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Prevalence and Haemato-biochemical studies on Hepato-renal dysfunction in pigs

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Abstract
The present study was conducted with an objective to study the prevalence of hepato-renal dysfunction in pigs. Blood samples were collected from a total of 90 clinically affected pigs in and around Guwahati, Assam. In this study, a total of 53 (58.8%) cases were recorded as positive out of which 25 (27.7%) for hepatic, 17 (18.8%) for hepato-renal and 11(12.2%) for renal dysfunctions respectively. Haemato-biochemical values of different parameters generated from the study were analyzed statistically. Haematological parameters *viz.* haemoglobin (g/dl). Packed cell volume (%), total erythrocyte count (10^6/mm^3), total leukocyte count (10^3/mm^3), differential leukocyte count (%), thromobocyte count (10^3/mm^3), Mean corpuscular haemoglobin (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) revealed that pigs suffered from hypochromic microcytic anaemia,. Biochemical marker included in liver and kidney function tests revealed that affected pigs showed moderate to severe dysfunction of liver and kidneys.

Keywords: Biochemical, haematological, hepato-renal, dysfunction, pig

Introduction
In India, pig husbandry is playing a significant role in rural livelihood programme, particularly among the tribal belt in the country. Pig husbandry contributes to economic growth through the generation of employment and foreign exchange. India possesses 11,133 pigs which contribute 2% of the world's pig population (19th livestock census). Eastern and North Eastern (NE) states of India possesses highest pig population. The pig population in Assam is 1.63 million which share 15.89% of total India's pig population. Liver and kidneys are two most important vital organs, that perform varieties of metabolic functions within the body. Liver and kidneys are most common vital organs subjected to various injuries and insults. Hepatic failure is a syndrome that results from inadequate hepatic function, may be the result of sudden massive hepatic destruction or more frequently the end point of progressive liver damage. Usually, liver failure is accompanied by the signs of kidney dysfunction such as anuria, oliguria and biochemical alterations. Pathogenesis of hepato-renal dysfunction is related to alteration of arterial blood volume due to reduce venous return and cardiac output (Jubb *et al*., 2007 and Jones *et al*., 2006). Increased compensatory vasoconstriction of renal circulation causes decrease rate of glomerular filtration and resultant renal failure. Kidneys might show minimal pathologic changes but prognosis is grave (Tari, 2016) [20].

Materials and Method
Blood samples were collected from clinically affected pigs from organized and unorganized farms located in and around Guwahati, Assam. The whole blood collected from clinically affected pigs were subjected to study different haematological parameters *viz.*, haemoglobin (g/dl), Packed cell volume (%), total erythrocyte count (10^6/mm^3), total leukocyte count (10^3/mm^3), differential leukocyte count (%), thrombocyte count (10^3/mm^3), Mean corpuscular haemoglobin (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) with the help of automated haematology cell counter model MS4e (Melet Schloesing Labaoratories -9 Chaussee Jules Cesar-Porte 402-95520 OSNY, France). Biochemical analysis was done by using kits for estimation of GD (Glutamate dehydrogenase), ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), Total protein, Glucose, Total bilirubin, Direct bilirubin, BUN (Blood urea nitrogen) & creatinine as per standard methods. Data obtained in the present experiment were analyzed statistically by Graph pad prism 7.0 [Analysis of variance (ANOVA, F test].

Results

To study the prevalence of Hepato-nephropathy in pigs, blood samples were collected from clinically affected pigs from organized and unorganized farms located in and around Guwahati. 53 pigs (Organized farms 41, Unorganized farms 12) were positive out of the 90 cases examined during the period from the month of April 2017 to March 2018. The prevalence for hepatic dysfunction was 25 (27.7%), renal dysfunction was 11(12.2%) and hepato-renal dysfunction was 17 (18.8%).On the basis of this survey, the overall positive cases was 58.8%.

