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Investigation of anti-diabetic potential of *Jasad bhasma* against streptozotocin induced diabetes in rat

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Abstract

The present study was undertaken to prepare ayurvedic preparation *Jasad bhasma* and evaluate its effect on streptozotocin induced diabetic rats along with their associated diabetic complications. *Jasad bhasma* was prepared as per the standard reference. The hypoglycemic effect of the *Jasad bhasma* was evaluated in normal rats by oral glucose tolerance. *Jasad bhasma* (100 mg/kg), (200 mg/kg) and Glibenclamide (10 mg/kg) caused 25.03%, 30.47% and 48.71% reduction ($P < 0.001$) in serum glucose levels respectively at 6 hours after glucose load. Diabetic rats treated with *Jasad bhasma* (100 mg/kg), (200 mg/kg) and Glibenclamide showed a significant antihyperglycemic effect with a reduction of 46.95%, 47.08% and 49.96% serum glucose level respectively. Both doses were efficient in reducing serum triglycerides, cholesterol, low density lipoprotein and very low density lipoprotein to normal values and there was a marked rise in high density lipoprotein level as compared to diabetic control group. Hence it can be effectively employed for the control of diabetes and its associated complications.

Keywords: *Jasad bhasma*; Streptozotocin induced diabetic; antihyperglycemic; diabetic complications

Introduction

Diabetes mellitus (DM) is a major endocrine disorder affecting nearly 10% of the population all over the World. In spite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem. Insulin therapy affords effective glycemic control, yet its drawbacks such as ineffectiveness on oral administration, short shelf life, need for constant refrigeration and hypoglycemia on excess dosage limits its usage [1]. Since time immemorial, patients with non-insulin dependent diabetes mellitus have been treated orally by folklore with a variety of plant extracts [2]. In the indigenous Indian system of medicine (Ayurveda), a mention was made on good number of plants for the cure of diabetes or 'madhumeha' and some of them have been experimentally evaluated and the active principles were isolated [3].

Jasad bhasma is a metal based ayurvedic herbomineral preparation obtained by purification and calcinations of zinc with herbal extracts used for treating various ailments, some diabetic forms and in muscle building [4]. Various synthetic procedures for the preparation of the bhasma are quoted in ancient ayurvedic text. *Yashada* (zinc) is one of the *putin Lohas* (metals with low melting points e.g. lead, zinc). Its bhasma (clax) has as its main indication, *prameha* (Diabetes). As early as 1930's, zinc was known to be important for the integrity of the crystalline structure of insulin [5]. In the presence of zinc within the beta cell, insulin monomers assemble to a dimeric form for storage and secretion as the zinc crystal. Dimeric insulin assembles further into a hexamer that is relatively more stable form of insulin [6]. Zinc not only prevents the degradation of insulin hexamer but also improves the binding of insulin to its receptors and inhibits degradation by liver plasma membranes [7]. These reported mechanisms might be working together to improve insulin action. In the 14th century, *Rasaka satva* (the metallic extract of zinc carbonate/ zinc oxide) was independently known by the name "*Yashada*". since then, its bhasma has been in therapeutic use for various disorders including *Prameha* (Diabetes), *Pandu* (Anemia), *Vatavyadhis* (Neuro muscular diseases) and *Netra vikaras* (Eye diseases) [8].

Material and methods**Preparation of *Jasad bhasma***

Jasad bhasma was prepared according to procedure described in [9]. Following steps were adopted for preparation of *Jasad bhasma*. According to Ayurveda, *Shodhana* is

the process through which the external as well as internal impurities of the metals are removed. Raw Jasad and raw phosphorus were purified by liquefying method. In this method raw jasad and raw phosphorus were heated in iron pan and dipped in sesa (*Sesamum indicum*) oil. The sample was then processed with cow's urine; this process was continued seven times. After *Shodhana* metal became more brittle. The jasad powder then levigated with mercury, aloe vera juice and lemon juice to form semisolid preparation (*Bhavana*). The process was repeated seven times. The pellets were prepared after drying of triturated of sample then subjected to incineration (*Marana*). The pellets were kept in the casseroles (*Sharav*). The sealing was done and subjected to heating by traditional method. The traditional method of heating was carried out using Kukkuput (40-60 cow dungs). The final form of bhasma was yellowish colour. The prepared bhasma was then analyzed by Scanning electron microscopy and X-Ray diffraction analysis.

