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TOXICOLOGICAL EVALUATION AND ORAL GLUCOSE TOLERANCE TEST OF *GANODERMA APPLANATUM* (PERS.) PAT. FROM KERALA

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ABSTRACT: Aim: The aim of the present study is to evaluate the toxicity and glucose tolerance potential of *Ganoderma applanatum* in Sprague Dawley rats. Settings and Design: Acute toxicity study: A single dose of extracts - 500, 1000 and 2000mg/kg body weight (b.wt.) were provided to animals and observed for a period of 14days. Sub-acute toxicity: 500 and 1000mg/kg b.wt. of extracts were provided alternatively for a period of 28days and after sacrifice, blood and tissue were evaluated for various investigations. OGTT: Oral glucose tolerance test was carried out in normal and hyperglycemic rats. Methods and Material: Acute and sub-acute toxicity study was conducted for a span of 14days and 28days respectively. The animals were examined for behavioral, physical, physiological and biochemical abnormalities. OGTT was conducted in normal and hyperglycemic rats by administrating 2g/kg b.wt. of glucose orally followed by extracts and standard drugs. Serum glucose level in blood was estimated periodically up to 120min. Statistical analysis: Results were analyzed using ANOVA with Tukey–Kramer multiple comparisons test. Results: No mortality observed in toxicity study. The rats appeared to be normal with no variations in cellular morphology, biochemical parameters and behavioral aspects. Oral administration of both extracts at 250mg/kg and 500mg/kg b.wt. significantly improve glucose tolerance by reducing blood glucose level in alloxan induced diabetic (hyperglycemic) rats. Conclusions: Study concludes the nontoxic nature of *G. applanatum* and potential use in glucose tolerance.

KEYWORDS: Alloxan, *Ganoderma applanatum*, Hyperglycemic, Medicinal Mushroom, OGTT, Sprague Dawley rat

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1. INTRODUCTION

Herbal medicines are getting substantial interest worldwide by people in developing as well as developed countries for their role in prevention and treatment of diseases without side effects [1]. Hence, there is enormous interest among the scientific community to explore therapeutic potential of various traditional folk medicinal plants and mushrooms [2–4]. Toxicological evaluation is the first step in validating the medicinal potential of these plants and mushrooms [5–7]. Thus, the organization for economic co-operation and development (OECD) mentions toxicity studies as key step in determining the safety of herbal drugs by gathering observational data and utilization of data to predict outcome using appropriate animal models. Oral glucose tolerance test (OGTT) is a standard procedure to identify persons at high risk for type-II diabetes [8]. It is a widely utilized method which has been used to evaluate the β-cell function and insulin resistance [9–12]. OGTT is a practical test to understand the body's ability to metabolize or oxidize glucose. Glucose tolerance impairs and decreases depending on various physiological or pathological conditions including Diabetes mellitus [13]. Type-II Diabetes Mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to increased hepatic glucose production, dysfunctional metabolism of carbohydrate, fat, protein and defects in β-Cell function and insulin action [14]. According to World Health Organization report in 2017, approximately 150million people are affected with diabetes mellitus worldwide and this number seems to double by the year 2025. The increase of this disease state is mainly contributed by population growth, ageing, obesity, improper or unhealthy diets and deskbound lifestyles. The increase in mortality rate by type-II diabetes is also due to the shortage of access to affordable insulin which remains a key obstacle to successful treatment. While oral hypoglycemic agents like sulphonylureas and biguanides are either too expensive or have undesirable side effects [15–17]. Hence, many efforts are relentlessly taken to discover various anti-diabetic drugs from nature which may reduce hyperglycemic conditions as well as combat diabetic complications [18]. Medicinal mushrooms are exemplary sources of untapped pharmacological compounds with various therapeutic properties and bioactivities including anti-microbial [19], anti-oxidant [19], anti-inflammatory [20], anti-cancer [21] and anti-diabetic properties [22]. They have been used for centuries as a folk medicine in oriental countries like China and Japan for medicinal and tonic properties [23–25]. Among medicinal mushrooms, the genus *Ganoderma* P. Karst has been evaluated, validated and well appreciated by the scientific community for its diverse properties. The wide range of properties of *Ganoderma* is due to the presence of various bioactive molecules like

polysaccharides, triterpenoids, steroids, alkaloids, flavonoids and phenols [26–28]. The specific applications and attributed health benefits of *Ganoderma lucidum* (Curtis) P. Karsten include control of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostatic and more [29]. Extensive studies have been carried out on the laccate species of *Ganoderma* particularly *G.lucidum* but very scanty or no efforts are taken to explore the non-laccate species of *Ganoderma* particularly *G.applanatum* (Pers.) Pat. which occurs widely in southern part of India. Hence, the present study investigates the toxicological and blood glucose lowering effect of methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum* with reference to biochemical parameters (hematology and serum analysis), histopathology and behavioral aspects in Sprague Dawley rats.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Alloxan was obtained from Sigma Chemical Company Inc., St. Louis, MO, USA. GOD-POD kit was purchase from Liquizone, Glucose - MR, Medsource Ozone Biomedicals Private Limited, Haryana, India while, glucometer was procured from One Touch® Glucometer, Kerala, India. EDTA (Ethylene diamine tetra acetic acid) and hypochlorite were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Glibenclamide and insulin were purchased from Pharmacy of Pushpagiri Hospital, Kerala, India. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

2.2 Collection and Identification of Mushrooms

Specimen was obtained from dead stump of an unknown tree at their sexual stage from Ranni, Pathanamthitta district of Kerala, India. Collected samples were kept in a zip lock polythene bag and brought in the laboratory for identification using morphological and molecular tools.

2.3 Extract preparation

Ganoderma fruiting body was shadow dried, cut in to small pieces and then powdered using a blender. About 100g of powdered mushroom was extracted three times separately with methanol and chloroform for 12hours at 60˚C. The extract was strained using gauze cloth further by Whatman filter paper No.1 under vacuum and condensed using rotary evaporator (IKA RV10, IKA India Private Limited, Karnataka, India) and finally lyophilized (FreeZone 2.5Liter -50˚C Benchtop Freeze Dryers, Labconco Corporation Kansas City, MO) at -40˚C.

2.4 Animals

Male Sprague Dawley rats weighing 180-260g were selected for the study. The animals were purchased from Kerala Veterinary College, Mannuthy, Kerala, India. Animals were hosted and acclimatized in Pushpagiri Animal House, Tiruvalla, Kerala, India with standard environmental controlled conditions of 23 ± 5ºC, 12h light-dark cycle, have free access to standard food (Krish Scientist's Shoppe, Bangalore, India) and UV sterile water. All animal experiments were carried out

with the prior approval of the Institutional Animal Ethics Committee and were conducted strictly adhering to the guidelines of CPCSEA (No.602/PO/Re/S/2002/CPCSEA) constituted by the Animal Welfare Division of Government of India.

2.5 Acute toxicity study

The acute toxicity of *G. applanatum* methanol extract (GAME) and Chloroform extract (GACE) was performed as per OECD-423 guidelines (acute toxic class method – OECD-423) [30]. The test was carried out using healthy young male Sprague Dawley rats weighing 180-200g by random sampling. Extract was oral administered in a single dose by using an oral gavage following a period of five hours fasting. The animals were divided into seven groups containing five rats each. The Group-1 served as control and was provided with distilled water. The Group-2, 3 and Group-4 received 500, 1000 and 2000mg/kg body weight (b.wt.) of GAME respectively while Group-5, 6 and Group-7 received 500, 1000 and 2000mg/kg b.wt. of GACE respectively. The animals were observed individually after dosing for toxic symptoms continuously for the first 4hours and thereafter, for a period of 14days. All the recordings of daily observation including toxic symptoms, behavioral change and weight of animals were maintained.