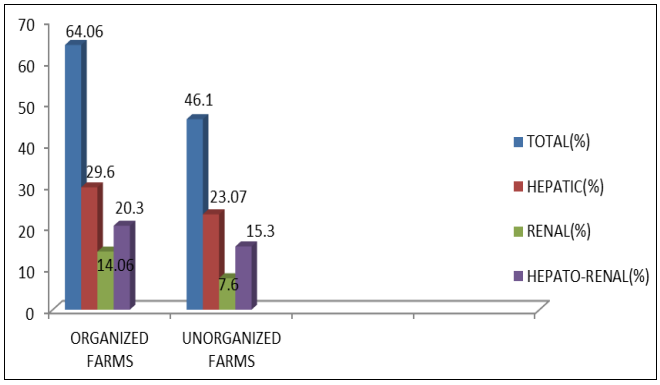

Fig 1: Prevalence of hepatic, renal and hepato-renal dysfunction from organized and unorganized farms

The animals were examined in four different seasons *viz*. pre-monsoon, monsoon, Post-monsoon and winter season. The highest rate of prevalence was observed for hepatic dysfunction in monsoon season (34.6%), renal dysfunction in winter season (27.3%) and Hepato-renal dysfunction in monsoon season (26.9%). The overall proportion of positive cases was highest in Monsoon seaon (73.1%). Regarding sex wise prevalence, the highest rate of hepatic dysfunction was observed in grower pigs (39.1%), renal dysfunction was observed in adult pigs of (20%) and hepato-renal dysfunction was observed in adult pigs (26.6%). The overall proportion of positive cases was highest among grower pigs (73.9%).In case of sex wise prevalence the prevalence rate of hepatic dysfunction was found to be higher in male pigs (32.2%) than in female pigs (19.4%). The prevalence rate of renal dysfunction was found to be higher in female pigs (12.9%) than in male pigs (11.9%). The prevalence rate of Hepato-renal dysfunction was found to be higher in male pigs (38.1%) than in female pigs (18.8%).

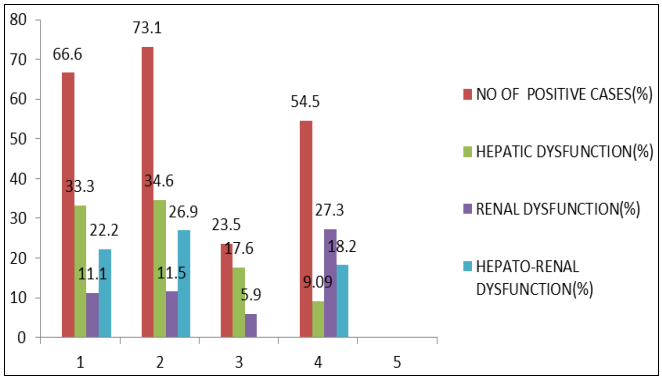

Fig 2: Season Wise Prevalence Rate of Hepatic, Renal and Hepato-Renal Dysfunctions in Pigs

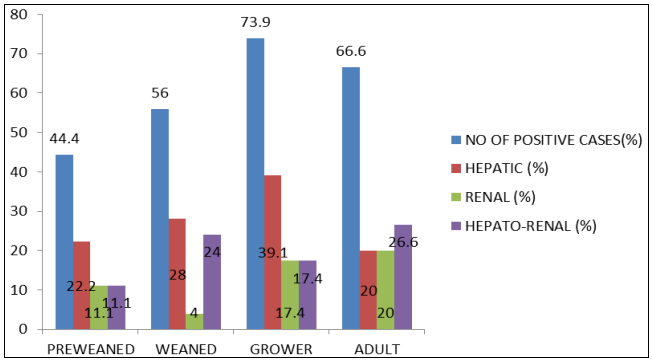

Fig 3: Age wise prevalence of hepatic, renal and hepato- renal dysfunctions in pigs

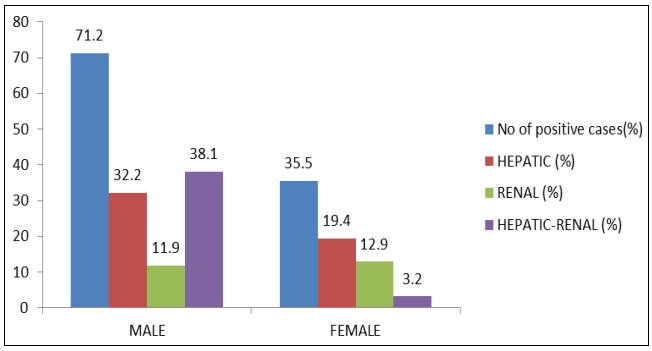

Fig 4: Sex wise prevalence of hepatic, renal and hepato-renal dysfunctions in pigs

The mean values of different haematological parameters with their standard error values of animals positive for hepatic, renal and hepato-renal dysfunctions were presented in the Table I.

