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All author's name and affiliations are given below, after references

Antidiabetic activity of herbal combined aqueous leaves extract of *Murraya koenigii* and *Aegle marmelos* on Alloxan induced diabetic rats

SS Bhosle, DP Patil, GD Ranvir, GR Gangane, PB Ghorpade, PR Suryawanshi, SV Londhe and SV Panchal

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Abstract
The present study was carried out to study the antidiabetic activity of herbal combination prepared using aqueous leaves extract of *Murraya koenigii* and *Aegle marmelos* on alloxan induced diabetic rats for a period 28th days. Fifty Wistar rats divided into five groups, each group comprising ten Wistar rats of either sex. Group I normal control was given pelleted feed and drinking water for 28th days. The Groups II, III, IV and V were given alloxan monohydrate @ 120 mg/kg BW. Intraperitoneal for induction of diabetes and hyperglycaemic rats were used for study. In Group III animals were treated with Metformin @100 mg/kg BW, group IV treated with herbal combination @ 130 mg/kg BW and group V treated with herbal combination @ 130 mg/kg BW concurrently administration with Metformin @ 100 mg/kg BW per orally once a day for 28th days. Significantly increased Blood Glucose Level, AST, ALT, BUN, Creatinine Phosphokinase (CPK), Total Cholesterol and Triglyceride level was observed in diabetic rats on 0 day. In treatment groups III, IV and V significantly reduced Blood Glucose Level, AST, ALT, BUN, Creatinine Phosphokinase (CPK), Total Cholesterol and Triglyceride level on 28th day compared with diabetic control group II animals on same day was observed. Gross pathology of liver, pancreas and kidney not showed any noticeable changes in any of the experimental rats. The wide range histoarchitectural change were predominantly noticed in liver, pancreas and kidney in diabetic control group II animal. Restoration of histoarchitecture of these organs was observed after treatment.

Keywords: *Murraya koenigii, Aegle marmelos,* diabetes, intraperitoneal, metformin, hypoglycaemic, alloxan monohydrate

Introduction
Hyperglycemia, the primary clinical manifestation of diabetes mellitus (DM), is a metabolic disturbance caused by a deviation in either insulin secretion, insulin action, or both (Stumvoll *et al*. 2005) [27]. Between 1980 and 2004, the incidence and prevalence of type 2 diabetes quadrupled worldwide as a result of obesity, sedentary lifestyles, and an ageing population, (Chatterjee *et al*., 2017) [5]. Type 2 diabetes incidence and prevalence vary by geography; more than 80% of patients live in low- to middle-income countries. However, every country has seen a general increase in the prevalence of diabetes since 1980 (Risk and Collaboration, 2016) [23]. Type 2 diabetes is characterised by a relative lack of insulin, which is caused by both malfunctioning of pancreatic β-cells and insulin resistance in target organs. Type 2 diabetes is characterised by hyperinsulinemia, insulin resistance, and pancreatic β-cell failure, which can cause up to 50% cell loss at diagnosis. (Holman *et al*., 2008) [10]. Similarly, the organs linked to the development of type 2 diabetes include the liver, skeletal muscle, kidney, brain, small intestine, adipose tissue, and the pancreas (β and α cells), (Defronzo, 2009) [6]. Adverse medication responses and interactions are among the dangers associated with long-term use of synthetic hypoglycaemic medicines. These days, using medicinal herbs in a range of therapies is recommended. Most plants include carotenoids, flavonoids, terpenoids, alkaloids and glycosides, many of which have anti-diabetic effects.

Corresponding Author:
DP Patil
Associate Professor, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

The anti-hyperglycaemic effects that often follow treatment with plants are due to their propensity to improve pancreatic tissue function, either by increasing insulin releases or by reducing intestinal absorption of glucose. (Kooti *et al*. 2016) [16] The prospective advantages of *Murraya koenigii* Spreng (commonly referred to as the curry leaf tree and belonging to the Rutaceae family) in enhancing glycemic control and managing diabetic conditions have been corroborated by a plethora of both *in vitro* and *in vivo* studies conducted in recent years. Commonly identified as sweet neem, this arboreal species proliferates across tropical regions and is routinely employed in the treatment of various ailments, encompassing diabetes, diarrhea, anemia, ulcers, obesity, and inflammation. (Rajkumar and Vinotha 2022) [22]. *Aegle marmelos* (Bel) represents one of the most extensively utilized medicinal and nutraceutical flora. The leaves, fruits, bark, stem, and roots of *Aegle marmelos* have been employed in ethnomedicine to harness its diverse therapeutic properties, which encompass antioxidant, antimicrobial, antidiarrheal, antidiabetic, antiproliferative, hepatoprotective, anti-inflammatory, and antihyperlipidemic effects, (kirtisharma *et al*. 2016) [17] and (Rajeswari *et al*. 2021) [21].

