An evaluation of the effects of the herbal supplements of amla (*Emblica officinalis*) and giloy (*Tinospora cordifolia*) on the liver function parameters of Magra lambs ~~living~~ in western Rajasthan's arid zone.

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Abstract

The aim of this study was to determine the effect of herbal feed additives amla (*Emblica officinalis*) and giloy (*Tinospora cordifolia*) on Liver Function Test parameters of Magra lambs in the extensive management system. The experiment was performed on twenty eight M~~m~~agra lambs of three to four months of age under the extensive system, which were randomly distributed into four experimental groups of seven lambs in each group in a randomized block design (RBD). Herbal feed additive amla (*Emblica officinalis*) fruit powder with seed and giloy (*Tinospora cordifolia*) stem powder were supplemented at level of 1.5g/kg body weight with concentrate as oral/feed supplemented in T_1 and T_2 group, respectively except control (C) group and T_3 in extensive management system. Group T_3 were supplemented with the combination of amla (*Emblica officinalis*) fruit powder and giloy (*Tinospora cordifolia*) stem powder at the level of 0.75g/kg body weight with concentrate as oral/feed. Therefore, it can be concluded that the experiment was safe and did not cause any damage to the liver.

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Comment [DK2]: Abbreviation for control group (C)

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Keywords:- LFT, SGPT, SGOT, Amla, Giloy

INTRODUCTION

Magra sheep is a native breed to Rajasthan, India. They are medium sized sheep with thick wool and are known for their docile temperament. They are also known for their ability to produce high-quality wool. Liver function tests (LFTs) play a crucial role in assessing the health and functionality of the liver, a vital organ with diverse metabolic, synthetic, and detoxification functions. This test measures the amount of enzymes and proteins in the liver. It is used to determine the functioning state of the liver and also helps to detect any underlying disorders or diseases. Liver enzymes, such as ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase), are measured in LFTs to assess liver cell damage. Elevated levels of ALT and AST may indicate liver diseases such as hepatitis or cirrhosis. Alkaline Phosphatase (ALP) is another enzyme measured in LFTs, providing information about bile duct health and bone metabolism. Abnormal ALP levels can indicate liver diseases or issues with the biliary system. Total bilirubin levels are assessed to identify problems with the liver's ability to process bilirubin, a waste product from the breakdown of red blood cells. Elevated bilirubin levels can suggest conditions like jaundice or other liver disorders.

Albumin and total protein levels are measured to evaluate the liver's synthetic capacity for producing proteins essential for various bodily functions. Reduced albumin levels may indicate chronic liver disease, malnutrition, or impaired synthetic function. Prothrombin time (PT) is a measure of blood clotting, and its assessment in LFTs helps gauge the liver's ability to produce clotting factors. Prolonged PT may suggest liver dysfunction, impacting the blood's ability to coagulate. LFTs are crucial in monitoring and managing chronic liver diseases, such as hepatitis, fatty liver disease, and cirrhosis. These tests are also essential before certain medical procedures or surgeries to assess the patient's liver health and potential risks.

MATERIALS AND METHODS

Blood samples from experimental lambs were collected in the morning hours before feeding and watering of lambs. Samples were collected at monthly interval by puncturing jugular vein following aseptic measures. The blood, so drawn was collected in sterilized test tubes containing adequate amount of anticoagulant. Biochemical studies were performed soon after collection of blood. For separation of serum, blood was collected in second tube, without anticoagulant, and kept in slanting position. These tubes were incubated for 1 h at 37°C. Blood clots were broken and tubes were centrifuged at 2500 rpm for 30 minutes. The serum was pipetted out in small pyrex tubes and kept for further analysis of serum albumin (ALB), serum globulin, total serum protein (TP), serum glucose, serum creatinine, serum cholesterol, serum triglyceride and blood urea nitrogen.

Comment [DK4]: Also write about experimental animals-number of animals, groupings, age of animals, health status
Management of animals- rearing system, feeding, housing.
Supplement- form, quantity and duration of supplement
Duration of experiment
Location of experiment
Method of estimation of various enzymes
Sampling – intrevals

Liver Function Test

ALT, AST, ALP and ACP were determined by Clinical Chemical Analyzer Model AGD2020.

