

Research Article**Evaluation of comparative antidiabetic efficacy of cow urine, A2 milk, wheatgrass juice and antidiabetic agent**

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Abstract

Objective: To investigate the role of cow urine, A2 milk and wheatgrass juice on alloxan induced diabetes in male Wistar rats and to compare it. Diabetes mellitus (DM) is a complex chronic illness associated with a state of high blood glucose level, or hyperglycemia, occurring from deficiencies in insulin secretion, action, or both. There are various herbal treatment available for treating diabetes like wheatgrass (containing chlorophyll), cow urine, A2 milk (milk obtained from Indian breed of cows). **Materials and methods:** Forty two animals were randomized into seven groups. Group 1-normal control; Group 2- diabetic control; Group 3 – cow urine(1ml/kg, twice a day); Group 4- A2 milk (as supplementary diet); Group 5- wheatgrass juice(30ml/kg, twice a day); Group 6- combination of all three; group 7- std.(glibenclamide,0.5 ml/kg, once a day). All the animals were sacrificed on 21st day and blood, serum and organs (liver, heart, and kidney) were collected for biochemical and histopathological study. **Results:** It was seen that wheatgrass juice and combination treatment showed better antidiabetic efficacy as compared to cow urine and A2 milk. **Conclusion:** Our results have showed that herbal treatment/ drugs have the potency to treat diabetes and are also non-toxic.

Keywords: Insulin, hyperglycemia, Glibenclamide, cow urine, A2 milk, wheatgrass juice

Introduction

Diabetes mellitus (DM) is a complex chronic illness associated with a state of high blood glucose level, or hyperglycemia, occurring from deficiencies in insulin secretion, action, or both. The chronic metabolic imbalance associated with this disease puts patients at high risk for long-term macro- and microvascular complications (Kalra and Agrawal, 2013).

In human history, plants and animal derived compounds have played a key role in treating human diseases like cancer, diabetes, and atherosclerosis i.e. wheatgrass, aloe vera, curcumin, alfalfa, garlic, ginger, German chamomile, grapefruit, green tea, milk, cow urine (Woodford, 2006). Wheatgrass juice is a rich source of chlorophyll as well as Vitamins A, C, E and B complex. It contains a plethora of minerals like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron, and molybdenum (Woodford,

2011). Chlorophyll is believed to be the pharmacologically active component in wheatgrass, acting as an anti-diabetic agent (Shirude, 2011). Cow urine contains nitrogen, sulphur, phosphate, sodium, manganese, iron, silicon, chlorine, magnesium, maleic, citric, tartaric and calcium salts, vitamin A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones and gold acids (Gosavi et al., 2011). Ingredients of cow urine are similar with human body. Hence consumption of cow urine is useful to maintain the balance of these substances and cures incurable diseases like diabetes, AIDS, cancer (Randhawa, 2010). Milk contains about 85% water. The remaining 15% is the milk sugar lactose, protein, fat and minerals. Beta-casein is about 30% of the total protein content in milk (Bell et al., 2006). Milk is divided into two groups A1 and A2. A2 milk is the milk that contains only the A2 type of beta-casein protein whereas A1 milk contains only A1 beta casein (Bech and Kristiansen, 1990). A1 milk produces BCM-7 on its digestion which is believed to be involved in autism, schizophrenia and other non-communicable disorders. A2 milk is harmless whereas A1 milk is harmful for health (Ng-Kwai-Hang and Grosclaude, 2002). A2 milk is found basically in indigenous cows and buffaloes of India (Laugesen and Elliott, 2003).

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Materials and methods

Materials and chemicals

Glibenclamide, Cow urine, A2 milk, Wheatgrass juice. All the chemicals were purchased from merck laboratories (potassium chloride, monosodium phosphate, disodium phosphate, Hydrogen peroxide, diethyl ether, sodium chloride, dichromate acetic acid, bovine serum albumin, NADPH, alloxan, glutathione, formaldehyde, haematoxyline dye, thiobarbituric acid, tricarboxylic acid, sodim phosphate buffer, dithinitrobenzene).

Animals

Male wistar rats (180-200 g) were obtained from the central animal facility of Shriram college of pharmacy Banmore and were maintained in polypropylene cages on rodent pellet condition of controlled temperature ($22\pm 2^\circ\text{C}$) and acclimatized to 12/12 h light/dark cycle. Free access to food and water were allowed until 2h before the experiment. The care and maintenance of the animals were as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". Food and water were provided 2h after the experiment. All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

Induction of diabetes

Diabetes was induced by administration of alloxan monohydrate (150 mg/kg body wt., i.p) 2% solution dissolved in 0.9% NaCl. After 72 hours, all the rats became diabetic. Weights of animals were recorded daily throughout the study period.