Materials and Methods

Collection and authentication of plant material

Department of Botany, Vasantrao Naik Marathwada Agricultural University, Parbhani, recognised the *Murraya koenigii* and *Aegle Marmeols* plant material. The healthy leaves of *Murraya koenigii* and *Aegle marmelos* are used in the present study were obtained locally from a single plant grown in Agriculture University Campus, Parbhani and transported in batches to the laboratory, where it was processed for further research.

Preparation of Aqueous extract

Aqueous extract of leaves of *Murraya koenigii* and *Aegle marmelos* were prepared by cold extraction method. *Murraya koenigii* and *Aegle marmelos* shade dried leaves were ground to powder with the help of an electric grinder. Aqueous solution was prepared by dissolving 200 grams powder mixture of both plant in double distilled water and the final quantity will be made to 1 liter. It was mixed thoroughly and allowed to soak for 48 hours, intermittent shaking was done for thoroughly mixing of powder and distilled water with an electrically operated flask shaker. The resulting solutions after 48 hours was first filtered by using muslin cloth and then by Whatman filter paper No 1. The filtrate obtained was then poured in the evaporating dishes and allowed for shed dry at room temperature. After complete drying the obtained extracts was weighed and stored in screw cap vial at 4°C in refrigerator and used when required.

Experimental animals

The present study was conducted on 50 Wistar rats of either sex, age 8-10 weeks and 150-200 g body weight. Animal were randomly selected after physical and behavioral examination; the live body weight range was within ±20 percent of the mean body weight. The detail of the experimental groups and their treatment are summarised in Table 1. The treatment was given once daily per orally for 28th consecutive days. In the experimental room of Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, all the rats were maintained as per specified laboratory conditions with ad-libitum water and feed throughout the experimental period. The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol, which met the national guidelines as per the guidelines of Committee for The Control and Supervision of Experiments on Animals (CCSEA) vide Resolution no. IAEC/138/25 dated 04/02/2025. The experimental animals were maintained under continual observation for at least five days before the experiment.

Experimental design

Table 1: Experimental design of groups and their treatments

Group No	Name of Group	Drug administration	No of Animals
I	Normal Control	Ad-libitum feed and water	10
II	Diabetic Control	Diabetic rats with Ad-libitum feed and water, no other treatment is given.	10
III	Diabetic + Standard Drug	Metformin @100mg/kg body weight, P.O.	10
IV	Diabetic + Herbal combination	Herbal combination @130mg/kg, P.O.	10
V	Diabetic + Herbal combination + Standard drug	Herbal combination @130mg/kg +Metformin @100mg/kg, P.O.	10

All the animal were maintained for the experimental period of 28 day

Induction of diabetes

Total 50 Wistar rats were used for the present investigation, amongst these 40 rats from group II to V were fasted overnight, diabetes was induced by intraperitoneal administration of Alloxan monohydrate @120 mg/kg body weight (Cheekati, *et al*. 2017) [5] in physiological saline as 5 percent W/V after one-week, diabetic status was confirmed in the Alloxan monohydrate administered rats estimating blood glucose level by using Glucometer.

Biochemical parameters

The blood sample collected from rat's retro-orbital plexus by using micro-capillaries at 0th, 7th, 14th and 28th days. The individual serum was separated from blood samples using clot activator tubes and stored at 4°C for further biochemical estimations. Biochemical estimations were carried out immediately for Blood Glucose level, Blood Urea Nitrogen (BUN), Total Cholesterol, Triglyceride, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Creatinine Phosphokinase (CPK). The biochemical estimation of these parameter was done by using Autoanalyzer.

Pathological Observations

All the rats in each group were sacrificed on the termination of the experiment and the organs were subjected to gross and microscopic examination.

Gross pathological investigations

After completion of experimental trial all the rats were sacrificed on 28th day. The abdomen of rat was operated to dissect out visceral organs like liver, kidney and pancreas.

Histopathological changes

The visceral organs like liver, kidney and pancreas were collected and processed for histopathological examination. The tissue was fixed in 10% Neutral Buffered Formalin (NBF). Fixed tissues of visceral organs were cut into proper size and processed to obtain paraffin sections of about 3-5 µ. The sections of liver, kidney and pancreas were stained by Mayer's Haematoxylene and Eosin staining method (Singh and Sulochana, 1997) [26] for histopathological studies.

Statistical analysis

The biochemical parameters in all five groups were analyzed by analyzing the data generated by Factorial Randomized Block Design (Panse and Sukhatme. 1967) [20]. The treatment means compared by critical difference by statistical method and analysis of variance.

Result and Discussion

The present experiment is conducted to assess the hypoglycaemic activity of herbal combination prepared using aqueous leaves extract of *Murraya koenigii* and *Aegle marmelos* on Alloxan induced diabetic rats. The result is interpreted in the topic.