The mean haemoglobin values were found highest for Hepatic dysfunction (19.77 g/dl) followed by renal dysfunction (7.7 g/dl) and then hepato-renal dysfunction (3.5 g/dl). The mean packed cell volume was found to be the highest for Hepatic dysfunction (53.4%) followed by renal dysfunction (23.68%) and then hepato-renal dysfunction (7.76%). The mean total erythrocyte count was found to be highest for hepatic dysfunction (10.26±0.26) followed by renal dysfunction (3.68±0.19) and hepato-renal dysfunction (1.84±0.21). The statistical analysis using F test revealed that the value for haemoglobin, Packed cell volume (PCV), Total erythrocyte count (TEC) differed significantly (P<0.0001) among hepatic, renal and hepato-renal dysfunctions respectively.

The mean thrombocyte count was found to be highest for hepato-renal dysfunction (1568±171.1) followed by renal (208.7±19.64) and hepatic (179.7±5.89). The statistical analysis using F-test revealed that the thrombocytes value for hepato-renal dysfunction differed significantly (P<0.001) with that for hepatic and renal dysfunction.

The MCV value was found to be highest for hepatic dysfunction (69.65±1.39) followed by hepato-renal (44.17±0.67) and renal dysfunction (43.6±0.56). The statistical analysis for MCV using F-test revealed that the MCV value for hepatic dysfunction differed significantly (P<0.0001) with that for renal and hepato-renal dysfunction.

The MCH value was found to be highest for hepatic dysfunction (20.45±0.64) followed by hepato-renal (16.63±0.45) and renal dysfunction (16.47±0.32). The mean value for MCHC was found to be highest for highest for

hepatic dysfunction (32.22±1.3) followed by renal (25±1.5) and hepato-renal dysfunction (20.85±0.51). The statistical analysis for MCH using F-test revealed that the MCH and MCHC value for hepatic dysfunction differed significantly (P<0.001) with that for renal and hepato-renal dysfunction respectively.

The mean total leukocyte count was found to be highest in hepatic dysfunction (35.22±4.36) followed by renal dysfunction (16.37±0.19) and hepato-renal dysfunction (16.32±0.09). The mean Neutrophil value was found to be highest for hepatic dysfunction (72.52±0.90) followed by renal (45.5±1.2) and hepato-renal (44.85±2.4) dysfunction. The statistical analysis using F-test revealed that the TLC and neutrophil values for hepatic dysfunction differed significantly (P<0.0001) with that for renal and hepato-renal dysfunction.

The mean lymphocytic value was found to be highest for hepatic dysfunction (50.38±2.13) followed by hepato-renal dysfunction (48.45±2.5) and renal dysfunction (47.27±1.82). The mean monocyte values were found to be highest for Hepato-renal dysfunctions (5.13±0.28) followed by hepatic dysfunction (4.51±0.28) and renal dysfunction (3.91±0.46). The mean eosinophil values were found to be highest for hepatic dysfunction (2.48±0.12) followed by renal dysfunction (2.31±0.13) and hepato-renal dysfunction (2.06±0.22). The statistical analysis using F-test revealed that there was no significant difference (P>0.05) between the average values of lymphocytes, monocytes and eosinophil for different dysfunctions respectively.

The mean values of different biochemical parameters with their standard error values of animals positive for hepatic, renal and hepato-renal dysfunctions were presented in the Table II.

The mean serum GD value was found to be highest for hepatic dysfunction (90.88±1.24) followed by hepato-renal (72.03±1.53) and renal dysfunction (42.85±0.76).The statistical analysis revealed that the value for GD differed significantly (P<0.0001) between the three dysfunctions.

The mean serum ALT value was found to be highest for hepatic dysfunction (115±18.2) followed by hepato-renal (85.23±1.36) and renal dysfunction (34.83±1.92). The statistical analysis revealed that the ALT values for renal dysfunction differed significantly (P<0.001) with that for hepatic and hepato-renal dysfunction.

The mean serum AST value was found to be highest for renal dysfunction (86.6±0.76) followed by hepatic dysfunction (86.3±1.17) and hepato-renal dysfunction (84.4±1).The statistical analysis revealed that there was no significant difference (P>0.05) between AST values for all the three dysfunctions.

The mean total protein value was found to be highest for renal dysfunction (5.68±0.08) followed by hepatic dysfunction (3.5±0.10) and Hepato-renal dysfunction (2.57±0.31) The statistical analysis revealed that the value for total protein differed significantly (P<0.0001) between the three dysfunctions.

The mean serum glucose values were found to be highest for hepatic dysfunctions (153.7±8.69) followed by hepato-renal (137±10.21) and renal dysfunction (111.9±1.54). The statistical analysis revealed that the glucose value for renal dysfunction differed significantly (P<0.01) with that for hepatic dysfunction but there was no significant difference between hepatic and hepato-renal dysfunctions; and also renal and hepato-renal dysfunctions.