2.6 Sub-acute toxicity study

The sub-acute toxicity study was carried out by administration of GAME and GACE according to OECD guideline-407 (OECD-407) [31]. Male adult Sprague Dawley rats weighing 180-200g were divided into six groups with five animals each and were placed under standard conditions. Group-1 was considered as control and the other four groups were considered as experimental groups with Group-2 and Group-3 were received the GAME and Group-4 and Group-5 were received the GACE at a dose of 500 and 1000mg/kg b.wt. respectively for 28 consecutive days. The blood was collected from the tail vein and hematological parameters like total erythrocytes count, total leukocytes count and hemoglobin were determined while Serum was used for the determination of liver function test by Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) [32] and alkaline phosphatase (ALP) [33], renal function tests such as urea by diacetyl monoxime (DAM) reagent method [34] and creatinine by alkaline picric acid method [35] and Cardiac markers like CK-MB [36] and LDH [37] were analyzed using serum analyzer (Agappe Diagnostics Ltd., Kerala, India). All the serum parameters were analyzed using test specific Kits from Agappe Diagnostics.

2.7 Histopathological analysis

A portion of liver, kidney and heart tissues were dissected after the sacrifice of animal under anesthesia (xylazine and ketamine) and washed in saline. Tissues were fixed in 10% formalin and embedded in paraffin wax for histological studies. A thin section of 5-micron thickness tissue piece were made using microtome and stained with haematoxylin-eosin (Hematoxylin 5% and Eosin 1% in water). Each section was observed under microscope (Leica DM750, Leica Biosystems,

Maharashtra, India) at 40X magnification and photographed (Leica ICC50 with Leica LAS software, Leica Biosystems Maharashtra, India).

2.8 Induction of Diabetes

Rats were made diabetic by a single intra-peritoneal injection of Alloxan (130mg/kg b.wt.) followed by an overnight fasting. Six hours after the alloxan challenge, animals were administrated orally with 2ml of 10% glucose to prevent fatal hypoglycemia [38–40]. The blood glucose was measured for a period of 48hours after the alloxan induction using the GOD-POD kit and confirmed by glucometer. Rats with fasting blood glucose level more than 150mg/dl were considered diabetic and hence, used for the experiment [18].

2.9 Oral glucose tolerance test (OGTT)

OGTT was performed in normal and diabetic rats. Both sets of animals were fasted overnight (12-16 hour) to carry out the procedure.

Experimental Design

In the present study a total sixty rats were taken. Out of which twenty-five rats were used in OGTT in normal rat study and thirty-five rats were made diabetic using Alloxan.

2.9.1 Effect of *G. applanatum* on OGTT in normal rats

OGTT was performed in overnight fasted normal rats. Normal rats were divided into five groups with five rats each.

Group-1: Normal Control: Rats were orally administered with water,

Group-2: Positive control: Glibenclamide (0.25mg/kg b.wt.) orally,

Group-3: Positive control: Insulin (0.5 U/kg b.wt.) intra-peritoneal (i.p.),

Group-4: Experimental Group: GAME (500mg/kg b.wt.) orally,

Group-5: Experimental Group: GACE (500mg/kg b.wt.) orally,

A total 2g/kg b.wt. of glucose [41] was orally administered 30min after the provision of extracts and standard drugs. Serum glucose level of blood sample from the tail was estimated by using GOD POD kit at 0, 30, 60, 90 and 120min and also confirmed by glucometer.

2.9.2 Effect of *G. applanatum* on OGTT in diabetic rats

Overnight fasted diabetic rats were separated in seven groups with five rats each.

Group-1: Diabetic Control: Diabetic rat + water orally,

Group-2: Positive control: Diabetic rat + Glibenclamide (0.25mg/kg b.wt.) orally,

Group-3: Positive control: Diabetic rat + Insulin (0.5U/kg b.wt.) intra-peritoneal (i.p.),

Group-4: Experiment Group: Diabetic rat + (250mg/kg b.wt. GAME) orally

Group-5: Experiment Group: Diabetic rat + (500mg/kg b.wt. GAME) orally

Group-6: Experiment Group: Diabetic rat + (250mg/kg b.wt. GACE) orally

Group-7: Experiment Group: Diabetic rat + (500mg/kg b.wt. GACE) orally

Glucose (2g/kg b.wt.) was fed 30min after the administration of extracts. Control animals were

administered with equal volume of water. Blood was withdrawn from the tail tip of rat at 0, 30, 60, 90 and 120min of glucose administration and glucose levels were estimated by GOD-POD kit and also by using glucometer.

2.10 Statistical analysis

The results were presented as mean ± SD of the studied group. Statistical analyses of the results were performed using ANOVA with Tukey–Kramer multiple comparisons test. A level of $P<0.01$ was taken as statistical significant.

3. RESULTS AND DISCUSSION

3.1 Collection of mushroom

The collected fungus was identified as non-laccate species of *Ganoderma applanatum* (Pers.) Pat. on the basis of its macro and micro morphology. This species is already described from this region on the basis of morphology [42–45] and molecular [46–49] characterization.

3.2 Extract preparation

The total yield obtained from methanol and chloroform extraction of *Ganoderma* was 4.7% and 5.3% respectively.

3.3 Acute toxicity study of Methanol and Chloroform extract of *G. applanatum*

Acute toxicity study conducted as per the guidelines of OECD-423 revealed that methanol and chloroform extract of *G. applanatum* showed no mortality or any drastic changes in body weight (table-1) throughout the span of 14days in any of the concentrations. There was no change in the eating habits, no sign of tremors, diarrhea, fatigue or sudden variation in body weight were observed. Also, the eyes and sleeping duration seems to be normal. LD50 for the oral dose of GAME and GACE was indeterminable being concentration in excess of 2000mg/kg b.wt. Thus, extracts were practically non-toxic hence testing at higher dose may not be necessary.

Table 1: Acute toxicity - Effect methanol (GAME) and chloroform (GACE) extract of *Ganoderma applanatum* on body weight of mice over a period of 14 days.

SN	Treatment days	Control	Extract 500mg/kg body weight		Extract 1000mg/kg body weight		Extract 2000mg/kg body weight	
			GAME	GACE	GAME	GACE	GAME	GACE
1	0	225.61± 5.04	226.61± 1.24	222.00±4. 51	222.77±2.5 5	222.05± 2.85	223.71± 1.5	223.53± 1.37
2	3	229.94± 3.98	229.94± 2.2	223.12±3. 47	222.93± 2.82	225.03± 3.08	222.75 ±2.65	227.72± 3.04
3	6	228.06 ± 2.84	230.06 ±3.04	225.44±2. 40	223.99± 1.70	229.60± 1.33	224.92± 3.83	229.92± 2.18
4	9	230.17 ± 0.91	232.17 ±4.16	226.54±1. 40	224.45± 3.07	232.18± 4.27	225.04 ±2.75	232.18± 4.27
5	14	225.17 ±1.08	235.17 ±1.08	235.66±1. 44	230.46± 2.76	233.84± 2.62	227.36 ±4.58	234.31± 2.09

3.4 Sub-acute toxicity study

The administration of both extracts to rats did not produce any significant change in the body weight, serum, hematological parameter and tissue morphology as compared to the normal control rats (table-2).