Biochemical Parameters

The various biochemical Parameter viz Blood glucose level, Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), Total Cholesterol, Triglyceride and Creatinine Phosphokinase (CPK) level analysed from blood collected at 0, 7th, 14th and 28th day of experimental period and values were observed.

Blood Glucose Levels

Significant reduction (p<0.05) in blood glucose level in group III, IV and V animals were observed on 28th day when compared with respective 0, 7th and 14th day values. The difference was observed to be significant when compared with group II animal values on 28th day. Also, the values on 28th day were observed at par in these groups when compared with group I which is normal control animals. A similar finding was found by Joel *et al*. (2014) [12]. The findings of present study corroborated with Vijayanand. (2015) [30] who reported that induction of diabetes occurs with single dose of alloxan (@120 mg/kg body weight). Our results were in accordance with Birudu *et al*. (2020) [3] who proved induction of diabetes with alloxan (@ 150 mg/kg BW). They also reported that oral administration of *Aegle marmelos* (L.) methanolic leaf extract at low dose (100 mg/kg), medium dose (250 mg/kg) and high dose (500 mg/kg) showed significant decrease in the blood glucose levels in the high-dose treated animals when compared to the diabetic control animals.

Serum Aspartate Aminotransferase (AST)

The treatment on 28th day was very much desirable. However, group III treated with Metformin shows better results followed by group V treated with concurrent administration of herbal combination with Metformin and group IV herbal combination. In groups III, IV and V treatment significantly reduced (p<0.05) Serum AST levels on day 28th was observed when compared to group II. The treatment of diabetes with Metformin or herbal combination or herbal combination with Metformin shows significantly reduced serum AST level on 28th day but normal Serum AST level was not achieved. The findings of present study corroborated with the of Mahran *et al*. (2023) [19] who reported significant increase in AST in diabetic control as compare to normal control group rats after administration of alloxan monohydrate @120 mg/kg BW intraperitoneally. The oxidative stress as a result of free radicals causes damage to the liver and high AST was produced. Ethanolic extract of *Murraya koenigii* @100 mg/kg, p.o significantly reduces the elevated serum levels of AST reported by Sangale and Patil. (2017) [24]. Our results were in accordance with Gandhi *et al*. (2012) [7] who reported positive effect of methanolic extract of *Aegle marmelos* bark (@400 mg/kg PO) on Streptozotocin @60 mg/kg IP induced diabetic rats. The AST level significantly reduced in plant treatment group when compared with diabetic control.

Serum Alanine Aminotransferase (ALT)

Significant reduction (p<0.05) in Serum ALT level in group III, IV and V animals was observed on 28th day when compared with respective 0, 7th and 14th day values. The difference was significant when compared with group II animals values on 28th day. Also, the values on 28th day were observed at par in these groups when compared with group I which is normal control animals. Alam *et al*. (2011) [1] and Khandelwal *et al*. (2015) [15] studied that alloxan administration in rats significantly increases alanine transaminase (ALT) when compared with normal rats. Gandhi *et al*. (2012) [7] found that administration of *Aegle marmelos* bark extract (@200 and 400 mg/kg) lowers the levels of alanine transaminase (ALT) in diabetic rats. Singh *et al*. (2024) reported that oral administration of hydroalcoholic extract of *Murraya koenigii* at different doses (@125, 250, 500 mg/kg), significantly decreases Serum ALT as compared to diabetic control group animals. Result obtained are in accordance with the present study.

Estimation of Blood Urea Nitrogen (BUN)

Mean BUN level in treatment group III, IV and V was significantly reduced (p<0.05) on 28th day when compare with 0, 7th and 14th day values. The difference was observed to be significant when compared with group II animals value on 28th day. Also, the values on 28th day were observed at par in these groups when compared with group I which is normal control animals. Kalita *et al*. (2017) [13] observed that, commercially available preparation of injectable gentamicin (40 mg/kg) given intraperitoneally (I.P.) induce nephrotoxicity and its treatment with aqueous leaf extract of *Aegle marmelos* (@500 mg/kg/day PO) significantly reduces BUN level. Arulselvan *et al*. (2006) [2] evaluated the anti-hyperglycaemic efficacy of *Murraya koenigii* in STZ induced diabetic rats. Ethanolic extract of *M. koenigii* at a dose of @ 200 mg/kg/BW/day for a period of 30 days significantly decreased (p<0.05) the levels of blood urea nitrogen compared with diabetic control group. Results obtained are in accordance with the present study.

