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Original Research Article (Experimental)

A study on toxicity and anti-hyperglycemic effects of *Abhrak Bhasma* in rats

Harish Gopinath, Murugesh Shivashankar[*]

Department of Chemistry, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India

ABSTRACT

Background: Abhrak bhasma (AB) is widely used in the treatment of diabetes mellitus (DM); however, no scientific study is reported till date on its efficacy and safety to prove it's pharmacological effects. Streptozotocin (STZ) is a gulcosamine–nitrosourea complex produced by *Streptomyces achromogenes*, which particularly induces DNA strand breakage in pancreatic β-cells causing DM. The damage caused by STZ to pancreatic β-cells is coupled with insulin release in the initial stage, subsequently leading to hyperglycemia owing to insulin deficiency.
Objective(s): In the present study, the safety and effectiveness of AB was evaluated by *in vivo* acute and sub-acute toxicity study and STZ-induced hyperglycemic activity in rats.
Materials and methods: Acute oral toxicity assessed as per OECD 425 guidelines by employing up-down procedure (UDP), sub-acute toxicity as per OECD-407 guidelines and anti-hyperglycemic activity using STZ method. The anti-hyperglycemic potential of AB in rats (40, 80, and 160 mg/kg body weight (b.w.)) was evaluated by determining the body weight, blood glucose, organ weight, lipid profile, and histo-morphological and histo-pathological investigations.
Results: The highest tolerated dose of AB was 2000 mg/kg b.w., and sub-acute toxicity of different AB doses showed no significant variation, when compared with the control. Interestingly, a noteworthy reduction in blood glucose, total cholesterol, and triglycerides levels were observed in AB-treated diabetic rats, along with a considerable increase in body weight, when compared with those noted in the disease control and normal control.
Conclusion: Effectiveness of AB as a potential safe and potent candidate for the treatment of diabetes was revealed as compared to the standard drug Metformin by STZ-induced method.

1. Introduction

Bhasma is a non-allopathic, natural substance used in Indian traditional medicine to produce economically inexpensive, safe medication having hepatoprotective and immunomodulatory effects for diseases like myocardial ischemia, and diabetes. However, its applicability is limited due to lack of scientific evidence. *Abhrak bhasma* (AB) is a red-colored powdery substance composed of oxides of iron, magnesium, calcium, silica, potassium, and aluminum. It is obtained by treating biotite (mica) with plant extract, which helps in converting the inactive material to active cellular

regenerator. AB mainly comprises of two types, namely, muscovite (hydrated aluminum potassium silicate [$KAl_2(AlSi_3O_{10})$ $(F,OH)_2$]) and phlogopite (potassium magnesium aluminum silicate hydroxide [$KMg_3AlSi_3O_{10}(F,OH)_2$]). Characterization studies based on scanning electron microscopy (SEM) and particle size analyzer have indicated the particle size of *bhasma* in nano range (200–1000 nm), which could be the result of incineration of mica at higher temperature. As a result of its particle size, *bhasma* exhibits enhanced bioavailability and penetration capacity [1–3]. Although the metallic elements present in *bhasma* could be useful for reducing oxidative stress induced by free radicals [6], an inappropriate dosing of *pseudo metals* might disrupt the metabolic processes in the human body. Besides, improperly processed mica might not be safe and may cause undesirable effects owing to the presence of toxic trace elements [4–7]. Hence, safety and toxicity studies on *bhasma* are necessary to develop appropriate standardization

* Corresponding author.
 E-mail: mshivashankar@vit.ac.in
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techniques for ascertaining effective dose, route and extent of exposure for treatment of acute or chronic illnesses [7–11]. In order to formulate an effective *bhasma,* favorable temperature and chemical exposure are necessary, including decontamination, detoxification, incineration and micronization performed according to *Rasashastra* [7,8].

Diabetes mellitus (DM) is a chronic disease caused by the lack of secretion of endocrine insulin from β-cells of pancreas, leading to elevated blood glucose level. Failure to maintain the blood glucose levels could lead to this life-threatening disorder [7], which has been predicted to affect around 350 million individuals by 2030 according to the World Health Organization [19]. Streptozotocin (STZ) is a gulcosamine−nitrosourea complex produced by *Streptomyces achromogenes,* which particularly induces DNA strand breakage in pancreatic β-cells causing DM. The damage caused by STZ to pancreatic β-cells is coupled with insulin release in the initial stage, subsequently leading to hyperglycemia owing to insulin deficiency [7]. *Bhasma* has the capability to induce insulin secretion from pancreatic β-cells, thereby reducing the blood glucose level by acting as a cellular regenerator [7]. Thus, in the present study focused on the *in vivo* acute and sub-acute toxicity study and STZ-induced anti-hyperglycemic activity of AB in rats.