~~**Alanine aminotransferase (ALT) (IU/L) SGPT**~~

~~The ALT (SGPT) test is a blood test used to measure the amount of the enzyme alanine transaminase (ALT) in the blood. This enzyme is found primarily in the liver, and high levels can indicate liver damage or disease.~~

~~**Aspartate aminotransferase (AST) (IU/L) SGOT**~~

~~AST (SGOT) is an enzyme found in many organs, but primarily in the liver. It is released into the blood when the liver is damaged. High levels of AST in the blood can indicate liver disease or injury, and can be used to monitor the effectiveness of treatment.~~

Comment [DK5]: Write the methodology for estimation

~~**Alkaline phosphatase (ALP) (IU/L)**~~

~~ALP is an enzyme that is primarily found in the liver and bones. It plays a crucial role in various physiological processes, including the metabolism of proteins and fats. Elevated levels of ALP in the blood may indicate liver damage or disease, such as hepatitis or cirrhosis. Additionally, ALP levels can also be elevated during pregnancy or due to certain medications. Therefore, measuring ALP levels through a liver function test can provide valuable insights into the overall health and functioning of the liver.~~

~~**Acid phosphatase (ACP) IU/L**~~

~~Acid phosphatase is an enzyme that is found in the livers of humans and other animals. It is used as an indicator of liver health, as its levels can indicate the presence of liver disease or inflammation. In a liver function test, acid phosphatase levels are measured to assess the functioning of the liver.~~

RESULTS AND DISCUSSION

Comment [DK6]:

Liver Function Test

Alanine aminotransferase (ALT) (IU/L) SGPT

The mean values of Alanine aminotransferase (IU/L) of lambs under various treatment groups at monthly intervals of experiment and overall mean for entire experimental period have been presented in Table 01.

The average values of ALT (IU/L) at 30 days in control, T_1, T_2 and T_3 treatment groups were found to be 10.61, 10.62, 10.82 and 10.91%, respectively, while at 60 days the values were found to be 10.62, 10.74, 10.71 and 10.33% for control, T_1~~1~~, T_2 and T_3 treatment groups in the semi-intensive system. At the 90 ALT (IU/L) in control, T_1, T_2 and T_3 treatment groups were found to be 10.53, 10.69, 10.56 and 10.61%, respectively which differed non significantly with each other. The overall mean contents of Alanine aminotransferase (IU/L) were found to be 10.59, 10.68, 10.70 and 10.62 in lambs of control, T_1, T_2 and T_3 treatment groups, respectively in the extensive system.

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Comment [DK7]: In which system are the animals reared?

The statistical analysis of variance revealed no significant effect of supplementation of herbal feed additives at each month of experimental period and also on overall mean Alanine aminotransferase (IU/L) concentration of experimental lambs in the extensive system.

Table 01: Average values of Alanine aminotransferase (IU/L) at different time intervals in different treatment groups of Magra lambs in the extensive system

Treatment groups	Period (months)				
	0	I	II	III	Mean
C	10.93	10.61	10.62	10.53	10.59
T_1	10.78	10.62	10.74	10.69	10.68
T_2	10.71	10.82	10.71	10.56	10.70
T_3	10.98	10.91	10.33	10.61	10.62
SEM	0.06	0.07	0.09	0.04	0.03

~~Note: Means with different superscripts in a column differ significantly~~

Comment [DK8]: Data are not-significant

The results obtained in extensive management system for ALT (IU/L) in control, T_1, T_2 and T_3 group lies within the normal range of 26-34 (IU/L) (Kahan, 2005 and Kaneko *et al*., 1997).

~~In the extensive system, the present findings are similar to the findings of Ding *et al*. (2021) who reported nonsignificant changes in ALT (IU/L) of hanlambs supplemented withdried *Allium mongolicum* Regel (AMR) powder.~~

~~These findings are not in agreement with Beigh *et al*. (2017) in which herb treated groups have significant improvement in ALT (IU/L).Niwas *et al*. (2012) for herbal additives, who also reported limited ALT activity in calves by feed additive supplementation. It could be concluded that supplementation of the herbal feed additives Amla (***Emblica officinalis***) and Giloy (***Tinospora cordifolia***) in the diets of Magra lambs had no adverse effect ALT (IU/L) in different management systems.~~

Comment [DK9]: Which system of management?
Data not significant, justification not required.

