

Comparative evaluation of A1A2 and A2A2 cow milk-containing diet on biochemical and histological parameters of healthy Wistar rats

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SUPPLEMENTARY FILE

Materials and methods

Wistar rats (80-120 g) of either sex were used for the present study and were acclimatized to the laboratory conditions for a period of 7 days before the onset of the experiment. The general behaviour, body weight, and feed-water intake of the rats were observed during the acclimatization period. The rats were maintained as per the Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the study was conducted as per the approved protocol (ethical approval No. 585/05/A/CPCSEA). The rats were kept on a proper diet and water throughout the study.

At the end of the study, the rats were painlessly sacrificed by the cervical dislocation method (Kumar *et al.*, 2022). The liver, kidney, and pancreas were isolated and washed with normal saline before being stored in a 5% formalin solution (Rankum, Thane, India) at room temperature until histological examination.

Results

Effect of diet on renal function

Table S1. Effect of different diets on renal profile (creatinine and uric acid levels) of rats

Day	Control group	Standard group	A1A2 group	A2A2 group
Creatinine (mg/dL)				
Basal (0) Day	0.61±0.32	0.69±0.18	0.64±0.12	0.74±0.23
15 th Day	0.64±0.28	0.67±0.22	0.6±0.15	0.8±0.35
30 th Day	0.71±0.29	0.67±0.32	0.69±0.14	0.82±0.26
45 th Day	0.63±0.23	0.64±0.14	0.79±0.29	0.79±0.33
60 th Day	0.65±0.24	0.72±0.30	0.81±0.33	0.78±0.21
75 th Day	0.63±0.21	0.75±0.27	0.82±0.33	0.75±0.45
90 th Day	0.61±0.18	0.71±0.26	0.88±0.32	0.74±0.22
Uric acid (mg/dL)				
Basal (0) Day	1.47±0.76	1.85±1.10	1.62±1.13	1.42±0.84
15 th Day	1.52±0.69	2.04±1.16	1.17±1.23	1.53±0.89
30 th Day	1.95±0.36	1.49±0.98	1.15±1.34	1.55±1.76
45 th Day	1.22±0.32	1.36±0.12	1.29±0.87	1.91±0.32
60 th Day	1.65±0.23	1.63±0.23	1.32±0.75	1.75±0.25
75 th Day	1.74±0.22	1.85±0.34	1.54±0.93	1.85±0.33
90 th Day	1.71±0.18	1.82±0.36	1.08±0.45	1.82±0.27

Effect of diet on histology of kidney, liver and pancreas

Histology of kidneys

Microscopic examination of sections of the kidney (Fig. S1 A-D) revealed no haemorrhages present in between intertubular spaces in all the groups. There are no degenerative changes of epithelial linings of tubules seen in all treated groups. No granuloma was seen in any of the treated rats.

Histology of liver

Microscopic examination of liver sections (Fig. S1 E-H) revealed the normal histological structure of hepatic lobules in all treated rats including the control group. The hepatocytes were arranged in cords radiating from the central vein and separated by blood sinusoids in all the groups. Hepatocytes were polyhedral in shape with slightly vacuolated granular cytoplasm and vesicular nuclei in all the groups similar to the normal control group. Bi-nucleated hepatocytes with no infiltration or granuloma were also seen in all the groups.

Histology of pancreas

The pancreatic sections of all groups (Fig. S1 I-L) showed normal architecture of the pancreas. The exocrine pancreas is composed of closely packed acinar cells and arranged into small lobules. Pancreatic lobules are separated by intact intra-lobular and interlobular connective tissue septa. The islet cells were seen interspersed between the acinar cells. Islets appeared more lightly stained than the surrounding acinar cells.

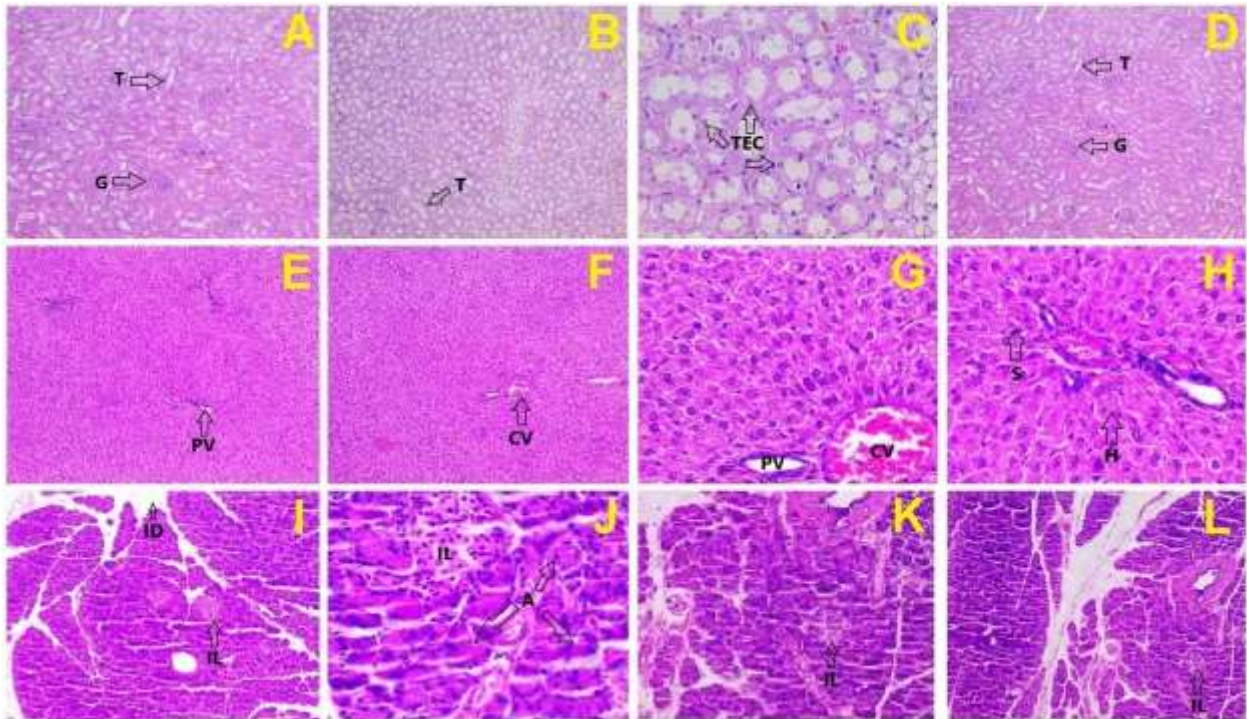


Fig. S1. Effect of experimental diets on histology of different organs in rats. Kidney histology of control group (A), standard group (B), A1A2 group (C), A2A2 group (D); liver histology of control group (E), standard group (F), A1A2 group (G), A2A2 group (H); Pancreas histology of control group (I), standard group (J), A1A2 group (K), A2A2 group (L); glomerulus (G), tubules (T), tubular epithelial cells (TEC), portal vein (PV), central vein (CV), sinusoids (S), hepatocytes (H), islets of Langerhans (IL), intercalated duct (ID), acini (A)

References

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- Kumar A, Negi AS, Chauhan A, Semwal R, Kumar R, Semwal RB, Singh R, Joshi T, Chandra S, Joshi SK and Semwal DK** (2022). Formulation and evaluation of SGLT2 inhibitory effect of a polyherbal mixture inspired from Ayurvedic system of medicine. *Journal of Traditional and Complementary Medicine* **12**, 477-487.