Drug treatments

Different drugs treatment was given to the different groups of rats after the induction of diabetes. The treatment includes:

- Cow urine (1ml/kg body weight) twice a day, orally.
- Wheatgrass juice (30ml/kg body weight) twice a day, orally.
- A2 milk (as a supplementary diet)
- Glibenclamide (0.5 mg/kg body weight) once a day, peritoneal cavity.

Grouping of animals

The animals were divided into seven groups. N = 7

- | | |
|-----------|--|
| Group I | : Normal control (vehicle only) |
| Group II | : Diabetes control (0.5 ml of normal saline) |
| Group III | : Diabetes induced + cow urine. |
| Group IV | : Diabetes induced + A2 milk |
| Group V | : Diabetes induced + wheatgrass juice |
| Group VI | : Diabetes induced + cow urine + A2 milk + |

wheatgrass juice

Group VII : Diabetes induced + standard drug(glibenclamide)

The body weights of the animals were recorded daily up to 21 days and the glucose level was checked on 0,7,14 and 21 days. After 21 days animals were anaesthetized with ether for collection of blood from retro orbital sinus, and then sacrificed by cervical dislocation for the removal of vital organ. Various hematological and biochemical analysis were carried out.

Hematological evaluation in blood

The hematological variables, viz., RBC, WBC, and Hb were measured.

Biochemical evaluation in Serum

Enzymes assay

Plasma SGPT, SGOT, ALT and ALP, creatinine and BUN were analyzed by using commercial kit.

Serum glutathione assay

Serum GSH was measured by the method of (Beutler et al. (1963). In which 0.2 mL fresh serum was collected from each animal and 1.8 mL distilled water was added to it and 3 mL of precipitating solution was added to mixture. The mixture was then allowed to stand for approximately 5 min and then filtered. 2.0 mL of filtrate was added to 8.0 mL of phosphate solution in cuvette and 1.0 mL DTNB reagent was added to cuvette and the optical density (OD) was measured at 412nm.

Serum MDA assay

Serum MDA was measured by the method of (Buege et al., 1978). 100µl serum was diluted to 500 µl of distill water. To the diluted sample 1ml of 0.67% thiobarbituric acid and 1ml of 20% tricarboxylic acid were added. The samples are kept for boiling in water bath for 15min. The reaction mixture is cooled and centrifuged at 12000 rpm for 5 min. the supernatant is taken and the optical density (OD) of the pink color is measured the 535 nm.

Biochemical evaluation in tissues

Lipid peroxidation assay

A portion of the liver, heart and kidney was used for biochemical estimation. Liver lipid peroxidation was determined by measuring the level of MDA according to the method of (Ohkawa et al., 1979). 2mL of suspension medium was taken from the supernatants of the 10% tissue homogenate in 1.15% KCl and centrifuged at 10,000 rpm. 1mL of 30% TCA followed by 1mL of 0.8% TBA were added to it. The tubes were covered with aluminum foil and

kept in shaking water bath for 30 minutes at 80°C, after 30 min, tubes were taken out and kept in ice cold water for 10 min. They were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against blank. Blank consisted of 2mL distilled water, 1mL TBA, and 1mL TCA.

GSH assay

Tissue GSH was determined by the method of (Sedlak and Lindsay, 1968). A portion of the reperused liver tissue (300-600 mg), heart tissue and kidney tissue homogenized in 5-8 mL of 0.02M EDTA and then 4mL of cold distill water was added to it. After mixing 1mL of 50% TCA was added to it and shaken for 10min and centrifuged at 6000 rpm for 15 min. 4 mL of 0.4 M tris buffer was mixed with 2 mL of supernatant and 0.1mL of 0.01M DTNB. The absorbance of this resulting mixture was read at 410 nm at room temperature against reagent blank.

Histopathological studies

Liver tissues of rats were removed and washed with normal saline. The cleared tissue was fixed in 10% natural buffered formalin solution (pH 7.0-7.2). After proper fixation tissue was processed for dehydration in ascending grade of ethanol, clearing with toluene, followed by impregnation in paraffin wax, then sections of 5 µ in thickness were cut with help of semi-automatic rotary microtome. Sections were stained with hematoxyline. Stained paraffin sections of liver were examined under phase contrast microscope and photomicrographs were taken. Representative area were captured and analyzed with the help of Lieca Qwin V3 digital image processing and analysis system.