Aspartate aminotransferase (AST) (IU/L) SGOT

The mean values of Aspartate aminotransferase (AST) (IU/L) of lambs under various treatment groups at monthly intervals of experiment and overall mean for entire experimental period have been presented in Table 02.

The average values of AST (IU/L) at 30 days in control, T_1, T_2 and T_3 treatment groups were found to be 69.69, 70.47, 70.32 and 70.05% respectively while at 60 days the values were found to be 70.52, 70.08, 70.30 and 70.18% for control, T_1, T_2 and T_3 treatment groups in the semi-intensive system. At the 90 AST (IU/L) in control, T_1, T_2 and T_3 treatment groups were found to be 69.95, 69.86, 69.86 and 70.16%, respectively.The overall mean contents of Aspartate aminotransferase (AST) (IU/L) were found to be 70.05, 70.14, 70.16 and 70.13 in lambs of control, T_1, T_2 and T_3 treatment groups, respectively in the extensive system. Analysis of data revealed no significant differences among the mean values.~~which differed non significantly with each other.~~

Table 02: Average values of Aspartate aminotransferase (IU/L) at different time intervals in different treatment groups of Magra lambs reared in the extensive system

Treatment groups	Period (months)				
	0	I	II	III	Mean
C	70.87	69.69	70.52	69.95	70.05
T_1	69.26	70.47	70.08	69.86	70.14
T_2	68.16	70.32	70.30	69.86	70.16
T_3	70.94	70.05	70.18	70.16	70.13
SEM	0.67	0.17	0.09	0.07	0.02

~~Note: Means with different superscripts in a column differ significantly~~

~~The overall mean contents of Aspartate aminotransferase (AST) (IU/L) were found to be 70.05, 70.14, 70.16 and 70.13 in lambs of control, T_1, T_2 and T_3 treatment groups, respectively in the extensive system.~~The statistical analysis of variance revealed no significant effect of supplementation of herbal feed additives at each month of experimental period and also on overall mean Aspartate aminotransferase (AST) (IU/L) concentration of experimental lambs in the extensive system. The results obtained in extensive management system for AST (IU/L) in control, T_1, T_2 and T_3 group lies within the normal range of 60-280 (IU/L) (Kahan, 2005 and Kaneko *et al*., 1997). ~~It could be concluded that supplementation of~~

~~the herbal feed additives amla (*Emblica officinalis*) and giloy (*Tinospora cordifolia*) in the diets of Magra lambs had no adverse effect AST (IU/L) in different management systems.~~

Alkaline phosphatase (ALP) (IU/L)

The mean values of Alkaline phosphatase (ALP) (IU/L) of lambs under different treatment groups at monthly intervals of experiment and overall mean for entire experimental period have been presented in Table 03.

The average values of ALP (IU/L) at 30 days in control, T_1, T_2 and T_3 treatment groups were found to be 70.11, 70.10, 70.03 and 70.21% respectively while at 60 days the values were found to be 71.29, 72.56, 73.33 and 71.83% for control, T_1, T_2 and T_3 treatment groups in the semi-intensive system.

At the 90 ALP (IU/L) in control, T_1, T_2 and T_3 treatment groups were found to be 71.75, 72.15, 72.40 and 72.72%, respectively which differed non significantly with each other. The overall mean contents of Alkaline phosphatase (ALP) (IU/L) were found to be 71.05, 71.60, 71.92 and 71.58 in lambs of control, T_1, T_2 and T_3 treatment groups, respectively in the extensive system.