The mean total bilirubin value was found to be highest for hepatic dysfunction (1.33±0.20) followed by hepato-renal (1.16±0.20) and renal dysfunction (0.25±0.01).The statistical analysis revealed that the total bilirubin value for renal dysfunction differed significantly (P<0.001) with that for hepatic and hepato-renal dysfunction.

The mean direct bilirubin value was found to be highest for hepatic dysfunction (0.49±0.08) followed by hepato-renal (0.41±0.05) and renal dysfunction (0.16±0.01).The statistical analysis revealed that the direct bilirubin value for renal dysfunction differed significantly (P<0.01) with that for hepatic and hepato-renal dysfunction.

The mean BUN values was found to be highest for renal dysfunction (36.53±1.02) followed by hepato-renal dysfunctions (33.18±0.86) and hepatic dysfunction (6.1±0.23). The mean creatinine values were found to be highest for renal dysfunction (6.45±0.12) followed by hepato-renal dysfunction (3.76±0.22) and hepatic dysfunction (0.88±0.02). The statistical analysis revealed that all the dysfunctions differed significantly (P<0.0001) in their BUN average values.

Table 1: Mean and Standard error Values of Different Haematological Parameters

No	Parameters (unit)	Normal range	Hepatic dysfunction	Renal dysfunction	Hepato-renal Dysfunction
1	Haemoglobin (g/dl)	10-17	19.77 ± 0.48^{A}	7.7 ± 0.18^{B}	3.5 ± 0.62^{C}
2	PCV (%)	32-50	53.4 ± 0.70^{A}	23.68 ± 1.3^{B}	7.76 ± 0.78^{C}
3	TEC (10^6/cumm of blood)	5-8	10.26 ± 0.26^{A}	3.68 ± 0.19^{B}	1.84 ± 0.21^{C}
4	Thrombocyte (10^3/cumm of blood)	250-750	179.7 ± 5.89^{A}	208.7 ± 19.64^{A}	1568 ± 171.1^{B}
5	MCV (fl)	50-68	69.65 ± 1.39^{A}	$43.6\pm.56^{B}$	44.17 ± 0.67^{B}
6	MCH (pg)	17-21	20.45 ± 0.64^{A}	16.47 ± 0.32^{B}	16.63 ± 0.45^{B}
7	MCHC (g/dl)	27-40	32.22 ± 1.3^{A}	25 ± 1.5^{B}	20.85 ± 0.51^{B}
8	TLC (10^3/cumm of blood)	11-20	35.22±4.36	16.37 ± 0.19^{B}	16.32 ± 0.09^{B}
9	Neutrophils (%)	28-62	72.52 ± 0.90^{A}	45.5 ± 1.2^{B}	44.85 ± 2.4^{B}
10	Lymphocytes (%)	35-64	50.38 ± 2.13^{A}	47.27 ± 1.82^{A}	48.45 ± 2.5^{A}
11	Monocytes (%)	2-10	4.51 ± 0.28^{A}	3.91 ± 0.46^{A}	5.13 ± 0.28^{A}
12	Eosinophils (%)	0.5-11	2.48 ± 0.12^{A}	2.31 ± 0.13^{A}	2.06 ± 0.22^{A}

P<0.05=*; P<0.01=**; P<0.001=***; P<0.0001**** P>0.05=NS

Means bearing different superscript bear statistically significant difference (p –value<0.05)

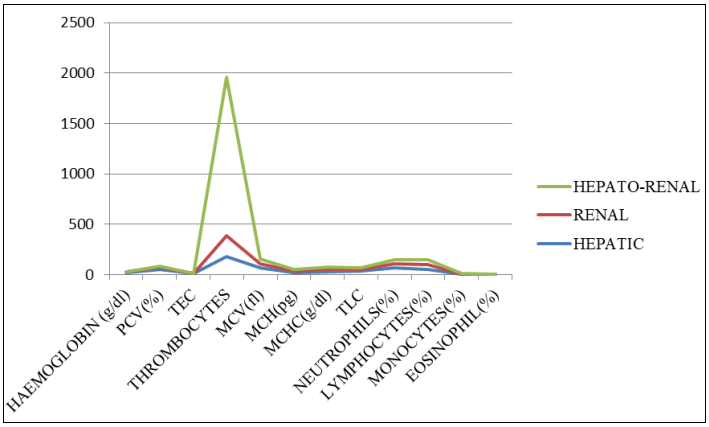

Fig 5: Graphical representation of mean values of different Hematological parameters