Table 2: Biochemical Parameter

Group	Blood glucose level (mg/dl)				AST (IU/L)			
Days	0 day	7 day	14 day	28 day	0 day	7 day	14 day	28th day
Group I	81.40^{c}±2.28	79.80^{d}±2.62	82.80^{e}±2.87	79.00^{e}±2.63	42.30^{c}± 2.05	43.20^{c}± 1.98	41.60^{d}± 2.09	42.2^{d}± 1.99
Group II	236.90^{b}±4.16	245.50^{a}±6.27	242.80^{a}±5.09	241.50^{a}±3.85	116.8^{abs}± 3.26	127.1^{ar}± 2.17	133.7^{aq}±3.28	156.3^{ap}±2.75
Group III	242.4^{abp}±2.99	197.60^{bcq}±5.83	151.80^{cr}±3.77	101.20^{cs}±2.52	116.4^{bp}± 2.38	100.9^{bq}± 4.73	86.10^{cr}±3.01	66.9^{cs}±2.45
Group IV	248.00^{ap}±3.26	209.50^{bq}±3.91	187.30^{br}±4.64	116.10^{bs}±3.26	116.1^{bp}±2.57	105.1^{bq}±2.53	95.50^{br}±3.19	73.1^{bs}±2.19
Group V	247.80^{ap}±3.68	190.20^{cq}±4.96	136.30^{dr}±3.42	92.40^{ds}±3.93	120.4^{ap}±3.05	103.8^{bq}±2.97	89.10^{cr}±3.49	68.4^{bcs}±2.71

ALT (IU/L)				BUN (mg/dl)			
0 day	7 day	14 day	28 day	0 day	7 day	14 day	28th day
27.80^{b}±2.55	28.30^{c}±1.61	29.40^{d}±2.02	28.60^{c}±2.17	28.50^{d}±2.37	28.10^{c}±1.44	27.80^{d}±2.32	26.80^{d}±1.94
104.60^{as}±4.12	109.60^{ar}±3.69	116.3^{aq}±3.74	122.0^{ap}±3.85	49.30^{cr}±2.08	53.20^{aq}±2.93	56.10^{aq}±2.18	63.20^{ap}±3.06
106.3^{ap}±3.68	95.90^{bq}±2.77	73.90^{cr}±4.19	64.40^{bs}±3.30	54.90^{bp}±2.71	49.80^{bp}±1.76	43.60^{cr}±2.57	38.10^{cs}±1.45
104.1^{ap}±4.24	96.70^{bq}±4.96	82.20^{br}±4.35	69.70^{bs}±4.25	64.10^{ap}±2.51	58.50^{aq}±2.63	50.90^{br}±2.70	42.40^{bs}±2.32
103.2^{ap}±2.76	91.10^{bq}±4.59	79.10^{cr}±3.32	66.50^{bs}±3.03	63.60^{ap}±2.57	58.10^{aq}±2.08	52.20^{abr}±2.19	40.80^{bcs}±1.67

Blood glucose level: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 4.94 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 5.52 (between different day on specific group) (p<0.05).

AST: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 3.56 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 3.99 (between different day on specific group) (p<0.05).

ALT: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 4.48 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 5.01 (between different day on specific group) (p<0.05).

BUN: Superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 2.84 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 3.17 (between different day on specific group) (p<0.05).

Table 3: Biochemical Parameter

Group	Serum Cholesterol (mg/dl)			
Days	0 day	7 day	14'day	28th day
Group I	66.90^{d}±2.07	68.10^{d}±2.18	70.20^{c}±2.60	67.10^{c}±2.59
Group II	122.60^{bs}±2.64	129.10^{ar}±2.91	134.90^{aq}±2.81	140.90^{ap}±3.44
Group III	130.90^{ap}±2.89	111.10^{bq}±4.31	91.20^{br}±4.58	71.80^{bcs}±3.09
Group IV	111.70^{cp}±3.77	105.90^{bcp}±3.42	95.10^{br}±3.60	75.20^{bs}±2.85
Group V	112.20^{cp}±3.01	102.40^{cq}±2.67	92.30^{br}±2.47	74.90^{bs}±3.25

Serum Triglyceride (mg/dl)			
0 day	7 day	14 day	28th day
129.10^{d}±4.64	125.90^{d}±6.92	122.70^{c}±5.59	120.10^{d}±5.98
287.70^{a}±5.92	277.10^{a}±5.46	266.60^{a}±6.94	274.20^{a}±6.54
265.30^{bp}±7.33	254.60^{bpq}±5.72	242.50^{bqr}±5.20	223.30^{br}±5.86
250.30^{bcp}±4.69	240.90^{bcp}±7.85	232.40^{bp}±6.39	207.90^{bq}±6.23
233.30^{cp}±6.36	228.50^{cp}±5.57	223.70^{bp}±7.62	176.60^{cq}±6.23

Serum Triglyceride (mg/dl)			
0 day	7 day	14 day	28th day
129.10^{d}±4.64	125.90^{d}±6.92	122.70^{c}±5.59	120.10^{d}±5.98
287.70^{a}±5.92	277.10^{a}±5.46	266.60^{a}±6.94	274.20^{a}±6.54
265.30^{bp}±7.33	254.60^{bpq}±5.72	242.50^{bqr}±5.20	223.30^{br}±5.86
250.30^{bcp}±4.69	240.90^{bcp}±7.85	232.40^{bp}±6.39	207.90^{bq}±6.23
233.30^{cp}±6.36	228.50^{cp}±5.57	223.70^{bp}±7.62	176.60^{cq}±6.23

Serum cholesterol: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 3.70 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 4.14 (between different day on specific group), (p<0.05).