Surface morphology

Surface morphology of prepared *Jasad bhasma* was viewed by means of Scanning electron microscopy (Philips XL 30, Japan). Sample was loaded on aluminium stub with carbon adhesive tape and on this enough gold had applied for electron conductivity. Samples were scanned at a voltage 10kV and the images were taken.

Induction of diabetes in rats

Male Wistar rat weighing 250-300 gram was used as experimental animal. Rats were divided into five groups (n=6) for five different treatments as shown in table 1. Adult rats (75-100 days old) were used for induction of diabetes. The rats were injected by streptozotocin (STZ) at the dose of 60mg/kg of the body weight intravenously [10]. STZ induces diabetes by destroying beta cells of islets of Langerhans within 3 days. Animals were kept in cage individually under feeding; Water consumption and urine volume was measured in terms of (ml) on a daily basis. Serum insulin, glucose levels were measured for 10 days.

Estimation of Serum glucose, triglycerides, lipoproteins and insulin level

Level of Serum glucose, triglycerides, lipoproteins and insulin in all five groups of rats after 7 days, 10 days and 15 days treatment were determined.

Result and discussions

Surface morphology and Crystallinity

SEM analysis of Particle size of *Jasad bhasma* was found to be irregular shape with particle size of 20 μm (Figure 1).

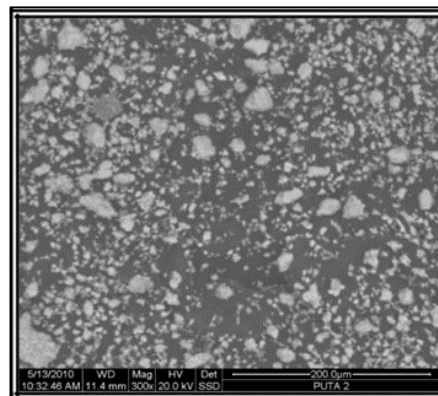


Fig 1: SEM image of Jasad bhasma

Effect of Jasad bhasma on serum glucose level in normoglycemic rats (oral glucose tolerance in normal rats)

Percent reduction SG in normal rats of all five groups is shown in table 1 and graphically in figure 2. Administration of glucose (2 gm/kg) produced significant change in SG level of normal rats. Treatment with lower dose of *Jasad bhasma* (100 mg/kg) and GLB (10 mg/kg) significantly ($P<0.01$; $P<0.001$) improve the glucose tolerance whereas, treatment with higher dose of *Jasad bhasma* (200 mg/kg) did not significantly reduced the SG compared to normal control group

Table 1. Effect of *Jasad bhasma* on SG levels in normoglycemic rats

Groups	0hr	2hr	4hr	6hr
Normal control	--	1.336 \pm 0.3097	0.3573 \pm 0.1557	1.001 \pm 0.2758
Diabetic control	--	0.7398 \pm 0.03524	1.123 \pm 0.1329	1.856 \pm 0.1345
Diabetic +GLB	--	7.766 \pm 0.2184 ^b	37.86 \pm 0.3671 ^b	48.71 \pm 0.5210 ^b
Diabetic +JB (100 mg/kg)	--	7.208 \pm 0.1015 ^a	13.53 \pm 0.3651 ^a	25.03 \pm 0.434 ^a
Diabetic +JB (200 mg/kg)	--	8.742 \pm 0.2783 ^c	22.99 \pm 0.2869 ^c	30.47 \pm 0.2987 ^c

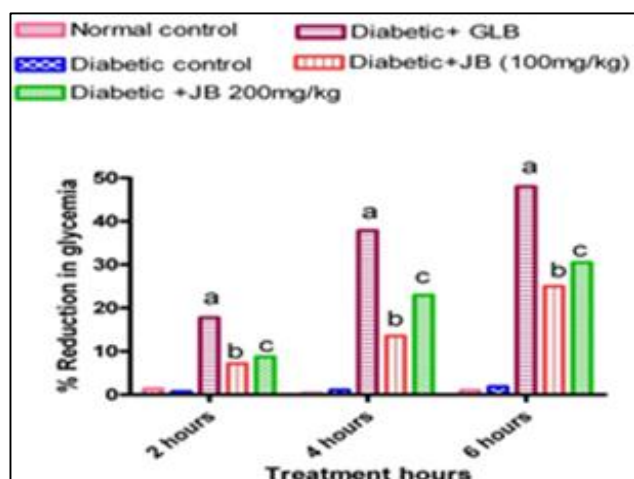


Fig 2: Percent reduction of SG levels in normoglycemic rats

Each value represents Mean \pm SEM, n=6, ^a $P<0.05$, ^b $P<0.001$, ^c $P<0.01$ as compared to diabetic control. One way Anova followed by Tukey's posttest.