Organ to Body Weight Indices (OBWI)

After sacrificing the animals, liver, heart and kidney was removed and the washed free of extraneous materials and weighed. The organ to body weight indices (OBWI) were calculated as per the formula given below:

$$OBWI = \frac{\text{Organ Weight}}{\text{Body Weight}} \times 100$$

Statistical analysis

Statistical evaluations were made using one-way ANOVA followed by Dunnett's test. A probability of 0.05 and less was taken as statistically significant. The analyses were carried out using sigma stat for windows version 2.03 (SPSS Inc. USA).

Results

Anti-diabetic effect

In figure 1 the body weight of wheatgrass and cow urine treated group significantly ($p < 0.05$) decreased in compared to control group. In A2 milk treated group body weight of diabetic animals have not significantly change but in combination group body weight significantly increased compared to control group.

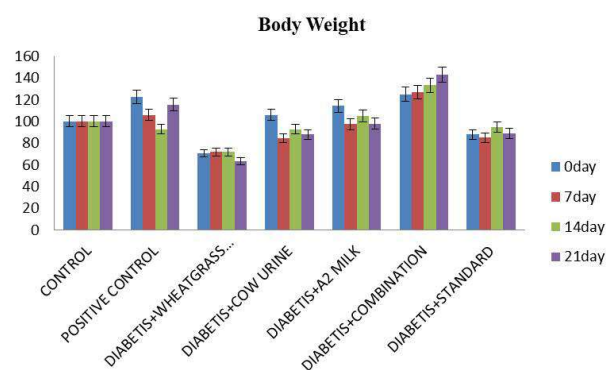


Figure 1. Effect of cow urine, wheatgrass juice, A2 milk, its combination and standard (glibenclamide) on body weight against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test).

In figure 2 showed the organ body weight index of diabetic animals of liver was decreased and that of heart and kidney was decreased. OBWI of liver have not significantly change in wheatgrass juice, cow urine, A2 milk and combination treated group. In heart and kidney OBWI was increased in wheatgrass juice, cow urine, A2 milk and its combination in compared to control group.

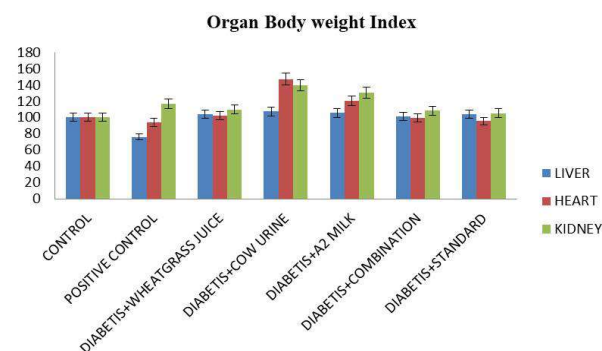


Figure 2. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on OBWI against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for liver OBWI 0.7401; Control values for heart OBWI 0.7457; Control values for kidney OBWI 0.7201.

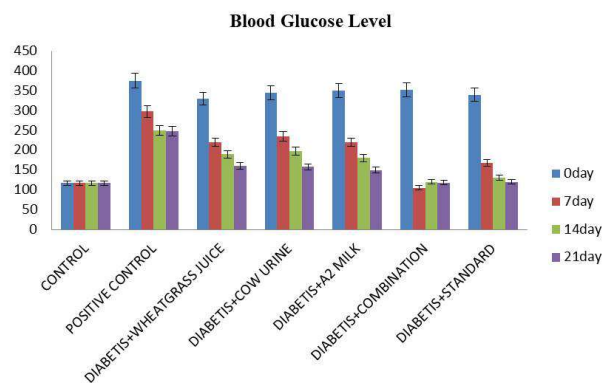


Figure 3. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on blood glucose level against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for blood glucose 70-100mg/dl

In figure 3 showed the blood glucose level of diabetic animals was increased in compared to control. In wheatgrass juice, cow urine, A2 milk and combination treated groups the blood glucose was increased initially and then decreased significantly ($p < 0.05$) in compared to control group.