Table 2: Sub-acute toxicity - Effect methanol (GAME) and chloroform (GACE) extract of *Ganoderma applanatum* on body weight of mice over a period of 28 days

SN	Treatment days	Control	Extract 500mg/kg body weight		Extract 1000mg/kg body weight		Extract 2000mg/kg body weight	
			GAME	GACE	GAME	GACE	GAME	GACE
1	0	223.53± 1.37	223.53± 1.37	225.89± 1.09	224.16±0 .16	223.09±1 .98	221.13±3 .10	225.03±2 .98
2	7	227.72± 3.07	227.72± 3.07	229.16± 3.01	226.18±1 .98	223.76±2 .76	225.98±5 .61	224.23±1 .05
3	14	230.46± 1.76	237.29 ±3.05	237.77± 3.49	231.58± 1.98	234.19± 3.53	228.42± 3.95	237.55± 3.19
4	21	235.21± 1.79	238.31 ±2.18	237.92± 3.60	233.66± 2.29	237.47± 1.52	230.71 ±2.09	238.79± 4.07
5	28	235.73± 2.82	239.59 ±4.08	235.09± 4.49	235.73± 4.33	235.62± 2.55	238.40± 4.09	238.85± 3.05

3.4.1 Hematological Parameter

There are no statistically significant differences in hematological parameter of groups administered with 500 and 1000mg/kg b.wt. of both extracts as compared to the normal rats (table-3).

Table 3: Hematological Parameters in Sprague Dawley rats after 28 days treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*

SN		WBC (10^9/L)	Lymphocyte %	Monocyte %	Hemoglobin (g%)	RBC (10^{12}/L)
1	CONTROL	11.50±1.04	53.250±5.150	4.0±1.080	16.4±0.24	9.23±0.09
2	GAME (500 mg/kg)	11.0±1.03	58.9±4.09	3.5±0.645	16.01±0.1	8.75±1.12
3	GAME (1000 mg/kg)	10.98±1.21	60.250±4.190	2.9±0.109	15.6±0.02	8.66±1.09
4	GACE (500 mg/kg)	11.21±1.4	59.16 ±3.18	3.5±1.190	15.77±0.1	7.95±0.07
5	GACE (1000 mg/kg)	12.09±1.17	58.250±2.600	3.75±0.76	15.01±0.09	7.88±0.5

3.4.2 Serum Parameters

Oral administration of methanol and chloroform extract of *G.applanatum* did not cause any significant changes in serum markers of liver, kidney and heart when compared to the normal non-treated control rats (table-4).

Table 4: Serum Parameters in Sprague Dawley rats after 28 days treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*

SN		UREA (mg/dl)	CREATININE (mg/dl)	ALT (IU/l)	AST (IU/l)	ALP (IU/L)	CK-MB (U/L)	LDH (U/L)
1	CONTROL	27.95±1.82	1.39±0.16	43.46±3.12	35.86±2.5	125.63±1.69	58.44±3.88	103.7±7.52
2	GAME (500 mg/kg)	25.62±3.36	1.17±0.11	40.83±2.04	35.73±1.3	123.036±4.50	56.75±4.26	107.1±0.32
3	GAME (1000 mg/kg)	27.47±1.96	1.33±0.14	43.50±2.42	35.86±2.7	126.187±2.09	57.74±7.21	108.4±4.30
4	GACE (500 mg/kg)	28.19±2.23	1.22±0.16	41.71±3.05	35.8±3.3	125.42±2.17	59.74±0.91	105.9±5.08
5	GACE (1000 mg/kg)	28.075±2.09	1.48±0.17	43.83±1.98	35.61±1.4	126.42±3.44	63.21±5.11	106.3±5.31

3.4.3 Histopathological analysis

Macroscopic investigation revealed that organs like liver, kidney and heart of the animals treated with both extracts showed no alterations in color as compared to control. Microscopic analysis of the organs depicted no noticeable differences between the control and experimental groups (fig.1-9). Methanol and Chloroform treated group did not exhibited any change in cellular morphology when viewed under the light microscope using 40X magnification power.