Serum Triglyceride: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 7.88 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 8.81 (between different day on specific group), (p<0.05).

CPK: superscripts a,b,c,d,e shows significant difference within the column CD: at 5% = 0.057 (between different groups on specific day) (p<0.05). Superscripts p,q,r,s shows significant difference within the row CD: at 5% = 0.063 (between different day on specific group) (p<0.05)

Serum Cholesterol

Serum cholesterol group V treated with concurrent administration of herbal combination and Metformin shows better results followed by group III treated with Metformin and group IV treated with a herbal combination. Groups III, IV and V show significantly reduced (p<0.05) Serum Cholesterol levels on day 28th when compared to group II animals. The treatment of diabetes with Metformin or herbal combination or herbal combination with Metformin significantly reduces Serum Cholesterol on 28th day but

normal Serum Cholesterol level was not achieved. Vinuthan *et al*. (2007) [31] They reported that significantly decreased (p<0.05) Serum cholesterol levels occurs due to presence of effect of tertiary and quaternary alkaloid, flavonoids and glycoside compounds which reduces lipid level. The findings of present study corroborated with Kesari *et al*. (2007) [14] who reported that oral administration of aqueous extract of *Murraya koenigii* leaves for 30 days in diabetic rats decreases the total cholesterol level, 30.8% fall was observed in the case of treated diabetic rats as compared with diabetic control. Garg *et al*. (2015) [8] reported significant reduction in total cholesterol after 30 days administration of ethanol and aqueous extracts of *Aegle marmelos* leaves in high fat diet (HFD) induced obese rats. At doses of 200 mg/kg, PO. Similar findings obtained in present study indicates plants hypolipidaemic effect.

Serum Triglyceride

Treatment group V treated with concurrent administration of herbal combination and Metformin shows better results followed by group IV treated with a herbal combination and group III treated with Metformin. Groups III, IV and V shows significant reduction (p<0.05) in Serum Triglyceride levels on day 28th when compared to group II animals. The treatment of diabetes with Metformin or herbal combination or herbal combination with Metformin shows significantly reduced Serum Triglyceride level on 28th day but normal Serum Triglyceride level was not achieved. Same observations of reduction in Serum triglyceride level values were reported by Gohil *et al*. (2010) [9] who Studied the antidiabetic and antilipidemic activity of combined aqueous extract of *Eugenia jambolana* seed and *Aegle marmelos* leaf in alloxan induced diabetic rats. Tembhurne and Sakarkar. (2012) [28] hypoglycaemic and anti-obesity activity of ethanolic leaf extract of *Murraya koenigii*. High fatty diet group shown increased total cholesterol and triglycerides levels as compared to control group.

Serum Creatinine Phosphokinase (CPK)

Significant reduction (p<0.05) in Serum CPK level in treatment group III, IV and V animals were observed on 28th day when compared with respective 0, 7th and 14th day values. The difference was significant when compared with group II animals' values on 28th day. Also, the values on 28th day were observed at par in these groups when compared with group I which is normal control animals. Group V treated with concurrent administration of herbal combination with Metformin shows better results followed by group IV treated with a herbal combination and group III treated with Metformin. Same observations of reduction in Serum CPK level values after treatment with *Murraya koenigii* root extract were observed by Lanjhiyana *et al*. (2011) [18] they reported hypoglycaemic activity of *Murraya koenigii* root in alloxan-induced diabetic rats using aqueous root extract dose (@ 200 mg/kg po) which significantly reduces (p<0.05) Serum CPK level compared to diabetic control. Similar observations were reported by Kesari *et al*. (2007) [14] which are in accordance to the present study.

Pathological changes

Gross Pathology

The gross pathological examination is concluded at the end of experimental period did not shows any appreciable gross changes seen by naked eyes in liver, kidney and pancreas of sacrificed rats in diabetic control as well as treatment groups.

Histoarchitectural changes

In addition to customized parameters, study was evaluated through histopathological examination of pancreas, liver and kidney at the end of trial.