2. Materials and methods

2.1. Chemicals

AB was procured from Delta Scientific, Vijayawada, AP, India. Honey (Dabur) and all other chemicals used in the study were of synthetic or analytical grade and obtained from Sisco Research Laboratories, India.

2.2. Test animals

5 healthy female Wistar rats (160−220 g) were obtained from and investigated at Central Animal Facility, SASTRA University, India. All animal experiments were approved by IAEC, SASTRA University (IAEC Approval No:352/SASTRA/IAEC/RPP), and performed at the Central Animal Facility according to the Control and Supervision of Experiments on Animals, Ministry of Forest and Environment, India. The rats were housed in ventilated cages and provided with standard rodent feed pellet (M/s. ATNT Laboratories, Mumbai, India) and reverse osmosis (RO) water *ad libitum*.

2.3. Preparation of test substance

The AB was dispersed in honey water at a ratio of 2:3 and administered at the dose of 2000 mg/kg body weight (b.w.) [14].

2.4. Acute oral toxicity assessment of AB by up-down procedure in rats

Acute oral toxicity of AB was assessed according to the OECD guidelines by employing up-down procedure (UDP) (OECD 425 guidelines) [15]. After acclimation for 5 days, 5 healthy female Wistar rats were treated with 2000 mg/kg b.w. AB and studied for 14 days [7,12]. Prior to AB treatment, the animals were abstained from food overnight. After AB treatment, the animals were returned to their cages immediately and feed was made available *ad libitum*. The body weights of the rats were recorded before the start of the experiment [33]. Administration of AB (2000 mg/kg b.w.) was achieved through oral gavage using appropriately sized syringe and stainless steel ball-tipped intubation needle [30]. After 14 days of exposure to the test substance, prominent parameters, such as percentage mortality, body weight, feed intake, adverse signs, and gross pathology, were evaluated [15,31].

2.5. Sub-acute toxicity assessment of AB in rats

The sub-acute toxicity of AB was examined on 6 to 8 week-old Wistar rats according to the OECD-407 guidelines [17]. A total of 60 rats were divided into 8 groups comprising 5 rats blinded for each sex. The first group was treated with honey water and served as the vehicle control, while the other groups were treated with three different doses of AB (80, 320 and 1280 mg/kg b.w., respectively) based on our acute toxicity assay. The satellite group was administered with both low dose and high doses of AB to check the reversibility of AB toxicity [25]. The test substance and vehicle were administered to respective groups for 28 days [24].

The body weight of the rats was measured weekly during dosing and on the day of sacrifice using digital weighing balance (Sartorius AG, Germany). The manifestations of AB toxicity such as change in weekly body weight and cage-side clinical observations were monitored. After 28 days, all the surviving animals were allowed to fast overnight, and their blood was collected through retro-orbital plexus under light anesthesia. The blood collected in EDTA-treated vials was used for hematological analysis and the serum from non-heparinized vials was collected to study the biochemical parameters [16]. The experimental rats were euthanized after blood collection and organs such as brain, heart, liver, spleen, thymus, kidneys, adrenals, testis, epididymis, and uterus were carefully dissected out, weighed, and observed for the presence of any gross lesions. The relative organ weight was determined by using the following equation:

$$\text{Relative organ weight} = (\text{Actual organ weight (g)/Rats body weight on the day of sacrifice (g)}) \times 100$$

The internal organs were first fixed in 10% buffered formalin solution and the tissues were fixed in paraffin, sectioned to a thickness of 5 μm and stained with dyes such as hematoxylin and eosin. The stained tissue sections were examined under a light microscope and clear photomicrographs were obtained.

2.6. Hematological parameters assay

The complete blood parameters were analyzed using hematology analyzer (Oxford science, USA). In addition, clotting time was ascertained manually using capillary tube method [20–23].