Effect of Jasad bhasma on serum glucose level in STZ induced hyperglycemic rats

Repeated administration of *Jasad bhasma* (100 and 200 mg/kg) for 15 days, showed significantly ($P<0.05$; $P<0.01$) reduced levels of SG compared to respective basal values (0 day) as shown in Table 2 and figure 3. On 15th day Rats treated with *Jasad bhasma* 100mg/kg and 200mg/kg showed 46.95% and 47.08% reduction in SG level. Rats treated with GLB showed little higher percent reduction in SG as compare to that of rats in *Jasad bhasma* treated group. Whereas rats in diabetic control group showed negative percent reduction in SG level at the end of 15 days.

Table 2. Effect of *Jasad bhasma* on SG levels in streptozotocin induced diabetic rats

Groups	0hr	7 th day	10 th day	15 th day
Normal control	--	-3.019±0.3732	-3.485±0.7569	-1.849±1.202
Diabetic control	--	-3.371±0.1022	-8.854±0.2155	-12.74±0.1096
Diabetic+GLB	--	24.44±0.2279 ^b	37.28±0.1350 ^b	49.96±0.1631 ^b
Diabetic+JB (100 mg/kg)	--	22.29±0.2821 ^a	36.92±0.1650 ^a	46.95±0.6203 ^a
Diabetic+JB (200 mg/kg)	--	24.67±0.06371 ^c	39.84±0.1529 ^c	47.08±3627 ^c

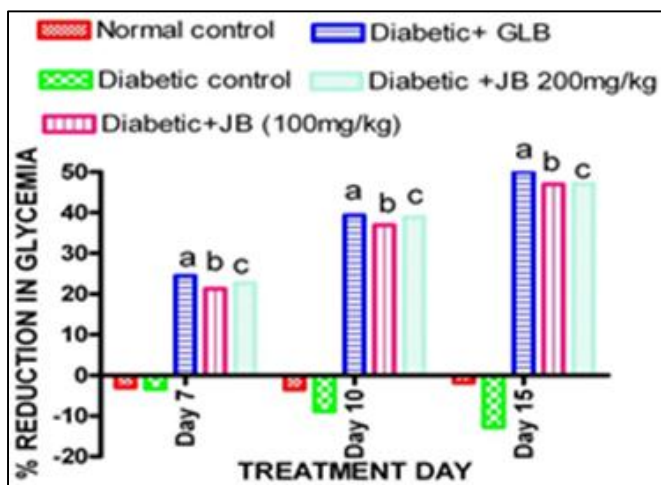


Fig 3: Percent reduction of SG levels in streptozotocin induced diabetic rats

Each value represents Mean ±SEM, n=6, ^aP<0.05, ^bP<0.001, ^cP<0.01 as compared to diabetic control. One way Anova followed by Tukey’s posttest.

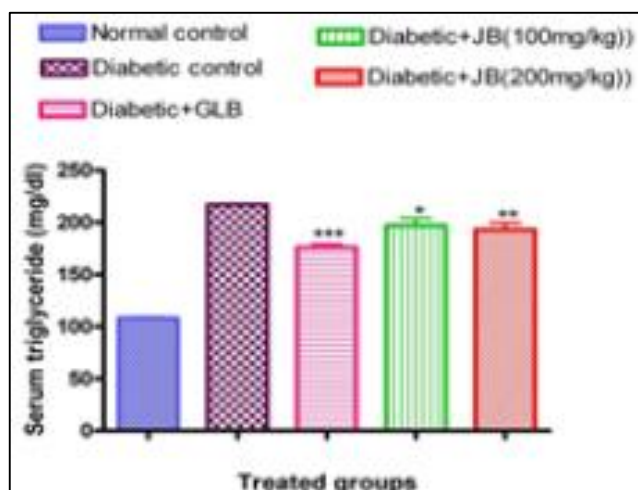
Estimation of Lipid parameter

Diabetes is often linked with abnormal lipid metabolism. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma [11]. Thus effect of *Jasad bhasma* treatment on plasma lipids was investigated. Lipid parameters such as STG, STC, VLDL-c, LDL-c, and HDL-c were estimated in all five groups of rats after 15 days treatment. Estimated parameters are shown in table 3. It was observed that Diabetic rats showed significantly (P<0.001) increased levels of STG, STC, VLDL-c and LDL-c levels, whereas HDL-c was decreased compared to normal rats. The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were significantly elevated in the diabetic group as shown in figure 4, 5 and 6.