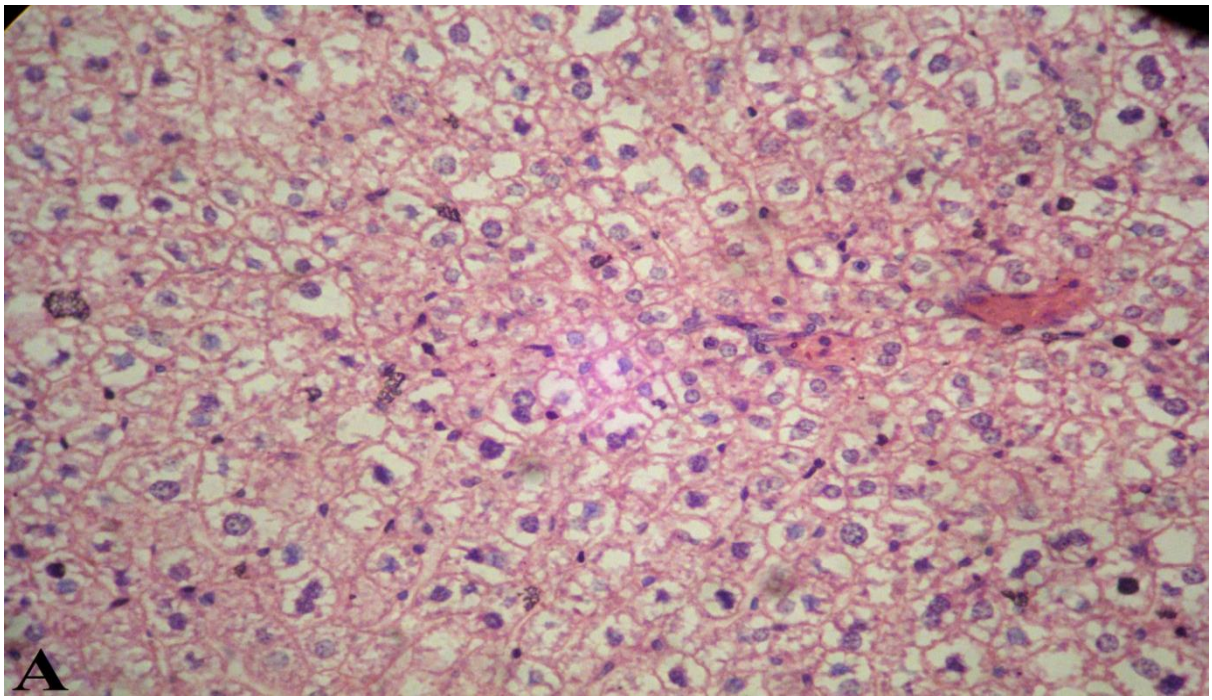

Fig.1: Histopathological sections of Liver: A) Control

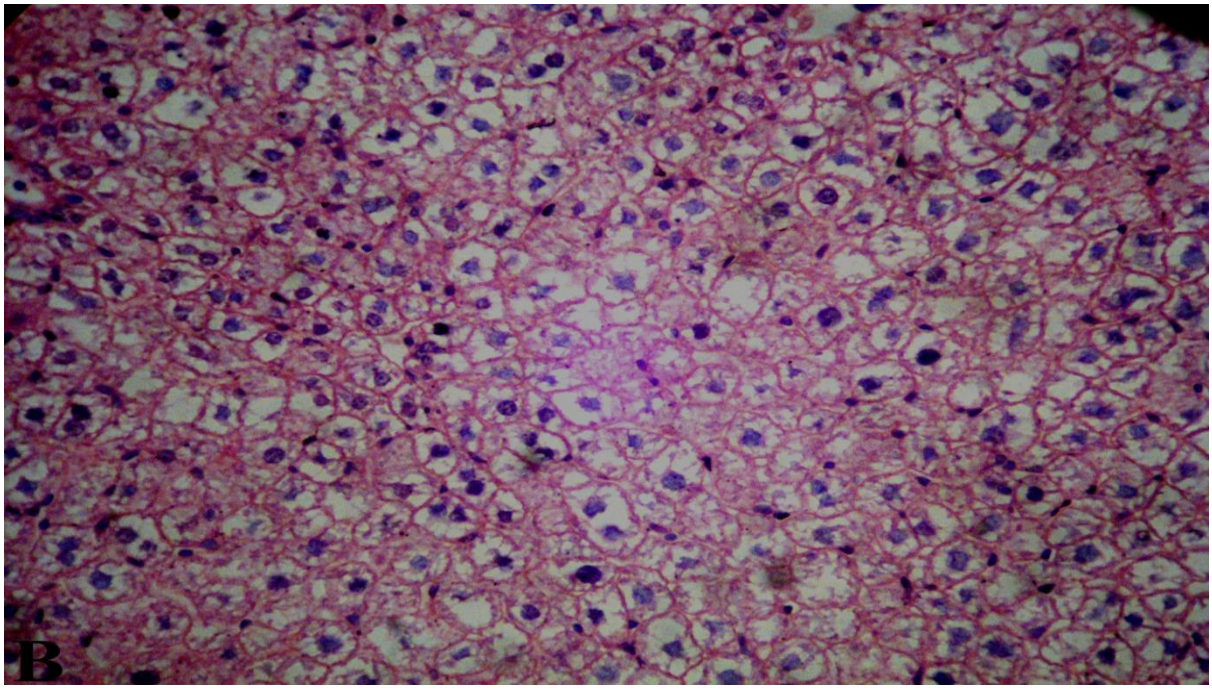

Fig.2: Histopathological sections of Liver: B) GAME (500mg/kg b.wt.)

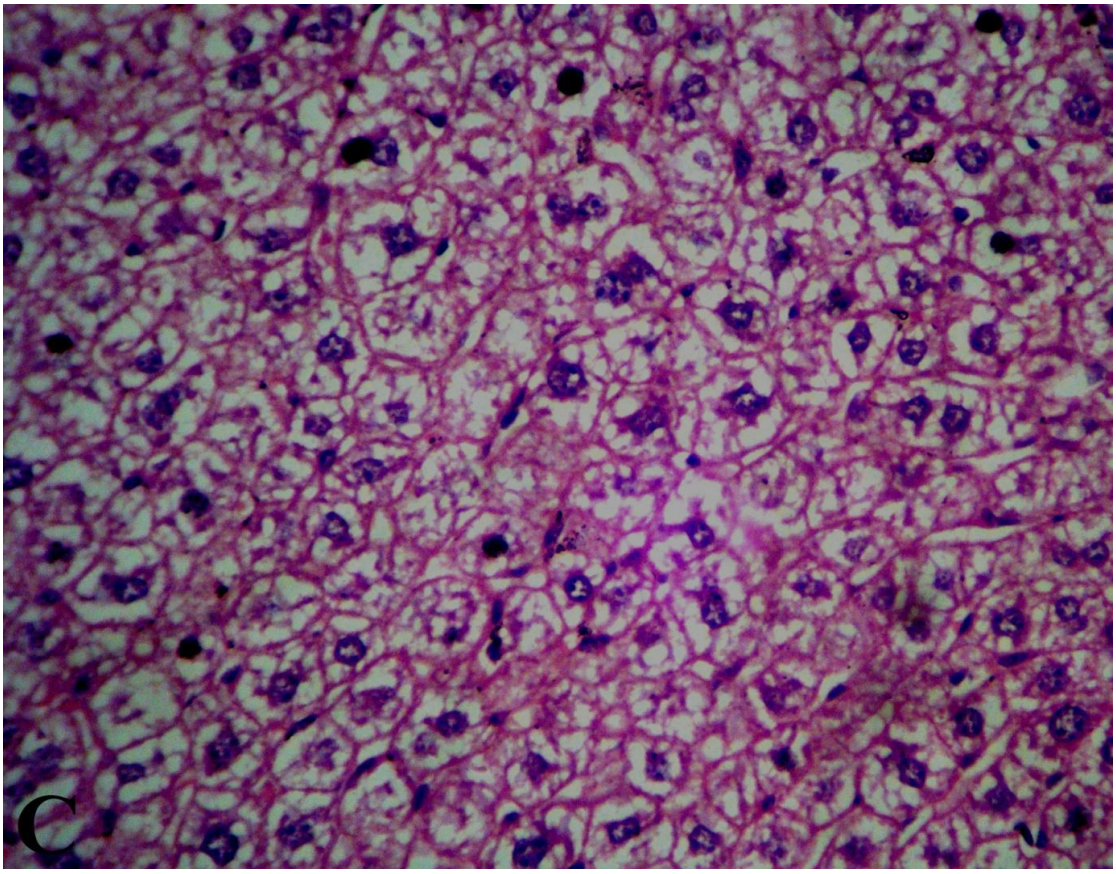

Fig.3: Histopathological sections of Liver: C) GACE (500mg/kg b.wt.)