2.7. Biochemical parameters analysis

The biochemical parameters such as albumin, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total cholesterol, creatinine, glucose, total protein, aspartate aminotransferase (AST), alanine transaminase (ALT), triglycerides (TGL), urea, and uric acid were evaluated using a semi-auto analyzer (ErbaChem5) [20,21].

2.8. AB treatment and anti-hyperglycemic investigation using STZ method

2.8.1. Inducing diabetes in rats using STZ

Prior to STZ administration, the rats were allowed to fast overnight. Subsequently, 65 mg/kg STZ (Batch No:14,653) (STZ dissolved in 0.1 M sodium citrate buffer at pH 4.5 and administered within 30 min of preparation) was injected intraperitoneally through vein of the rats. The control rats received the vehicle alone, whereas the diabetic groups received STZ 65 mg/dL. After 48 h of
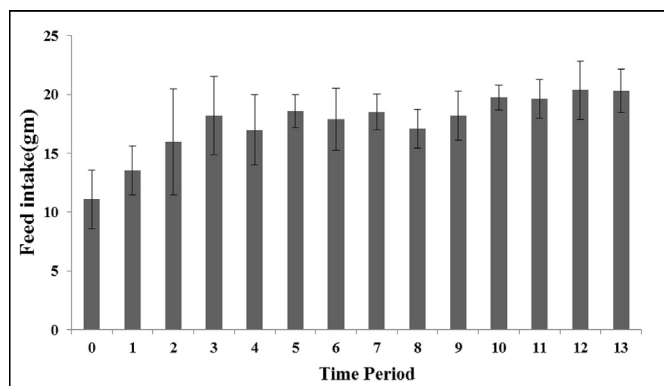
Fig. 1. Daily feed intake (g) during acute toxicity studies.

intraperitoneal STZ administration, the blood glucose level was measured using a glucometer (Aspen AP Plus) [18,29].

2.8.2. Experimental design

A total of 48 Wistar rats were divided into 6 groups comprising 4 rats blinded for each sex. Group I (control) was administered with 0.9% sodium chloride; Group II (disease control) was treated with STZ (65 mg/kg b.w.); Group III (STZ-treated + standard diabetic) was administered with STZ and oral Metformin (100 mg/kg, b.w.); Group IV (STZ-treated + low dose AB) was treated with STZ and administered with low dose of AB (40 mg/kg b.w.); Group V (STZ-treated + medium dose AB) was treated with STZ and administered with medium dose of AB (80 mg/kg b.w); and Group VI (STZ-treated + high dose AB) was treated with STZ and administered with high dose of AB (160 mg/kg b.w.).

The blood glucose level in all the rats was determined every week with a glucometer (Aspen AP Plus) using strip method by collecting blood from the tip of the tail after cutting under general anesthesia. After 28 days, blood sample was obtained from retro-orbital sinus under light anesthesia and the serum total cholesterol and TGL [20] levels were estimated using semi-auto analyzer (Erba Chem 5) [7,13,29].

2.8.3. Statistical analysis

The results are presented as Mean ± SEM. Statistical analysis was conducted by using GraphPad Prism software [32], Version 6

[27] and one-way ANOVA followed by Tukey's multiple comparisons test was performed. The values were considered as statistically significant at $P \leq 0.05$.

2.8.4. Histopathological investigation

Liver tissue from the experimental rats was dissected out and fixed in 10% neutral buffered formalin and processed for blind histopathological evaluations [26]. Similarly, a part of the pancreatic tissue was dissected out and fixed in 10% neutral buffered formalin and processed as previously reported [20,28].

3. Results and discussion

3.1. Investigation of acute toxicity of AB

The results of acute oral toxicity investigation revealed no considerable changes in the mortality of female rats treated orally with 2000 mg/kg b.w. AB, when compared with those in other treatment groups. Throughout the observation period of 2 weeks, the animals were evaluated for their body weight, feed intake (Fig. 1), and vital toxicity signs (Table 1) which were all found to be generally normal and the gross pathology was typical. Although animals treated with AB showed significant changes in body weight gain on days 7 (191.56 ± 5.91) and 14 (195.99 ± 4.47) when compared with that on day 0 (179.88 ± 3.86), the daily feed intake of rats remained unaffected throughout the experimental period. No explicit signs of neuronal toxicity, such as changes in autonomic nervous system, central nervous system, and behavioral pattern, were observed during the entire observation period. All gross observations were agonal and presented no relation to AB treatment and all animals survived until the end of the study.