Hematological parameters in blood

In figure 4 showed the diabetic animals the RBC_s level does not have significant change in compared to control. In wheatgrass juice, cow urine, A2 milk and combination treated groups the level of RBC_s do not have any significant change in compared to control groups. In diabetic animals the level of WBC_s was significantly increased in compared to control. In wheatgrass juice and combination treated groups the level of WBC_s was significantly ($p < 0.05$) increased in compared to control groups. In cow urine and A2 milk the level of WBC_s was decreased in compared to control groups.

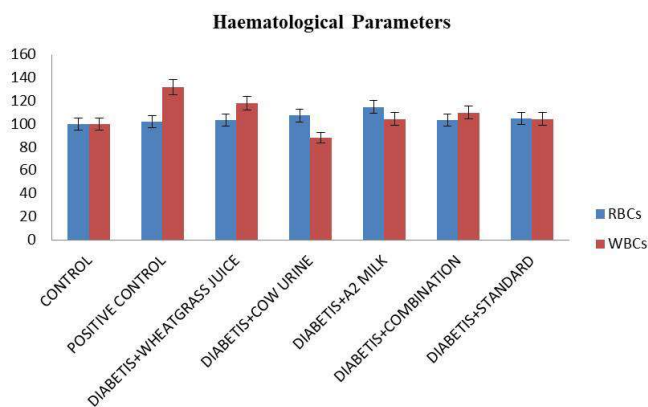


Figure 4. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on haematological parameters against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for RBC_s $6.76-9.75 \times 10^6 \text{ mm}^3$; Control values for WBC_s $6.6-12.6 \times 10^3 \text{ mm}^3$

Biochemical parameters in Serum

In figure 5 showed the level of SGOT was significantly decreased in diabetic animals compared to control groups. In A2 milk and combination treated groups the levels of SGOT was decreased as compares to control groups. In wheatgrass juice and cow urine treated animals the levels of SGOT was significantly ($p < 0.05$) decreased as compared to control groups. The level of SGPT was significantly decreased in diabetic animals compared to control groups. In wheatgrass juice, A2 milk and combination treated groups the levels of SGPT was decreased as compares to control groups. In cow urine treated animals the levels of SGPT was significantly ($p < 0.5$) decreased as compared to control groups.

In figure 6 there is no significant change in the diabetic animals compared to control groups. The level of creatinine significantly ($p < 0.05$) increased in wheatgrass treated group as

compared to control group whereas in cow urine, A2 milk and combination treated animals the level of creatinine slightly decreased as compared to control groups.

The level of BUN significantly ($p < 0.05$) increased in the diabetic groups compared to control groups. In wheatgrass juice and A2 milk treated groups the level of BUN increases as compared to control groups. In cow urine and combination treated groups the level of BUN significantly ($p < 0.05$) increases as compared to control groups.

Evaluation Of Biochemical Parameters In Serum (enzyme assay in liver)

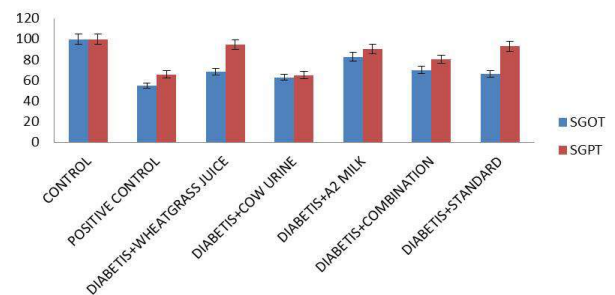


Figure 5. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on SGOT SGPT against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for SGPT 54.54g/dl; Control values for SGOT 44.45g/dl

Evaluation Of Biochemical Parameters In Serum (enzyme assay in kidney)

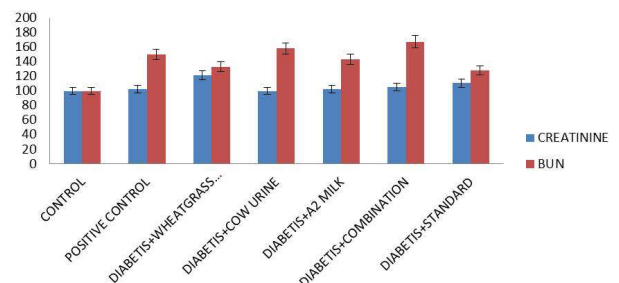


Figure 6. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on Creatinine, BUN against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for CREATIINE 0.2-0.8 mg/dl; Control values for BUN 15-21 mg/dl

In figure 7 the level of MDA significantly ($p < 0.05$) increases in diabetic groups as compared to control groups. In wheatgrass and combination treated groups the level of MDA increases as compared to control groups. In A2 milk and cow urine treated groups do not have any significant change as compared to control groups.