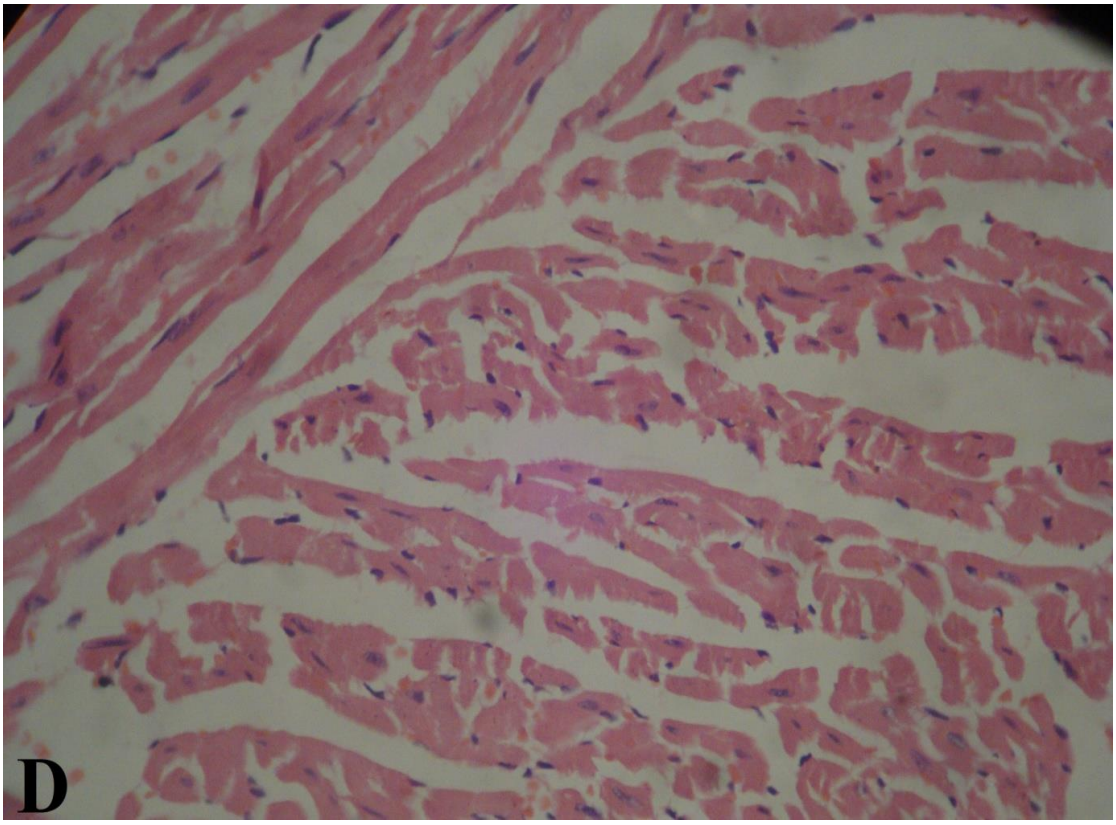

Fig.4: Histopathological sections of Heart: D) Control

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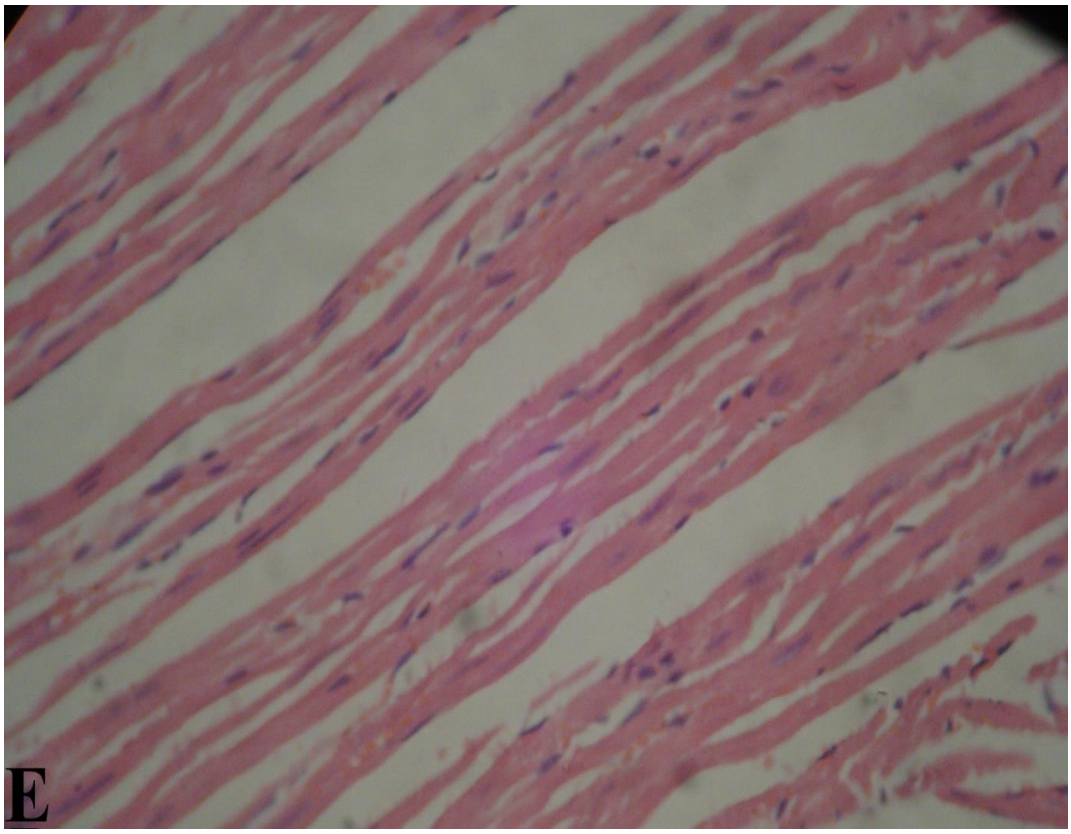

Fig.5: Histopathological sections of Heart: E) GAME (500mg/kg b.wt.)

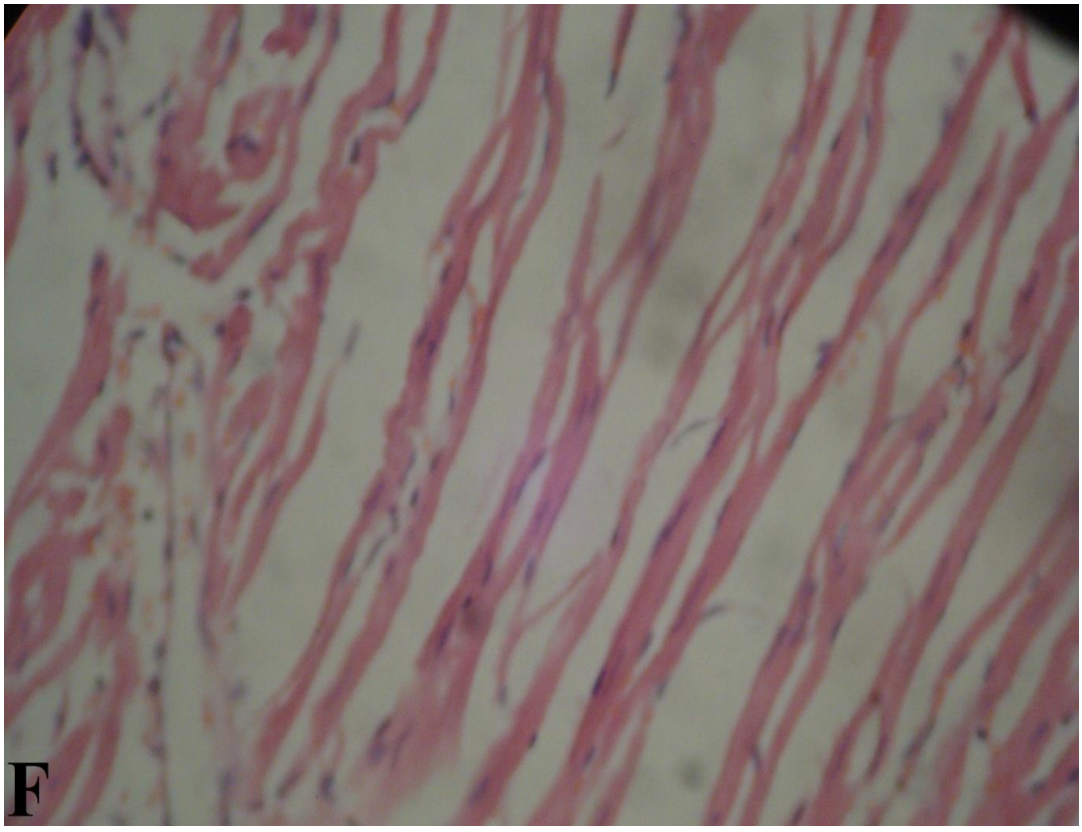

Fig.6: Histopathological sections of Heart: F) GACE (500mg/kg b.wt.)