Liver

On histomorphological assessment, the section of liver from the rats of control group and Metformin treatment groups did not reveal any treatment related appreciable changes, except, in few section there were minimal focal degenerative changes as an incidental finding. The section of liver from rats of diabetic group revealed mild to moderate, focal to multifocal degenerative, necrotic changes in hepatocytes and focal congestion. Occasionally, sinusoidal space found to be distended. In few sections, there were scanty circumscribed vacuolation in hepatic parenchyma denoting fatty changes. Section of liver from the rats in all treatment groups found to be restored architecturally as evidenced by regeneration and restoration of hepatic parenchyma. Upadhye *et al*. (2019) [29] revealed focal and multifocal degeneration in hepatocytes as compared to diabetic control group the result are in accordance with the result of present study (Plate No 1-5).

Pancreas

The section of pancreas from rats of diabetic group showed minimal degeneration, derangement and necrotic changes in β cells. There was occasional vacuolation in islet of Langerhans, in few sections. Occasionally, there was mild to moderate congestion in pancreatic tissue. In treatment groups such as group III, IV and V, the sections of pancreas showed structural restoration, except, in few sections of pancreas from rats of herbal treatment group, there was congestion and minimal focal degenerative changes in β cells. The sections of pancreas from rats of control and Metformin treatment groups didn't showed any treatment related appreciable change. Similar observations are reported by, Vijayanand. (2015) [30] concluded that the vacuolation in islets of Langerhans and moderate congestion in diabetic group compare to treatment group was observed. He also reported that treatment of herbal combination showed structural restoration of pancreas (Plate No 6-10).

Kidney

On histomorphological assessment, the sections of kidneys from the rats of control group and Metformin treatment groups did not reveal any treatment related appreciable changes, except, in few sections, there were minimal focal degenerative changes as an incidental finding. Kidney from diabetic control group on histoarchitectural study revealed mild to moderate congestion, cellular swelling of tubular epithelial cell, focal cystic degenerative change, mild to moderate MNC infiltration and mild necrotic change in tubular epithelial cells. In few sections, the congestion in corticomedullary junction was appreciable. Occasionally, there were mild to moderate hydropic degenerative changes in kidney tubules. The sections of kidney from the rats of group III, IV and V found to be repaired as evidenced by the histoarchitecture of the studied organs. Ibrahim *et al*. (2022) [11] Goes well with the result of present study (Plate No 10-15).

Conclusions

Alloxan @ 120 mg/kg BW by intra-peritoneal injection induces diabetes in Wistar rats. Use of aqueous leaves extract of *Murraya koenigii* and *Aegle marmelos* herbal combination

is effective tool in treatment of diabetes. Herbal combination was observed to be effective for the elevated blood glucose level in alloxan induced diabetic rats. Herbal combination have positive effect on lipid profile of diabetic rats. The use of herbal combination with standard antidiabetic drug Metformin showed better effect in controlling blood glucose level, AST, ALT, BUN, total cholesterol, triglyceride and creatinine phosphokinase. Herbal combination revealed restoration of normal histoarchitectural structure in liver pancreas and kidneys of diabetic rats.

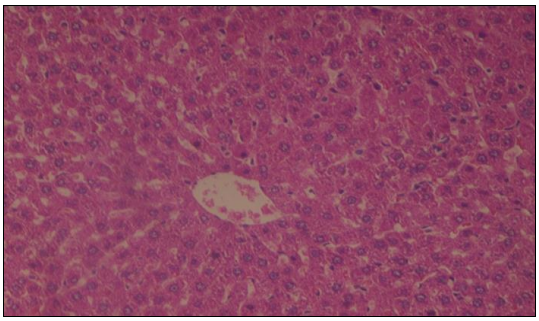

Plate 1: Section of liver from rat of group I showing normal histological features (H and E 400X)

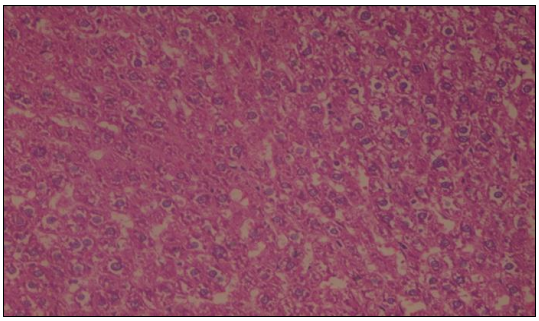

Plate 2: Section of liver from rat of group II showing mild to moderate, focal to multifocal degeneration, necrotic changes & fatty changes in hepatocytes (H and E 400X)

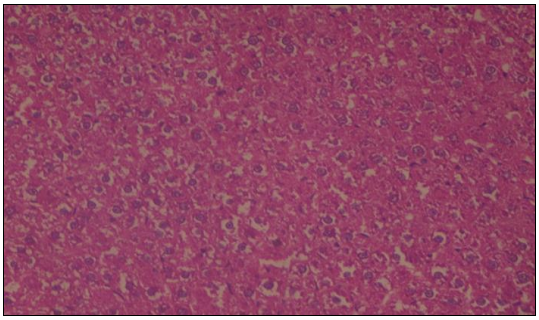

Plate 3: Section of liver from rat of group III showing minimal degenerative changes in hepatocytes (H and E 400X)