In figure 8 the level of MDA in the liver tissue significantly ($p < 0.05$) increases in diabetic group compared to control groups. In wheatgrass juice, cow urine, A2 milk and combination treated groups the level of MDA in liver tissue increases as compared to control groups. In diabetic groups

the level of MDA in **heart** tissue slightly increases as compared to control groups. In wheatgrass juice treated groups the level of MDA in heart tissue slightly increases as compared to control group. In cow urine, A2 milk and combination treated groups the level of MDA in heart tissue does not have any significant effect as compared to control groups. In diabetic groups the level of MDA in **kidney** tissue significantly ($p < 0.05$) increases as compared to control groups. In wheatgrass juice treated animals there is no significant change in the level of MDA in kidney as compared to control groups. In cow urine, A2 milk and combination treated groups the level of MDA in kidney slightly increases as compared to control groups.

Evaluation Of Biochemical Parameters In Serum (MDA)

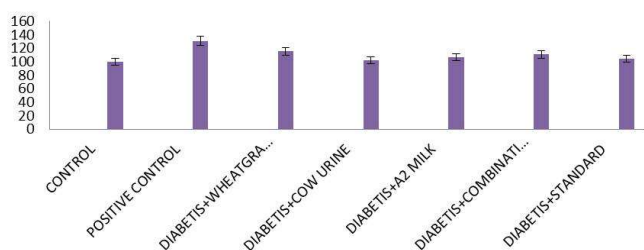


Figure 7. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on MDA in serum against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test).

Evaluation Of Biochemical Parameters In Tissue (MDA)

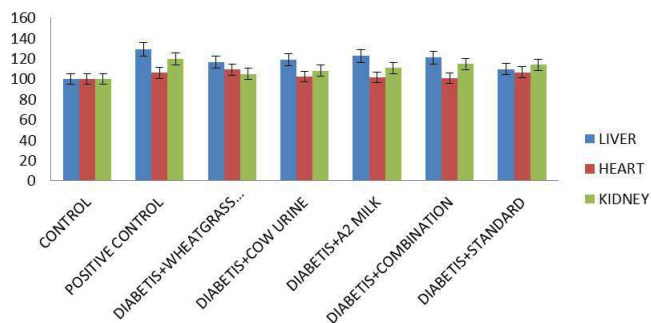


Figure 8. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std. (glibenclamide) on MDA in tissue against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for MDA in liver 21.7 ± 5.66 nmol/g; Control values for MDA in heart 48.99 ± 4.40 nmol/g; Control values for MDA in kidney 41.32 ± 4.79 nmol/g

Biochemical parameters in tissues

In figure 9 showed the level of GSH in **liver** tissue decreased in diabetic group compared to control group. The level of GSH in liver tissue significantly ($p < 0.05$) decreased in wheatgrass juice and combination treated group compared to control group. The level of GSH in liver tissue slightly increased in cow urine and A2 milk treated group compared to control group. The level of GSH in **heart** tissue increased in diabetic group compared to

control group. The level of GSH in heart tissue significantly ($p < 0.05$) increased in wheatgrass juice and combination treated group compared to control group. The level of GSH in heart tissue increased in cow urine and A2 milk treated group compared to control group. The level of GSH in **kidney** tissue increased in diabetic group compared to control group. The level of GSH in kidney tissue significantly ($p < 0.05$) decreased in wheatgrass juice, cow urine, A2 milk and combination treated groups compared to control.

Evaluation of Biochemical Parameters in Tissue(GSH)

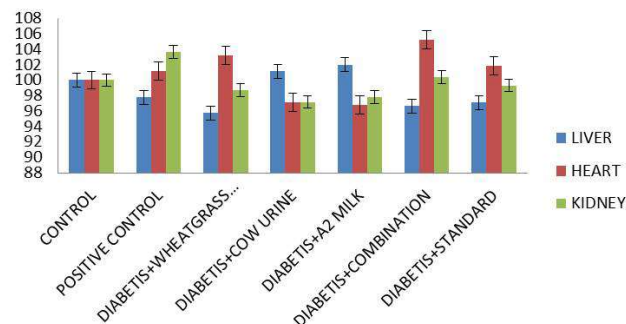


Figure 9. Effect of cow urine, wheatgrass juice, A2 milk, its combination and std.(glibenclamide) on GSH in tissue against alloxan induced diabetes in rats. $P < 0.05$, as compared to control group (One Way ANOVA followed by Dunnett's t test). Control values for GSH in liver 124.24 ± 1.39 mg/g; Control values for GSH in heart 98.2 ± 3.67 mg/g; Control values for GSH in kidney 101 ± 2.38 mg/g.

