farah-Saeed, Noor-Jahan, Mansoor Ahmad.Effects of a. Mellifica extract on the hematological, biochemical, urine and histopathological parameters of white albino rabbits. *International Journal of Pharmacy and Pharmaceutical Sciences*., 2015:7(5);127-129(S1-S5).

Iqbal Z, Lateef M, Jabbar A, KhanMK. Anthelmintic Activity of *Vernonia anthelmintica* Seeds against Trichostrongylid Nematodes of Sheep. PharmaceuticalBiology, 2006:44(8); 563–567.

Alam MF, Chopra AK, Mohammed M. Safhi1 and Dua VK. Toxicity of *Vernonia anthelmintica* Linn. (Asteracea) seeds against mosquitoes vectors. Journal of Applied and Natural Science, 2010: 2 (2): 190-193.

Gunstone FD.Fatty acids. Part II. The nature of the oxygenated acid present in *Vernonia anthelmintica* (Willd.) seed oil. *J. Chem. Soc.*, 1954, 1611-1616.

Ablajan Turak, Yongqiang Liu, Haji AkberAisa.Elemanolide dimers from seeds of *Vernonia anthelmintica*. *Fitoterapia*, 2015; 104, 23–30.

Rajani Srivastava, Amita Verma, Alok Mukerjee, and NeetuSoni. Phytochemical, Pharmacological and Pharmacognostical Profile of *Vernoniaanthelmintica*: An Overview. *RRJPP*, 2014: 2 (2); 22-28.

Richard AH, Denise RF. (2011). Lippincott's Illustrated Reviews: Biochemistry Fifth Edition,Lippincott Williams & Wilkins, a Wolters Kluwer business, pp 64-66; 288.