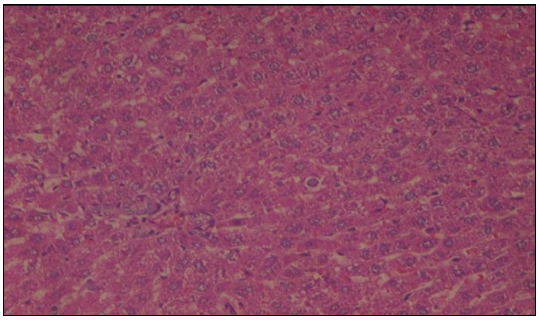

Plate 4: Section of liver from rat of group IV showing minimal focal degenerative changes in hepatocytes and MNC infiltration (H and E 400X)

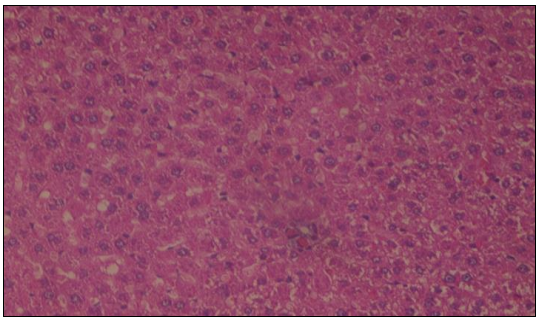

Plate 5: Section of liver from rat of group V showing minimal focal degenerative changes (H and E 400X)

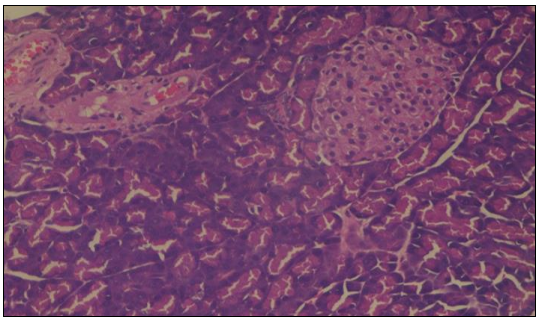

Plate 6: Section of pancreas from rat of group I within histological limits (H and E 400X)

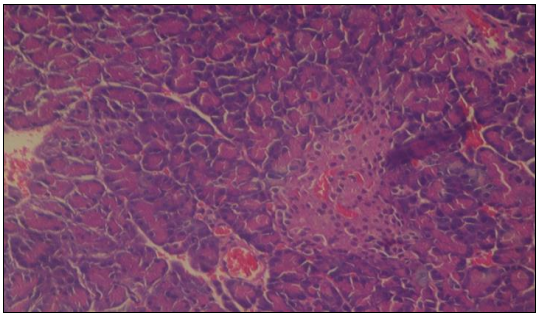

Plate 7: Section of pancreas from rat of group II showing congestion, degeneration and focal necrotic changes in β-cells (H and E 400X)

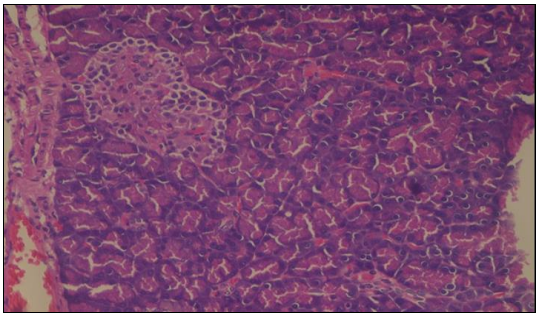

Plate 8: Section of pancreas from rat of group III showing normal histological appearance (H and E 400X)

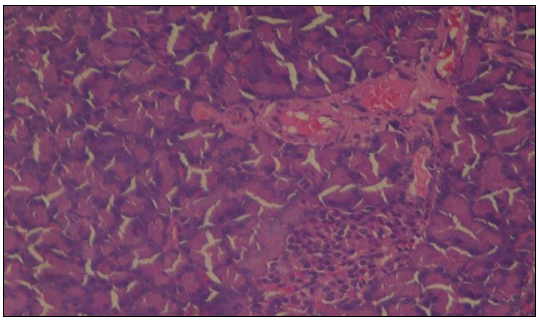

Plate 9: Section of pancreas from rat of group IV showing mild congestion and focal degenerative changes in β-cells (H and E 400X)