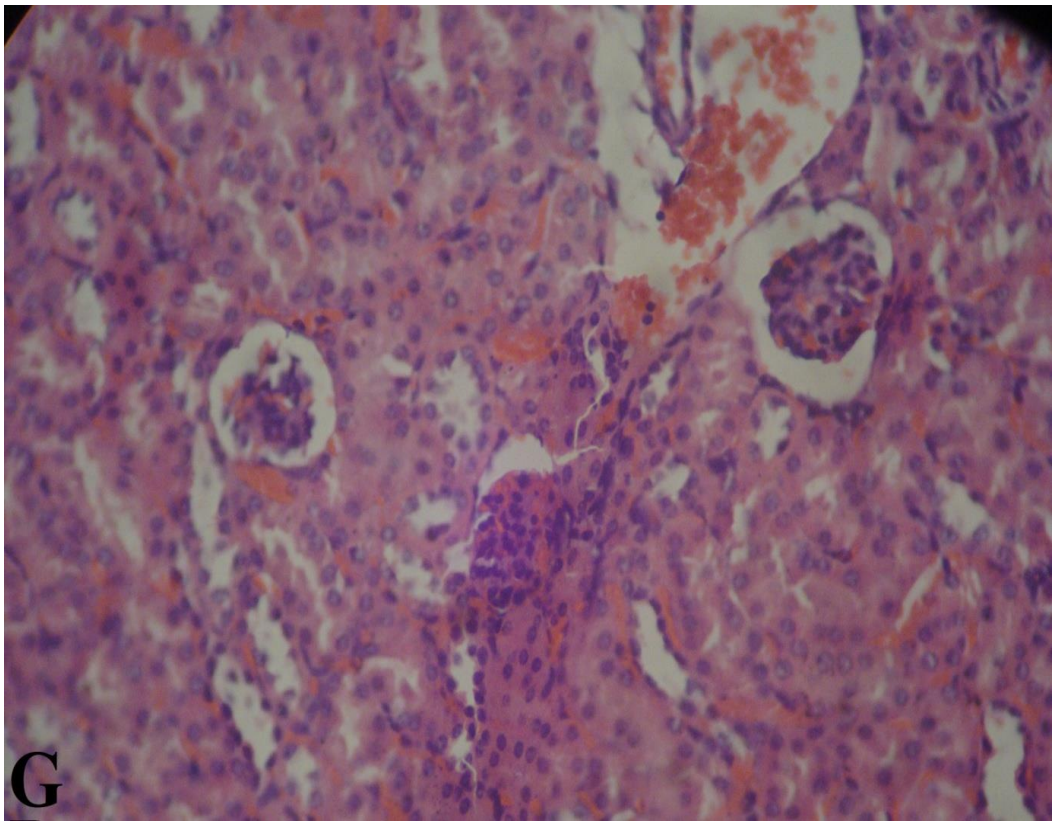

Fig.7: Histopathological sections of Kidney: G) Control

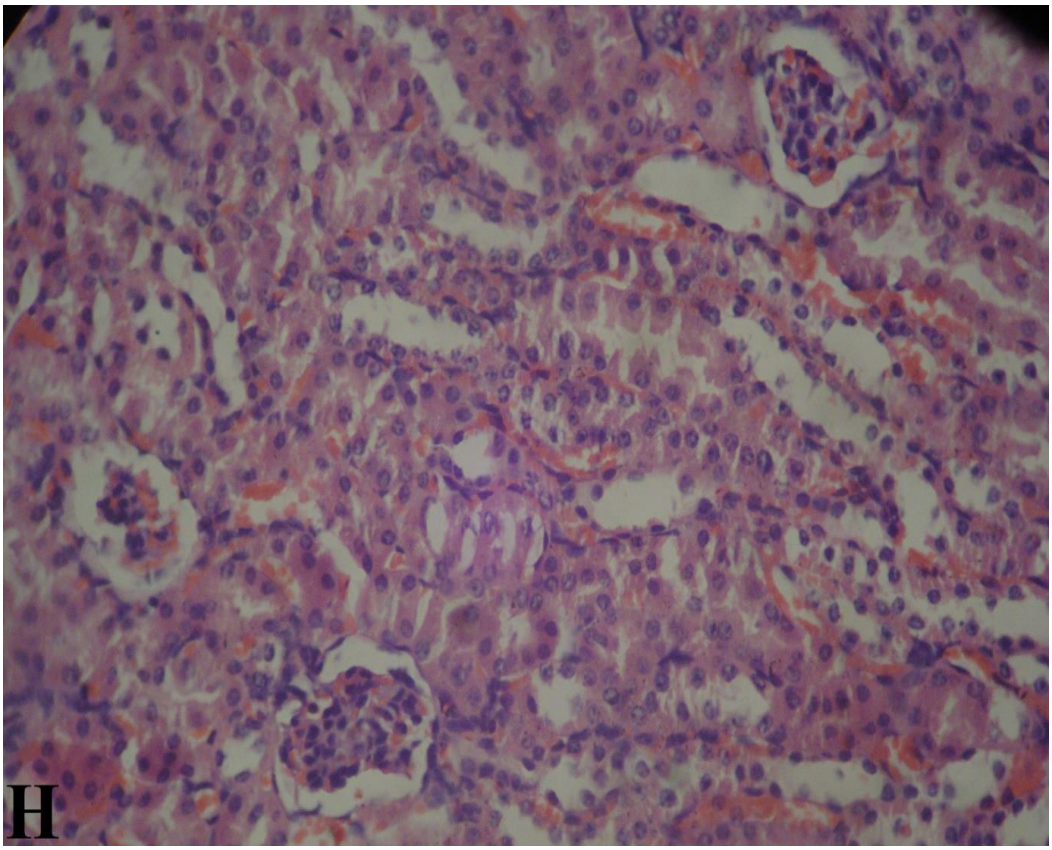

Fig.8: Histopathological sections of Kidney: H) GAME (500mg/kg b.wt.)