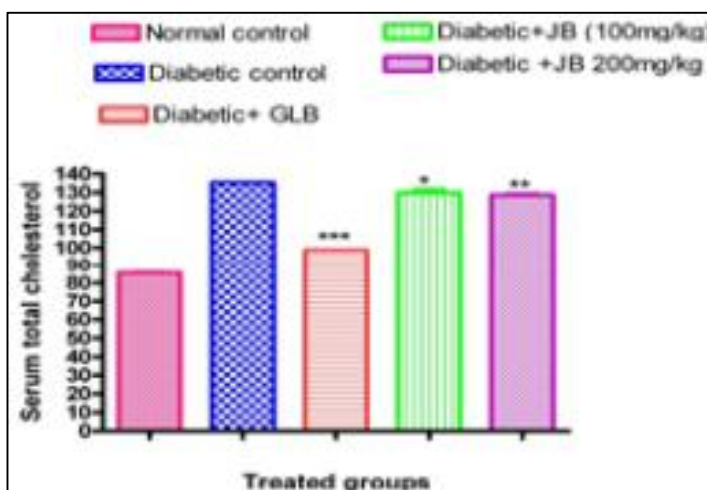
Table 3. Effect of *Jasad bhasma* on serum lipid content in STZ induced diabetic rats

Parameter	Normal control	Diabetic control	Diabetic +GLB	Diabetic +JB (100 mg/kg)	Diabetic +JB (200 mg/kg)
STG	108.2±1.142	217.3±0.7991	176.2±3.154***	197.1 ±6.837*	192.9 ±6.593**
STC	85.42±0.6091	135.3±0.3458	98.72±0.3652***	129.8±2.090*	128.7±0.9355**
HDL-c	36.71±0.1661	15.63±0.5421	31.21±0.4962***	19.63±1.114*	20.80±1.457**
VLDL-c	26.82±0.4274	48.57±0.479	33.77±0.4336***	46.07±0.4954*	45.02±0.8375**
LDL-c	35.06±0.43	76.21±0.6046	36.18±0.5073***	70.88±1.315*	69.71±1.744**
TC/HDL-c	2.331±0.023	8.706±0.2913	3.167±0.0522***	6.737±0.4494*	6.387±0.5853**
LDL-c/HDL-c	0.9547±0.01	4.902±0.1629	1.161±0.029***	3.675±0.2333*	3.452±0.3002**

Each value represents Mean ±SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 as compared to diabetic control. One way Anova followed by Tukey’s posttest.

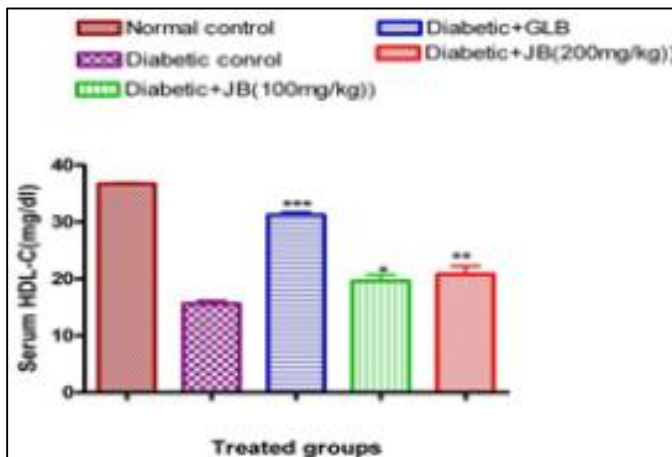


4(a)