3.2. Investigation of sub-acute toxicity of AB

3.2.1. Effect of AB on body weight

The body weight of all the rats was determined on days 0, 7, 14, 21 and 28 as shown in and Table 3. The body weight of the control group at the end of day 28 was 300.2 ± 22.54 g for male rats (M) and 204.86 ± 12.58 g for female rats (F). However, groups treated with 80, 320 and 1280 mg/kg b.w. AB showed body weight of 301 ± 33.29 g (M) and 192.47 ± 10.4 g (F), 298.16 ± 17.1 g (M) and 189.99 ± 9.77 g (F), and 288.85 ± 30.81 g (M) and 195.51 ± 8.1 g (F),

Table 1
Histo-morphological observations in STZ induced hyperglycemia.

Group	Pancreas	Liver	Kidney
Control	Within normal limits	Granuloma, multifocal, random, moderate, Necrosis, hepatocellular, multifocal, random, minimal, Infiltrates, mononuclear, periportal, diffuse, minimal	Infiltrates, interstitial, mononuclear, multifocal, minimal
STZ induced[a]	Islet cell atrophy/degeneration, diffuse, marked	Granuloma, multifocal, random, moderate, Necrosis, hepatocellular, multifocal, random, minimal, Infiltrates, mononuclear, periportal, diffuse, minimal Necrosis, hepatocellular, focal, marked	Infiltrates, interstitial/perivascular, mononuclear, multifocal, mild
STZ + STD**	Islet cell atrophy/degeneration, diffuse, moderate	Granuloma, multifocal, random, minimal, Necrosis, hepatocellular, multifocal, random, minimal, Infiltrates, mononuclear, periportal, diffuse, minimal	Vacuolar degeneration, tubular, cortical, bilateral, multifocal, mild
STZ + 40 mg/kg.B.w	Islet cell atrophy/degeneration, diffuse, mild	Granuloma, multifocal, random, minimal, Necrosis, hepatocellular, multifocal, random, mild Infiltrates, mononuclear, periportal, diffuse, mild	Vacuolar degeneration, tubular, cortical, bilateral, multifocal, minimal
STZ + 80 mg/kg.B.w	Islet cell atrophy/degeneration, diffuse, moderate	Granuloma, multifocal, random, minimal, Necrosis, hepatocellular, multifocal, random, minimal Infiltrates, mononuclear, periportal, diffuse, minimal	Vacuolar degeneration, tubular, cortical, bilateral, multifocal, mild
STZ + 160 mg/kg.B.w	Islet cell atrophy/degeneration, diffuse, moderate	Granuloma, multifocal, random, minimal, Necrosis, hepatocellular, multifocal, random, minimal Infiltrates, mononuclear, periportal, diffuse, minimal	Vacuolar degeneration, tubular, cortical, bilateral, multifocal, moderate Pigment accumulation, interstitial, multifocal, minimal

[a] 65 mg/kg 35 + 30 48 h gap ip hyperglycemia checked by glucose, ** Metformin 100 mg/kg 28 days oral, Histo-morphological observation of Rats pancreas exposed to STZ, compared with that of the standard control and bhasma treated.

Table 2
Relative organ weight of Male rats and Female rats in sub-acute toxicity study.