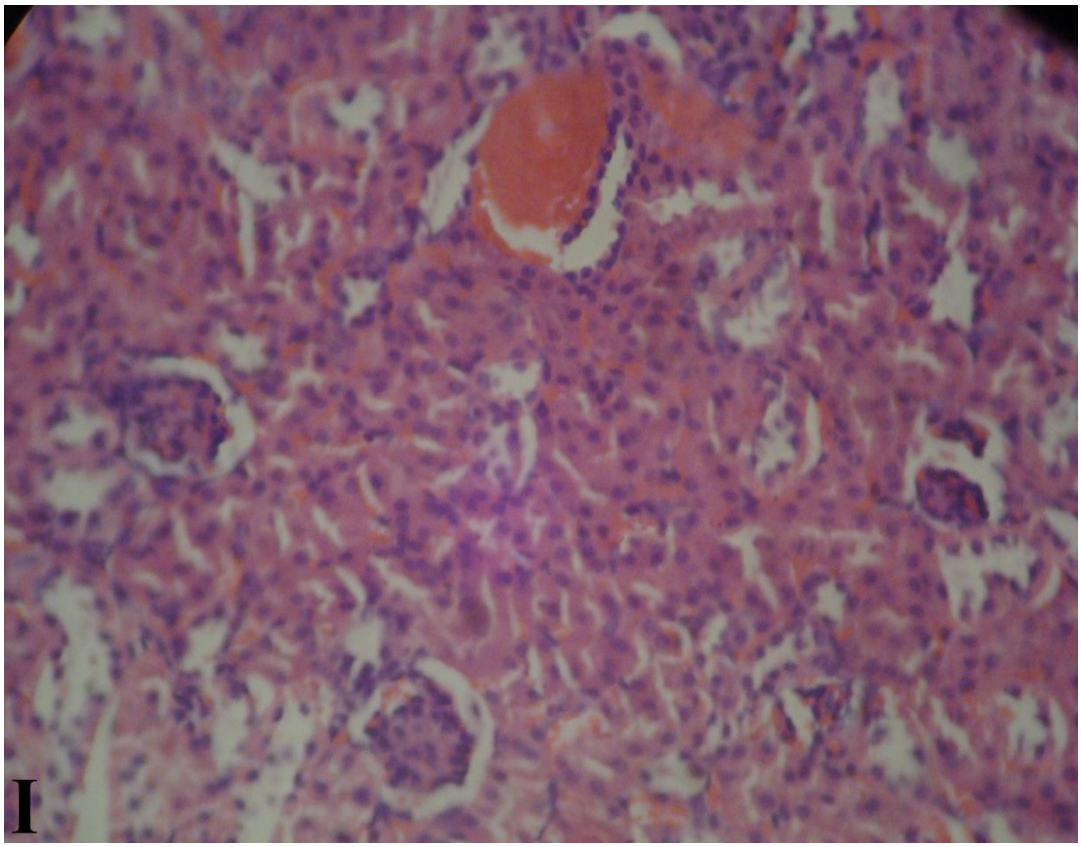

Fig.9: Histopathological sections of Kidney: I) GACE (500mg/kg b.wt.)

3.5 ORAL GLUCOSE TOLERANCE TEST (OGTT)

3.5.1 Effect of *G. applanatum* on OGTT in normal rats

Oral administration of glucose increased the blood glucose levels of the normal rats which, reached peak value at 60min and gradually decreased to the pre-prandial level at 120min (table-5). GAME administered Group-4 significantly suppressed rise in blood glucose level of rats at 60min and 90min while, GACE administered Group-5 significantly suppresses rise in blood glucose level at 60min as compared to control group. Ultimately both extracts decreased and significantly attenuated blood glucose at 120min almost near to the pre-prandial level. Both positive controls Group-2 and Group-3 administered with Glibenclamide and Insulin respectively decreased blood glucose level significantly as compared to control group after the administration of an oral glucose load (fig.10).

Table 5: Oral Glucose Tolerance Test in normal rats after treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*

SN	GROUP	0 MIN	30 MIN	60 MIN	90 MIN	120 MIN
1	CONTROL	82.28±3.09	127.43±4.31	156.36±2.09	117±5.04	92.75±2.09
2	GLIBENCLAMIDE CONTROL (0.25mg/kg b.wt.)	81.69±2.70	105.12±3.61***	111.81±6.01***	91.15±2.12***	82.07±4.67***
3	INSULIN CONTROL (0.5U/kg b.wt.)	87.3±3.53	100.76±4.7***	106.66±2.05***	89.03±3.07***	87.31±3.89**
4	GAME (500mg/Kg b.wt.)	87.69±1.70	113.90±4.91***	126.41±5.04***	100.82±5.09***	88.82±3.08**
5	GACE (500mg/Kg b.wt.)	86.56±3.95	120.97±1.41^{ns}	132.25±4.07***	110.09±2.53^{ns}	92.58±2.09^{ns}

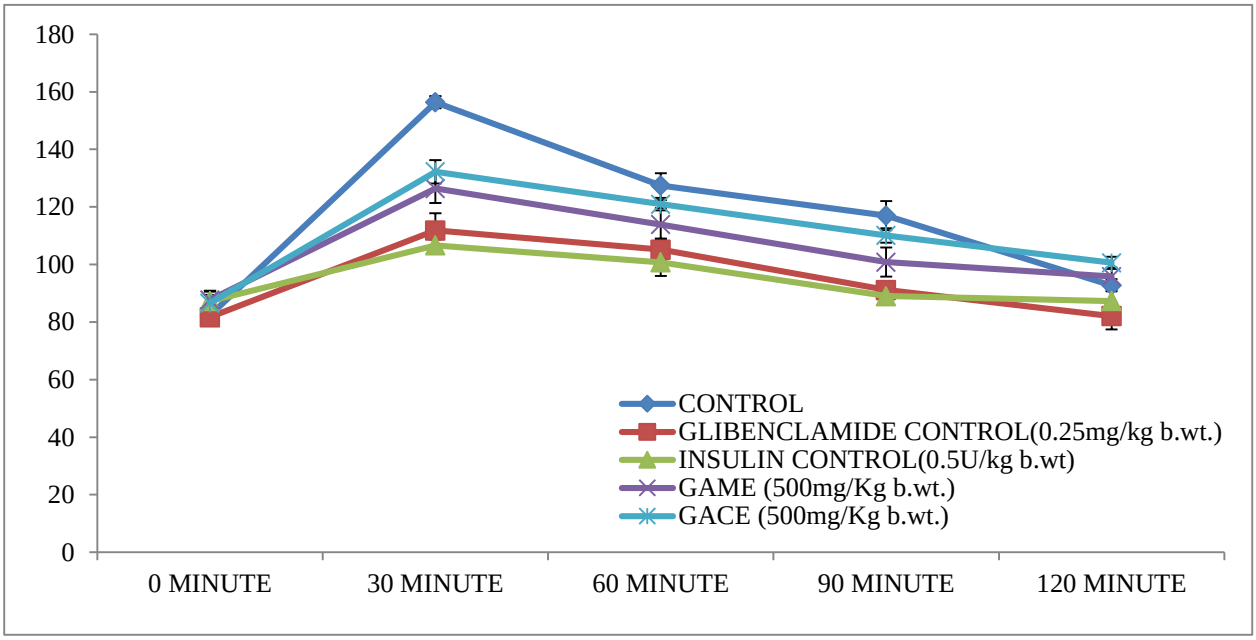

Fig 10: Oral Glucose Tolerance Test in normal rats after treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*.

3.5.2 Effect of *G. applanatum* on OGTT in diabetic rats

The fasting blood glucose levels of diabetic rats were 2-3times higher than that of the normal rats. At a dose of 250mg/Kg b.wt. and 500mg/Kg b.wt., GAME (Group-4 and 5) and GACE (Group-6 and 7) produced a significant attenuation of the blood glucose at 120min after the oral glucose load as compared to diabetic control Group-1 (table-6). Both positive controls i.e. Glibenclamide (Group-2) and Insulin (Group-3) caused significant attenuation at 60min, 90min and 120min when compared to the diabetic control group. The reduction in the blood glucose levels by GAME and

GACE treated groups were found to be dose-dependent at 120min after glucose administration (fig.11).