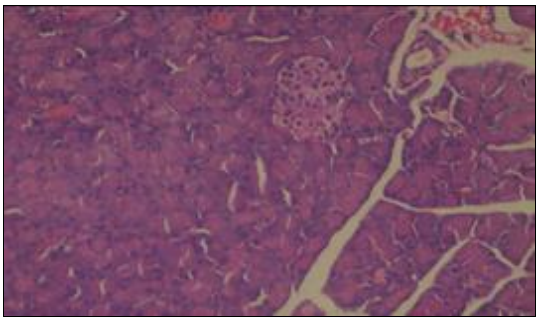

Plate 10: Section of pancreas from rat of group V showing congestion (H and E 400X)

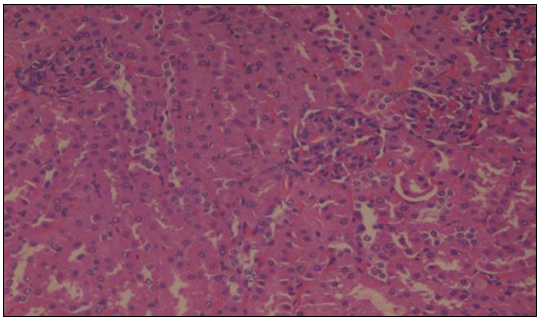

Plate 11: Section of kidney from rat of group I showing minimal focal congestion as a incidental change (H and E 400X)

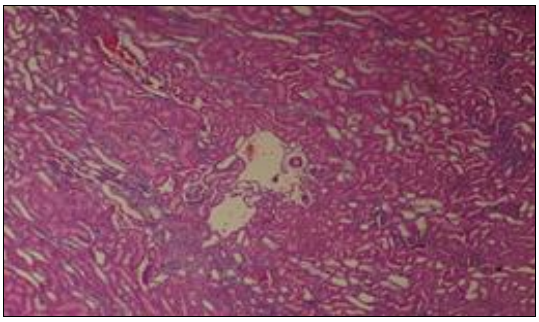

Plate 12: Section of kidney from rat of group II showing cellular swelling in PCT, Cystic degenerative changes and MNC infiltration (H and E 100X)

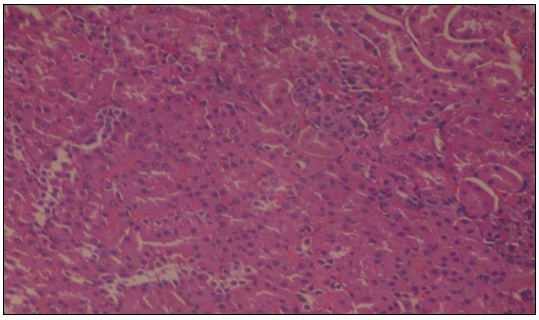

Plate 13: Section of kidney from rat of group III showing minimal focal congestion and MNC infiltration (H and E 400X)

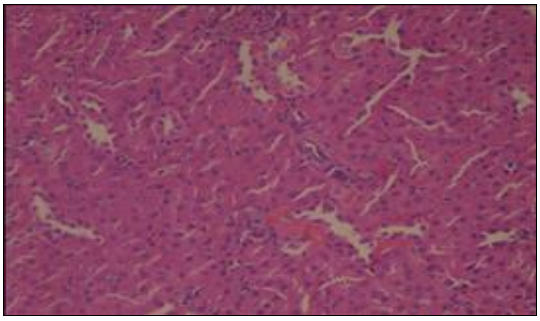

Plate 14: Section of kidney from rat of group IV showing minimal focal congestion and MNC infiltration (H and E 400X)

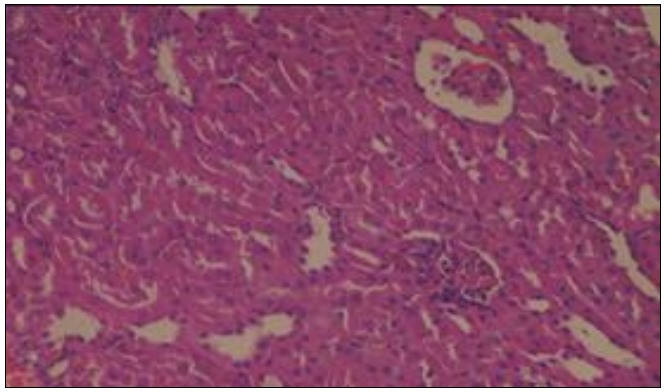

Plate 15: Section of kidney from rat of group V showing minimal MNC infiltration and degenerative changes in tubular epithelial cells (H and E 400X)

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Conflict of Interest
Not available

Financial Support
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Author's Name and Details

SS Bhosle
PG Scholar, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

DP Patil
Associate Professor, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

GD Ranvir
Associate Professor, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

GR Gangane
Associate Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

PB Ghorpade
Assistant Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

PR Suryawanshi
Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

SV Londhe
Assistant Professor, Department of Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

SV Panchal
PG Scholar, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

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