Parameter	Control group (Honey)	Dose		
		80 mg/kg	320 mg/kg	1280 mg/kg
Sex: Male rats				
Brain	1.93 ± 0.08	1.91 ± 0.12	1.84 ± 0.06	1.84 ± 0.04
Heart	1.14 ± 0.05	1.09 ± 0.08	1.02 ± 0.07	1.07 ± 0.11
Liver	11.14 ± 0.83	10.42 ± 1.42	10.31 ± 0.96	10.01 ± 1.47
Spleen	1.60 ± 0.29	1.70 ± 0.18	1.69 ± 0.31	1.68 ± 0.45
Thymus	0.36 ± 0.04	0.34 ± 0.10	0.39 ± 0.08	0.32 ± 0.05
Kidney (R)	1.25 ± 0.08	1.22 ± 0.18	1.17 ± 0.10	1.21 ± 0.20
Kidney (L)*	1.28 ± 0.11	1.19 ± 0.14	1.14 ± 0.07	1.22 ± 0.22
Adrenals	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01
Testis (R)*	1.46 ± 0.17	1.32 ± 0.41	1.43 ± 0.13	1.46 ± 0.10
Testis (L)	1.44 ± 0.24	1.51 ± 0.07	1.43 ± 0.15	1.47 ± 0.10
Epididymis	1.07 ± 0.07	1.03 ± 0.28	1.11 ± 0.10	1.09 ± 0.10
Sex: Female rats				
Brain*	1.69 ± 0.07	1.70 ± 0.07	1.78 ± 0.12	1.70 ± 0.05
Heart*	0.89 ± 0.19	0.83 ± 0.10	0.75 ± 0.06	0.77 ± 0.09
Liver*	7.43 ± 0.79	6.66 ± 0.43	6.68 ± 0.12	7.09 ± 0.57
Spleen*	1.16 ± 0.06	1.07 ± 0.11	1.00 ± 0.15	1.25 ± 0.19
Thymus*	0.35 ± 0.13	0.25 ± 0.08	0.30 ± 0.05	0.30 ± 0.03
Kidney (R)	0.79 ± 0.06	0.76 ± 0.04	0.77 ± 0.08	0.82 ± 0.07
Kidney (L)	0.81 ± 0.08	0.77 ± 0.08	0.77 ± 0.07	0.78 ± 0.04
Adrenals	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Gonads	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.02
Uterus	0.47 ± 0.15	0.39 ± 0.10	0.42 ± 0.17	0.41 ± 0.05

The above data was expressed as mean ± SEM, with normality $n = 10$, Organ weight unit: grams. *Significant change was observed.

respectively. The body weight of female rats in all AB treatment groups remained lower, when compared with control and satellite group but did not present any significant differences. Overall, AB did not produce any effective changes in the body weight of the experimental animals.

3.2.2. Effect of AB on relative weight of vital organs

The relative weight of the vital organs of the experimental animals, including brain, heart, liver, spleen, thymus, kidneys (right and left), adrenals, testis (right and left) and epididymis, following treatment with 80, 320 and 1280 mg/kg b.w. AB for 28 days was determined. In line with the total body weight, the average relative organ weight of the animals (both male and female rats) also showed no statistically significant variations, when compared with that noted in the control (Table 2).

3.2.3. Effect of AB on hematological parameters

Treatment with AB for 28 days produced no significant effect on white blood cells (WBC) population (monocyte, eosinophil, basophil, and hematocrit), red blood cells (RBC) (hemoglobin, MCV, MCH, MCHC, RDW%, and RSD) and platelet cells (MPV and PDW%), when compared with those noted in the control. Thus, the hematological parameters were normal without any abnormalities following AB treatment compared with control and satellite group (Tables 4 and 5).

3.2.4. Effect of AB on biochemical parameters

The biochemical parameters, including albumin, ALP, total bilirubin, direct bilirubin, total cholesterol, creatinine, glucose, total protein, AST, ALT, TGL, urea and uric acid levels, exhibited no clinical changes after 28 days of AB treatment, when compared with those noted in the control. The lack of significant increase in the levels of AST, ALT and ALP after AB treatment indicated that the test substance had no effect on the functioning of liver. The values obtained for the biomarkers of liver injuries such as AST and ALT were comparable between the control and treatment groups, implying no important functional abnormalities after AB treatment. Similarly, the levels of urea, uric acid and blood glucose in both male and female rats presented no significant changes. Billirubin is normally excreted in urine and bile, and the total bilirubin and direct

Table 3
Mean Body Weight (SD) body weight changes in rats.