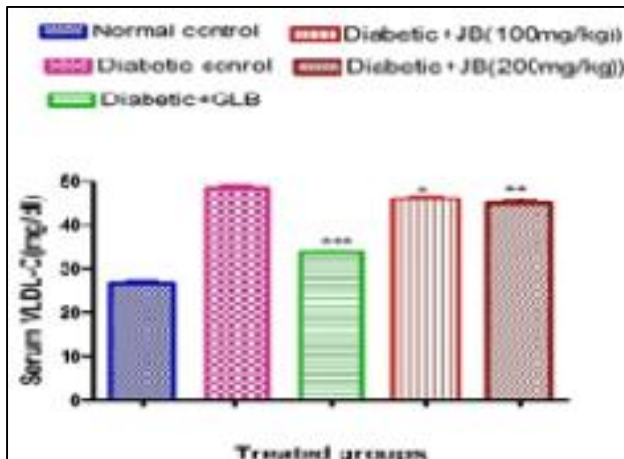


4(b)

Fig 4: Effect of *Jasad bhasma* on serum (a) triglyceride (b) total cholesterol level

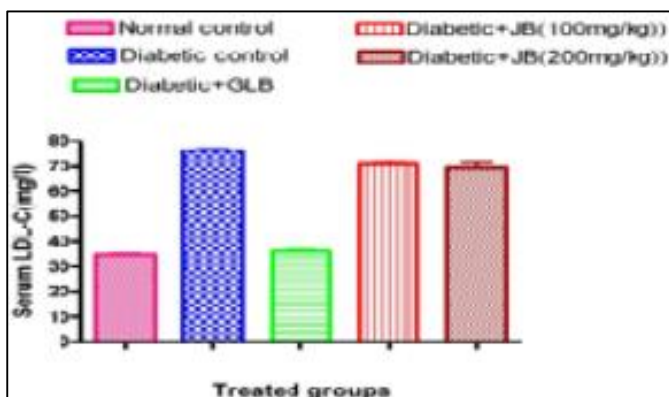


5(a)

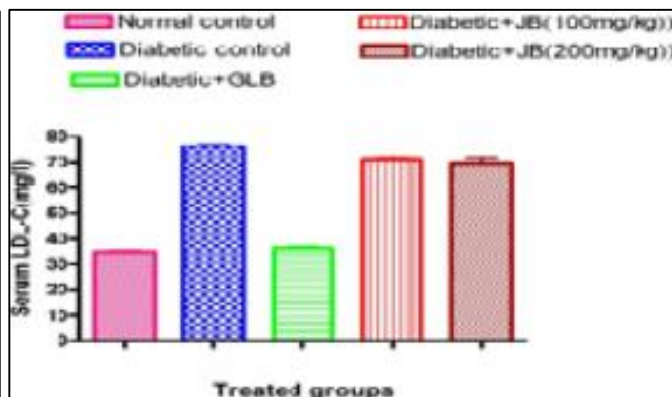


5(b)

Fig 5: Effect of *Jasad bhasma* on serum (a) HDL-C (b) VLDL-C level



6(a)



6(b)

Fig 6: Effect of *Jasad bhasma* on serum (a) LDL- (b) insulin level

Oral administration of different doses of *Jasad bhasma* for fifteen-days exhibited significant reduction ($P < 0.001$) in all tested lipid parameters and restoring them to near-normal values. These effects may be due to low activity of cholesterol biosynthesis enzymes or low levels of lipolysis.

Estimation of serum insulin level

Serum insulin of rats in all five groups was determined and shown in Figure 6(b). It was observed that serum insulin level of normal rats was 23.10 mg/dL. The marker of dyslipidemia such as serum insulin level was significantly reduced in the diabetic control group. Oral administration of different doses of *Jasad bhasma* for fifteen-days restored serum insulin level to near-normal value.

Investigation of diabetic complications

The streptozotocin injected rats developed not only diabetes as indicated by increased fasting blood glucose values, but also showed external signs of retinopathy by 10 days [12]. Figure 7 depicts the morphological changes in retinopathy. The eyes of diabetic rats looked opaque even from outside. Therefore the retina was observed as mentioned under methods with a special camera and the photographs showed clear signs of retinopathy as indicated by the presence of dilated vessels and laser spots (Figure 7). After treatment for 16 weeks, the eyes of the *Jasad bhasma* treated group of rats appeared normal from outside. Photograph of the retina indicated that the laser spots, disappeared (Figure 8), pointing out that the treatment reversed the changes in the eye, that is, abnormal changes of retinopathy almost disappeared. Thus

showed efficacy of *Jasad bhasma* to overcome diabetic complications.