Table 03: Average values of Alkaline phosphatase (ALP) (IU/L) at different time intervals in different treatment groups of Magra lambs reared in the extensive system

Treatment groups	Period (months)				
	0	I	II	III	Mean
C	70.68	70.11	71.29	71.75	71.05
T_1	69.58	70.10	72.56	72.15	71.60
T_2	69.28	70.03	73.33	72.40	71.92
T_3	71.83	70.21	71.83	72.72	71.58
SEM	0.58	0.04	0.44	0.21	0.18

~~Note: Means with different superscripts in a column differ significantly~~

The statistical analysis of variancerevealed no significant effect of supplementation of herbal feed additives at each month of experimental period and also on overall mean ALP (IU/L) concentration of experimental lambs in the extensive system. The results obtained in extensive management system for ALP (IU/L) in control, T_1, T_2 and T_3 group lies within the normal range of 68-387 (IU/L) (Kahan, 2005 and Kaneko *et al.*, 1997).

~~It could be concluded that supplementation of the herbal feed additives Amla (*Emblica officinalis*) and Giloy (*Tinospora cordifolia*) in the diets of Magra lambs had no adverse effect ALP (IU/L) in different management systems.~~

Acid phosphatase (ACP) IU/L

The mean values of Acid phosphatase (ACP) (IU/L) of lambs under different treatment groups at monthly intervals of experiment and overall mean for entire experimental period have been presented in Table 04.

The average values of ACP (IU/L) at 30 days in control, T_1, T_2 and T_3 treatment groups were found to be 1.26, 1.27, 1.27 and 1.25% respectively while at 60 days the values were found to be 1.23, 1.24, 1.26 and 1.23% for control, T_1, T_2 and T_3 treatment groups in the semi-intensive system. At the 90 ACP (IU/L) in control, T_1, T_2 and T_3 treatment groups were found to be 1.22, 1.23, 1.28 and 1.25%, respectively which differed non significantly with each other. The overall mean contents of Acid phosphatase (ACP) (IU/L) were found to be 1.23, 1.25, 1.27 and 1.24 in lambs of control, T_1, T_2 and T_3 treatment groups, respectively in the extensive system.

The statistical analysis of variance revealed no significant effect of supplementation of herbal feed additives at each month of experimental period and also on overall mean Acid phosphatase (ACP) (IU/L) concentration of experimental lambs in the extensive system.

The results obtained in extensive management system for ACP (IU/L) in control, T_1, T_2 and T_3 group lies within the normal range of 0-12 (IU/L) (Kahan, 2005 and Kaneko *et al*., 1997). ~~It could be concluded that supplementation of the herbal feed additives Amla (*Emblica officinalis*) and Giloy (*Tinospora cordifolia*) in the diets of Magra lambs had no adverse effect ACP (IU/L) in different management systems.~~

Table 04: Average values of Acid phosphatase (ACP) (IU/L) at different time intervals in different treatment groups of Magra lambs reared in the extensive system

Treatment groups	Period (months)				
	0	I	II	III	Mean
C	1.29	1.26	1.23	1.22	1.23
T_1	1.24	1.27	1.24	1.23	1.25

T_2	1.22	1.27	1.26	1.28	1.27
T_3	1.31	1.25	1.23	1.25	1.24
SEM	0.021	0.005	0.007	0.014	0.008
~~Note: Means with different superscripts in a column differ significantly~~					

These findings of Liver function test are not in agreement with Beigh *et al.* (2017) who reported that the supplementation of feed additives had a significant impact on mean A:G ratio ($P<0.01$) and ALT levels ($P<0.05$), while a significant effect on total mean total serum proteins was recorded in the T_2 and T_3 groups.

Moreover, the results of our study also indicated that the serum enzymes AST, ALT, ALP and ACP were within the ranges of normal physiological ranges, indicating that the serous enzymes are functioning normally. Because of this, no gastrointestinal issues or pathological organ lesions were noted. (Van Putten *et al*., 2013; Al-Hadithy*et al*., 2013). In thefinding of our study no significant differences in activities of AST and ALT in all treatment groups, indicating no damage to liver tissue or function (Thapa and Walia, 2007).

CONCLUSION

It could be concluded that supplementation of the herbal feed additives Amla (***Emblica officinalis***) and Giloy (***Tinospora cordifolia***) in the diets of Magra lambs had no adverse effect on LFT parameters in the extensive management system. Liver function test in sheep revealed no abnormalities, suggesting that sheep are tolerant to the toxic compounds of the herbs.

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