Group ID	I	II	III	IV
Group description	Sex: Male rats			
	Honey	80 mg/kg	320 mg/kg	1280 mg/kg
Day 0	211.45 ± 16.06	232.99 ± 24.32	228.88 ± 18.28	216.62 ± 16.29
Day 7	265.99 ± 22.92	273.33 ± 21.02	268.85 ± 22.1	265.23 ± 22.25
Day 14	285.66 ± 23.57	290.08 ± 28.22	289.71 ± 21.22	276.42 ± 29.71
Day 21	302.26 ± 25.85	303.57 ± 32.75	301.52 ± 18.98	289.45 ± 28.08
Day 28	300.2 ± 22.54	301.03 ± 33.29	298.16 ± 17.1	288.85 ± 30.81
Group description	Sex: Female rats			
Day 0	176.77 ± 8.88	165.09 ± 7.8	164.77 ± 6.3	170.23 ± 6.09
Day 7	197.9 ± 15.7	184.28 ± 9.52	183.66 ± 11.51	186.65 ± 6.86
Day 14	205.76 ± 13.1	187.87 ± 10.7	187.17 ± 8.97	192.46 ± 6.36
Day 21	207.27 ± 15.11	198.61 ± 13.17	190.83 ± 8.3	196.06 ± 7.28
Day 28	204.86 ± 12.58	192.47 ± 10.34	189.99 ± 9.77	195.51 ± 8.1

Data are expressed as mean ± SEM, $n = 10$.

Table 4
Haematological parameter of male rats showing normal values after treatment with *Abhrak bhasma* (n = 10, Mean ± SD).

S.No	Hematological parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
		Normal (Honey)	Dose			Std.Normal	Std. high
			80 mg/kg	320 mg/kg	1280 mg/kg		
	Hemoglobin (g %)	14.5 ± 1.1	14.6 ± 2.4	14.9 ± 0.4	14.6 ± 1.7	13.6 ± 0.0	13.9 ± 0.85
	HCT %	53.7 ± 4.2	52.1 ± 8.3	52.9 ± 3.2	53.3 ± 7.1	49.6 ± 1.2	50.7 ± 3.0
	RBC (10^{12}/L)	9.4 ± 0.5	8.8 ± 1.3	9.4 ± 0.5	9.2 ± 1.2	8.6 ± 0.4	8.9 ± 0.7
	Red blood cell distribution (RDW %)	13.5 ± 0.9	14.3 ± 0.7	13.9 ± 0.8	13.4 ± 0.4	12.8 ± 0.8	13.6 ± 0.6
	RSD fL	7.7 ± 0.3	8.5 ± 0.5	7.8 ± 0.4	7.7 ± 0.2	7.4 ± 0.4	7.8 ± 0.4
	PLT (10^9/L)	786	740.5	627	655	546	764
	PCT %	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.0
	WBC (10^9/L)	14.8 ± 0.06	12.6 ± 0.08	14.2 ± 0.61	15.4 ± 2.11	16.3 ± 5.37	10.9 ± 3.3
	Platelet count MPV(fL)	7.6 ± 0.1	7.2 ± 0.3	7.7 ± 0.1	7.8 ± 0.3	7.6 ± 0.2	7.6 ± 0.3
	PDW %	33.0 ± 0.2	33.1 ± 0.1	33.9 ± 0.1	33.9 ± 0.2	33.4 ± 0.2	33.0 ± 0.8
Differential Leucocytes count							
	Neutrophil %	6.0 ± 2.48	15.1 ± 1.21	9.1 ± 1.82	9.0 ± 6.17	8.2 ± 5.21	14.6 ± 2.86
	Lymphocyte %	87.83 ± 2.1	81.92 ± 2.0	87.38 ± 2.5	86.3 ± 4.0	87.33 ± 3.7	82.15 ± 1.9
	Eosinophil %	0.04 ± 0.05	0.03 ± 0.01	0.20 ± 0.22	0.07 ± 0.1	0.29 ± 0.39	0.01 ± 0.01
	Basophil %	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	Monocyte %	6.12 ± 0.42	2.97 ± 0.77	3.35 ± 0.91	4.57 ± 2.20	14.63 ± 12.16	3.23 ± 1.99
Blood Indices							
	MCV (μm^3)	57.3 ± 1.7	59.0 ± 0.6	56.2 ± 0.4	57.6 ± 0.2	57.5 ± 1.0	57.3 ± 1.1
	MCH (pg)	15.5 ± 0.4	16.5 ± 0.3	15.9 ± 0.4	15.8 ± 0.3	15.8 ± 0.6	15.7 ± 0.3
	MCHC (g/dL)	27.0 ± 0.0	28.0 ± 0.1	28.3 ± 0.9	27.5 ± 0.5	27.5 ± 0.6	27.5 ± 0.1

The above data was expressed as mean ± SEM, with normality $n = 10$.

bilirubin levels were within the normal range of <1.23 and 0.1—0.3 mg/dL, respectively, in the AB-treated and control groups. The total protein concentration was <6—8 g/dL and the albumin concentration was low (5.5 ± 0.5 g/dL) in both AB-treated and control groups, satellite group suggesting normal functioning of liver in both male and female rats after AB treatment (Tables 6 and 7).