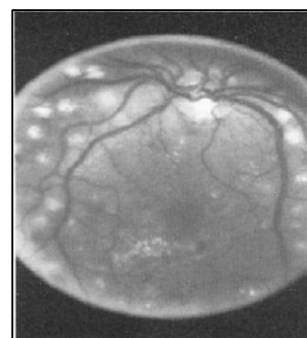


Fig 7: Photographs of retina of eye in Diabetic control rat (b)



Fig 8: Photographs of retina of eye in Diabetic control rat after *Jasad bhasma* treatment.

Glomerular damage in diabetic kidney was due to the increased production of Kallikrein and prostaglandin E2 which may be caused due to hyper filtration and vasodilatation in diabetes [13]. Thus effect of *Jasad bhasma* on nephropathy was evaluated.

Photomicrograph of 3 microns thick PAS stained paraffin section from the medulla of kidney of all five groups were taken. The gross examination of kidney showed no changes in the color on the external surface of the kidneys taken from animals in groups (1), and moderate changes in group 2,3and

4). Figure 9(a) represents section of kidney from group '1' (Normal control) showed no signs of pathology in the cortex and the medulla. In figure 9(b) sections of kidneys from group '2' (Streptozotocin treated) showed mild infiltration of the lymphocytes in the interstitial spaces. Some of the glomeruli appeared to be distorted and slightly expanded. There were signs of tubular necrosis with loss of their brush border in some of the sections of proximal convoluted tubules.

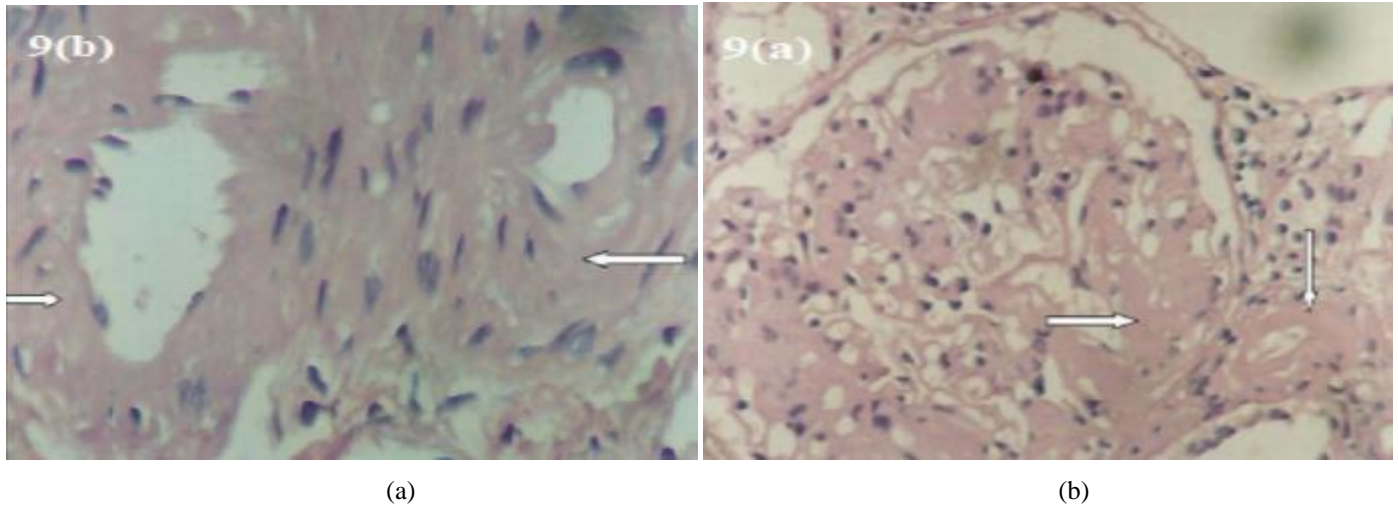


Fig 9: Photomicrograph of 3 microns thick H & E stained paraffin section from the cortex of kidney of rat in (a) group 1 (b) group 2

Glibenclamide treated rat (group-3) showed normal tubular atrophy and glycogen vacuolization of renal tubular epithelial cells. Some of the arterioles at the vascular poles also showed hyaline change. Glomerular membrane also appeared without affected by hyaline change as shown in figure 9(c). Figure 9(d) represents section of kidney from Group-4 (*Jasad*

bhasma treated rat) showed a glomerulus with diffused glomerulosclerosis, decreased mesengial matrix and thinning of capillary wall. Group-5 (*Jasad bhasma* treated rat) showed recovery of tubular basement membrane, lymphocytic infiltrate and tubular atrophy as represented in Figure 9 (e).