Histopathological observations

The observation showed in figure 10, that:

- The liver section of normal control rats shows normal architecture with well preserved cytoplasm, prominent nucleus, central vein and compact arrangement of hepatocytes.
- Liver of alloxan treated rats showing fatty changes in hepatocytes, central vein was seen in inflammatory cells.
- In the liver section of rats administered with alloxan and wheatgrass showed mid fatty changes and mild sinusoidal congestion.
- Animals administered with alloxan and cow urine exhibit protection of liver against alloxan induced liver damage.
- The section of liver taken from animals treated with A2 milk showed normal hepatic architecture with presence of very few inflammatory cells.
- Liver section treated with combination showed normal hepatic cords and architecture.
- Liver treated with std. showed hepatic protection with few inflammatory cells.

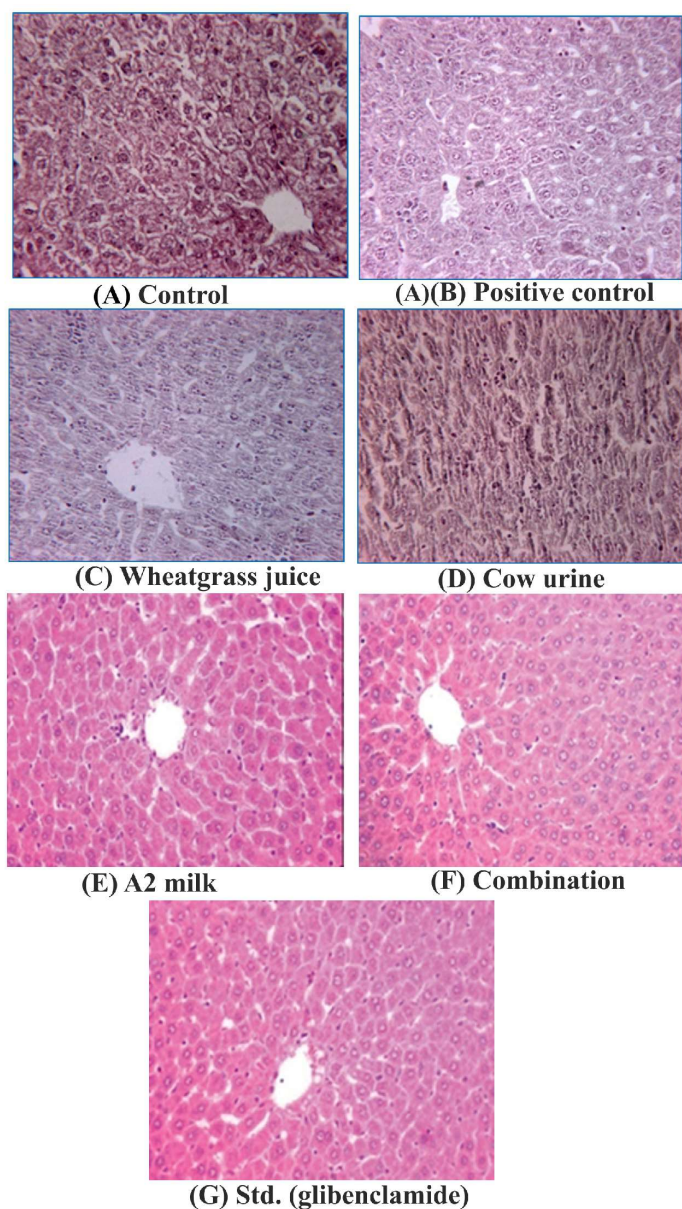


Figure 10 (A-G). Photomicrographs of the treatment of wheatgrass juice, cow urine, A2 milk, combination and std. drugs on rat liver (Histopathological study)

Conclusion

Results concluded that the wheatgrass juice, cow urine, A2 milk and its combination showed antidiabetic efficacy and thus can be used as an antidiabetic agent in treating diabetes.

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Conflicts of interest: Nil

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