Table 2: Mean and Standard error Values for Biochemical Parameters

Sl. No	Parameters (Unit)	Normal Range	Hepatic dysfunction	Renal dysfunction	Hepato-renal Dysfunction
1	GD(U/L)	0-8	90.88 ± 1.24^{A}	42.85 ± 0.76^{B}	72.03 ± 1.53^{C}
2	ALT (U/L)	31-58	115 ± 18.2^{A}	34.83 ± 1.92^{B}	85.23 ± 1.36^{A}
3	AST (U/L)	32-84	86.3 ± 1.17^{A}	86.6 ± 0.76^{A}	84.4 ± 1^{A}
4	Total protein (g/dl)	7.9-8.9	3.5 ± 0.10^{A}	5.68 ± 0.08^{B}	2.57 ± 0.31^{C}
5	Glucose(mg/dl)	85-150	153.7 ± 8.69^{A}	111.9 ± 1.54^{B}	137 ± 10.21^{AB}
6	Total bilirubin (mg/dl)	0-1	1.33 ± 0.20^{A}	0.25 ± 0.01^{B}	1.16 ± 0.20^{A}
7	Direct bilirubin(mg/dl)	0-0.3	0.49 ± 0.08^{A}	0.16 ± 0.01^{B}	0.41 ± 0.05^{A}
8	BUN(mg/dl)	10-30	6.1 ± 0.23^{A}	36.53 ± 1.02^{B}	33.18 ± 0.86^{C}
9	Creatinine (mg/dl)	1-2.7	0.88 ± 0.02^{A}	6.45 ± 0.12^{B}	3.76 ± 0.22^{C}

$P<0.05$=*; $P<0.01$=**; $P<0.001$=***; $P<0.0001$**** $P>0.05$=NS

Means bearing different superscript bear statistically significant difference (p –value<0.05)

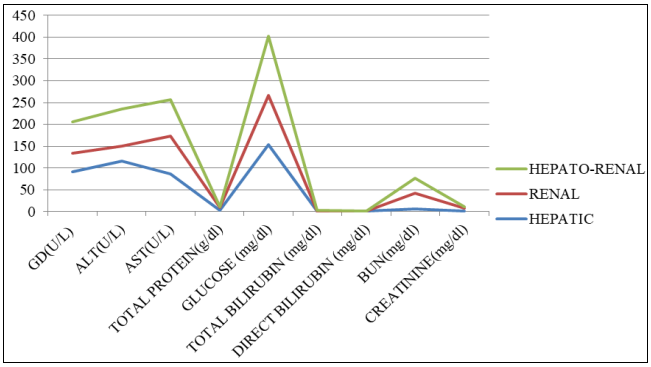

Fig 6: Graphical representation of mean values of different biochemical parameters

Discussion

As per the available literature, the prevalence study of hepato-nephropathy in pigs has not been carried out in this region of the country till now. However, the hepato-renal dysfunction in dogs was reported, it was observed that out of 511(5.34%) positive cases 148(1.55%) suffered from liver dysfunction, 182(1.82%) renal dysfunction and 181(1.89%) from hepato-renal dysfunctions [20].

In the present investigation, a detailed study was conducted on hepato-renal dysfunction in pigs on the basis collected blood samples from clinically affected pigs in which 58.8% were found to be positive for hepato-renal dysfunction, of which 27.7% were positive for hepatic dysfunction, 12.2% for renal dysfunction and 18.8% for hepato-renal dysfunction.

In the present study, for hepatic dysfunction the prevalence was higher in monsoon season 34.6% and lower in winter season 9.09%. For renal dysfunction the higher prevalence was seen in winter season 27.3% and lower in post-monsoon season. For hepato-renal dysfunction the prevalence was higher in monsoon season (26.9%) and post-monsoon was negative. The probable cause of higher prevalence during monsoon season for hepatic dysfunction might be due to higher ambient temperature with increase in the relative humidity which seems conductive for substantial growth and multiplication of infectious factors and vectors. The present observation of higher prevalence of factors causing hepato-renal dysfunction during hot and humid weather was in agreement with the earlier observation [18, 20].

In this study renal dysfunction was found to be higher in winter season (27.3%). This was in accordance with previous study [5, 20].