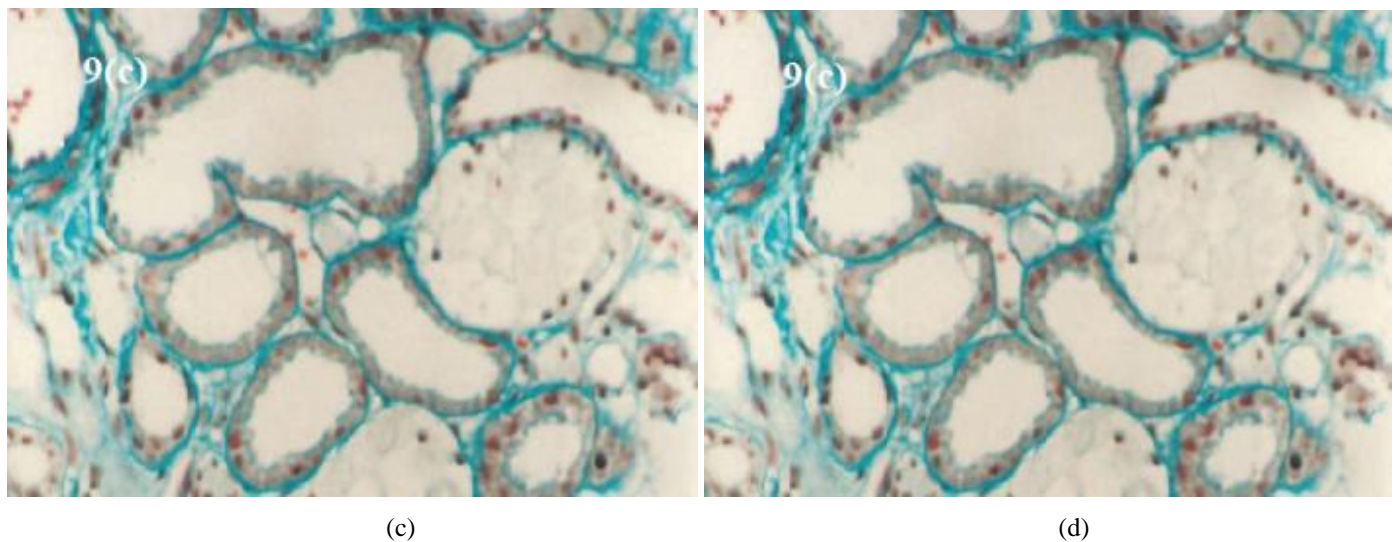
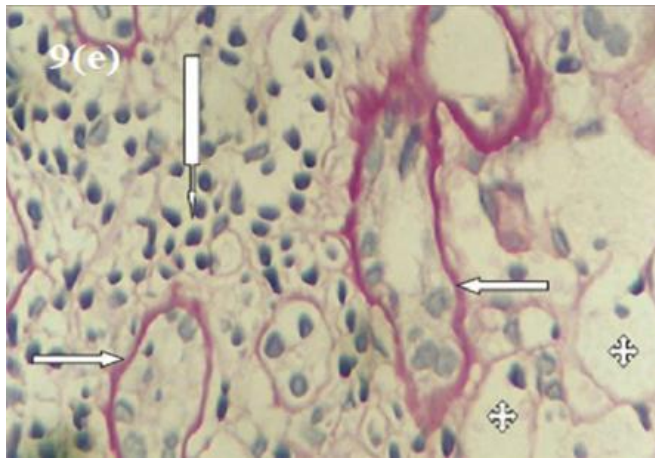


Fig 9: Photomicrograph of 3 microns thick H & E stained paraffin section from the cortex of kidney of rat in (c) group 3 (d) group 4



(e)

Fig 9: Photomicrograph of 3 microns thick H & E stained paraffin section from the cortex of kidney of rat in (e) group 5.

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