Table 6: Oral Glucose Tolerance Test in diabetic rats after treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*

SN	GROUP	0 MIN	30 MIN	60 MIN	90 MIN	120 MIN
1	DIABETIC CONTROL	249.12 ±0.7	352.3±2.12	420±1.41	328.84±4.94	313.1±1.85
2	DIABETIC+GLIBENCLAMIDE (0.25mg/kg b.wt.)	251.40 ±3.5	304.61±1.4***	332.72±7.07***	282.69±3.53***	253.46±2.0***
3	DIABETIC+INSULIN (0.5U/kg b.wt.)	243.33 ±6.1	334.61±5.0***	377.57±1.04***	307.45±5.90***	247.10±3.5***
4	DIABETIC+GAME (250mg/kg b.wt.)	249.81 ±5.0	328.65±2.0***	346.02±5.01***	319.09±2.12*	290.09±1.3***
5	DIABETIC+GAME (500mg//kg b.wt.)	269.52 ±2.7	316.76±1.9***	339.19±0.44***	301.09±2.22***	275.51±4.3***
6	DIABETIC+GACE (250mg/kg b.wt.)	256.95 ±0.7	335.32±3.5***	370.52±6.01***	325.58±2.12^{ns}	300.10±1.6***
7	DIABETIC+GACE (500mg//kg b.wt.)	242.09 ±3.5	329.84±7.7***	365.98±3.53***	320.51±7.07*	289.63±3.8***

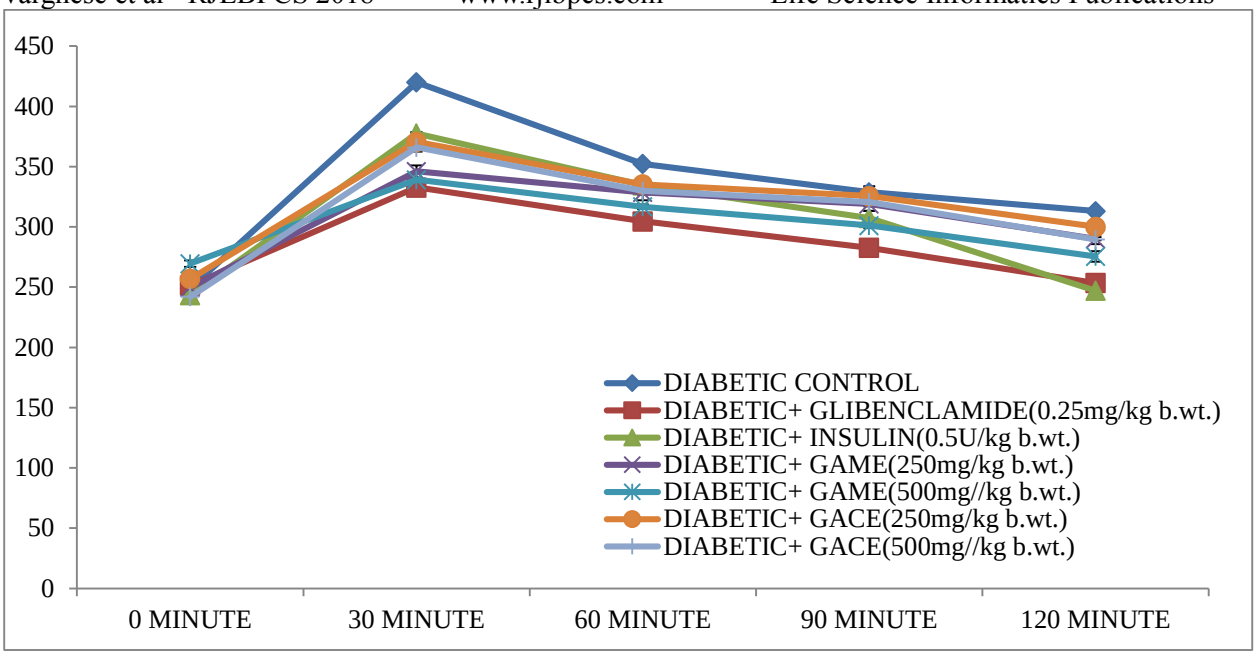

Fig 11: Oral Glucose Tolerance Test in diabetic rats after treatment with methanol (GAME) and chloroform (GACE) extracts of *Ganoderma applanatum*.

DISCUSSION

Though various types of glucose lowering or anti-hyperglycemic agents are commercially available along with the insulin for treating diabetes mellitus and various researches are undertaken for betterment of the efficacy of these drugs, still there is a great deal of increase in the demand for usage of natural products with anti-diabetic activity. This is mainly due to the unavailability of oral insulin and adverse side effect of the oral anti-diabetic drugs [50-51]. Many scientific findings stated that natural products are ideal anti-hyperglycemic agents with no adverse side effects and have good rejuvenating power. Herbal drugs are widely preferred and been prescribed even when their biologically active compounds are unknown [52]. Medicinal mushrooms are one such herbal drug with exemplary source of bioactive compounds. Hence, traditionally it has been consumed over centuries for its culinary and therapeutic value. Over the years various researchers endeavored to explore its anti-hyperglycemic property because these fungi not only reduce blood glucose, but also reduces cholesterol levels and have rejuvenating power which in turns help the organs like liver and pancreas to stimulate insulin and other hormones thereby promoting proper metabolic functioning [53-54]. However, the need for toxicological evaluation is essential as it will help to understand the effect of major bioactive molecules involved, range of doses to be used for the animal studies and probable clinical signs and symptoms evoked by the test compounds under investigation [55-56]. The first part of the present study focused to determine the acute and sub-acute toxicity studies of methanol and chloroform extract of *G.applanatum* (GAME and GACE) on Sprague Dawley rats. Animals showed no unfavorable signs and symptoms and no mortality throughout the experimental period which is similar to earlier reports where 2000mg/kg b.wt. dose of *G.boninense* methanol

extract showed no significant differences in body weight or behavioral aspects [57]. No significant hematological, morphological and serum level variation by GAME and GACE administered groups in sub-acute toxicity study were observed as compared to control animals. This observation is in congruence with earlier reports were 1000, 2500 and 5000mg/kg b.wt. of triterpenes isolated from *G.lucidum* showed no significant changes in hematological or serum parameters of swiss albino mice [58]. Hence, acute and sub-acute toxicity study revealed the non-toxic nature of the methanol and chloroform extract of *G.applanatum*. Oral glucose tolerance test (OGTT) is the earliest diagnosis for type–II diabetes mellitus which measures the body's ability to use glucose, the body's main source of energy. The present result suggested that OGTT analysis of GAME and GACE (500mg/kg b.wt.) extracts treated Group-4 and 5 maintained the glucose homeostasis by inhibiting the rise in plasma glucose levels compared to post versus pre-prandial plasma glucose levels which is nearly similar to normal control rats Group-1 and was comparable to that of positive control groups. This result is agreeable to the earlier studies where petroleum ether and methanol extracts administration of *G.lucidum* (180mg/kg b.wt.) significantly increased the glucose tolerance in Long Evans rats [59]. In hyperglycemic rats, GAME and GACE were significantly improvised the post prandial glucose homeostasis as compared to the diabetic control and the effects were dose-dependent. While, the suppression of blood glucose level post glucose administration at higher concentrations (500mg/kg b.wt.) by both extracts were comparable to that of positive controls which is in congruence with earlier studies were 75, 250 and 450mg/kg b.wt. of *G.lucidum* extract treated rats dose dependently increases the glucose utilization when compared to diabetic control mice [60]. OGTT is a clinical practice and research to identify how administered drug efficiently clears off exogenous glucose from the body. After glucose administration, uptake of glucose solely depends on insulin secretion and insulin sensitivity of the pancreatic β-cells. In our study, both extracts efficiently reduce the blood glucose level post administration of exogenous glucose dose dependently. This may be due to the presence of various secondary metabolites which helps them to restore the capacity of pancreatic β-cells by stimulating insulin secretion and glucose uptake by the peripheral tissues. Further detail studies at cellular and molecular level are required to confirm anti-diabetic potential of methanol and chloroform extract of *G.applanatum*.

4. CONCLUSION

The present study concludes non- toxic nature of methanol and chloroform extract of *G. applanatum* and also by the pharmacological point of view this mushroom can be used in the treatment for hyperglycemia.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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