Age wise prevalence of hepatic, renal and hepato-renal dysfunctions were reported by previous workers [18, 20]. The highest prevalence was recorded (73.9%) among grower pigs and lowest in preweaned pigs (44.4%). The probable cause of higher prevalence among grower pigs might be due to separation from mother, changing food habit and amount of feed consumed. Similarly, the probable cause of lower prevalence among preweaned pigs might be due to maternal antibody which protects piglets from any type of infections.

In regards to sex wise prevalence, higher prevalence was recorded in male animals. More prevalence was reported in female dogs [20]. In the present study, the prevalence of renal dysfunction was found to be higher in female pigs (12.9%) because females suffer more from chronic kidney diseases and incidence increases with age [13].

The average thrombocytes count was found to be lower in hepatic and renal dysfunction *viz.* 179.7(10^3/cumm of blood) and 208.7(10^3/cumm of blood) respectively. There was no significant difference ($P>0.05$) seen statistically in between these values. Thrombocytopenia is seen in hepatic and renal dysfunction which has long been associated with the concept of Hypersplenism, where portal hypertension was thought to cause pooling and sequestration of all platelates in the enlarged spleen. But sometimes secondary thrombocytosis is

another condition which is seen in iron deficiency anemia which causes plate late count to rise [6, 8. 19]. Similar findings were also recorded in the present study in animals suffered from hepato-renal dysfunction where the average thromobocyte count was higher i.e 1568 (10^3/cumm of blood). The mean MCV (69.65 fl) value was slightly higher in hepatic dysfunction but MCH (20.45pg) and MCHC (32.2g/dl) is within the normal range which is indicative of macrocytic normochromic anemia seen in liver diseases [23]. The mean MCV (43.6fl), MCH (16.47pg) and MCHC (25g/dl) for renal dysfunction and MCV (44.17fl), MCH (16.63pg), MCHC (20.85g/dl) for hepato-renal dysfunction was found to be lower, which is indicative of Microcytic hyporchromic anemia seen in chronic diseases and renal failure [17].

The mean TLC (35.22 10^3/cumm of blood) and neutrophils count (72.52%) for hepatic dysfunction was recorded higher is indicative of a variety of disorders including infections, injuries, inflammatory disorders, certain drugs [22]. Other parameters were within the normal range, but it doesn't conclude the cause of hepatic dysfunction. Similarly, in renal and hepato-renal dysfunction also TLC and DLC values were within normal range, no significant difference was seen (P>0.05). Hence, it can be concluded that leukocyte count cannot be used as a diagnostic tool in the detection of hepato-renal dysfunction in pigs. Hepato-renal dysfunction in humans were studied by previous workers [2, 24]. and didn't consider leukocyte count and differential leukocyte as a means of diagnosis of hepato-renal dysfunction.

In the present study the mean total protein values for hepatic (3.5), renal (5.6) and hepato-renal (2.57) were significantly lower (P<0.0001). There was decrease in total protein level in kidney dysfunction [6]. This was in accordance with the present study. Diffuse and chronic liver diseases (cirrhosis) decreases protein concentration and in renal diseases where there is excessive loss of protein due to reduction of glomerular filtration rate results in low protein level [1, 11].

The mean values for serum bilirubin were significantly higher (P<0.001) for hepatic and hepato-renal dysfunction as compared to renal dysfunction. Bile pigments are synthesized and excreted by the hepatobiliary system; hence there is alteration in their blood levels during any dysfunction of this system [1, 6, 12].

Alteration in the serum levels of enzymes were also seen along with the above parameters. The levels of enzyme like ALT and GD (Glutamate dehydrogenase) were significantly higher (P<0.0001) in hepatic and hepato-renal dysfunctions when compared with renal dysfunction. Similar kind of observations were also recorded by previous workers in dogs [20]. Largest ALT were observed in hepatocellular inflammation and necrosis [12]. Elevated GD levels had been reported in ruminants with hepatic necrosis [10]. In the present study also, similar kind of elevated GD levels (90.88 U/L) were recorded in animals suffered from hepatic dysfunction. However, there was no significant difference (P>0.05) of AST values in all the three dysfunctions. AST activity is higher in kidneys, liver and skeletal muscles so considered as not specific [12].

In the present study the mean values of BUN and creatinine were significantly higher (P<0.0001) in renal and hepato-renal dysfunctions when compared with that in hepatic dysfunctions. Similar changes were documented by previous workers [1, 6, 12]. in renal dysfunction caused due to various factors. Increased level of BUN and Creatinine in dogs suffered from renal and hepato-renal dysfunction [20].

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