3.2.5. Histopathological analysis of sub-acute toxicity of AB in rat liver

No obvious histopathological changes were noted between rats treated with 80, 320 and 1280 mg/kg b.w. AB and the control animals. The liver sections from animals treated with high dose of AB (1280 mg/kg b.w.) showed completely normal phenotypic features of hepatocytes. Furthermore, although increase in the number of Kupffer cells was observed owing to local proliferation, no evidence of inflammatory lesions was detected [21,22]. These findings indicated that normal liver architecture was maintained without any obvious pathological changes following AB treatment (Fig. 2).

3.2.6. Antihyperglycemic effect of AB in STZ-induced hyperglycemic rats

The effect of AB on the blood glucose level of the experimental animals is shown in Table 8. The fasting blood glucose level in the normal control and STZ-induced untreated disease control did not change during the experimental period of 4 weeks. In contrast, in the AB-treated groups, the glucose levels were 182.13, 220.63 and 267.25 mg/dL following 40, 80, 160 mg/kg b.w. AB treatment, respectively, which were significantly lower than that noted in

Table 5
Haematological parameter of female rats showing normal values after treatment with *Abhrak bhasma* (n = 10, Mean ± SD).

S.No	Hematological parameter studied	Group I	Group II	Group III	Group IV	Group V	Group VI
		Normal (Honey)	Dose			Std. Normal	Std. High
			80 mg/kg	320 mg/kg	1280 mg/kg		
	Hemoglobin (g %)	15.9 ± 0.8	15.4 ± 1.2	13.2 ± 1.0	14.2 ± 1.3	13.8 ± 2.1	12.0 ± 0.3
	HCT %	58.3 ± 6.0	54.3 ± 4.5	48.4 ± 3.8	51.9 ± 5.6	50.8 ± 7.8	44.5 ± 1.6
	RBC (10^{12}/L)	9.8 ± 0.7	9.1 ± 0.4	8.1 ± 0.3	8.9 ± 0.8	8.7 ± 1.8	7.9 ± 0.6
	Red blood cell distribution (RDW %)	12.1 ± 0.7	11.8 ± 0.2	12.4 ± 0.3	12.4 ± 1.0	12.2 ± 0.8	11.9 ± 0.0
	RSD fL	7.2 ± 0.2	7.0 ± 0.4	7.4 ± 0.1	7.2 ± 0.4	7.1 ± 0.8	6.7 ± 0.3
	PLT (10^9/L)	772	611	790	758	688	1118
	PCT %	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.8 ± 0.3
	WBC (10^9/L)	13.9 ± 3.8	11.3 ± 5.5	10.8 ± 2.6	11.5 ± 0.3	15.6 ± 5.0	14.3 ± 1.5
	Platelet count MPV(fL)	7.3 ± 0.2	7.7 ± 0.5	7.6 ± 0.5	7.5 ± 0.1	7.5 ± 0.1	6.9 ± 0.7
	PDW %	33.5 ± 0.1	34.1 ± 0.6	33.4 ± 0.6	34.0 ± 1.1	33.2 ± 0.6	32.6 ± 0.9
Differential Leucocytes count							
	Neutrophil %	14.9 ± 1.5	7.5 ± 1.6	6.9 ± 3.3	12.1 ± 4.5	10.5 ± 0.9	17.4 ± 7.1
	Lymphocyte %	83.0 ± 1.5	85.9 ± 0.8	86.7 ± 1.2	85.2 ± 5.2	84.9 ± 2.7	79.6 ± 9.3
	Eosinophil %	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Basophil %	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	Monocyte %	2.1 ± 0.0	6.5 ± ± 0.8	6.3 ± 2.2	2.7 ± 0.7	4.6 ± 3.6	2.9 ± 2.2
Blood Indices							
	MCV (μm^3)	59.2 ± 2.1	59.7 ± 2.3	60.0 ± 2.3	58.2 ± 1.1	58.5 ± 3.2	56.5 ± 2.6
	MCH (pg)	16.2 ± 0.2	16.9 ± 0.6	16.4 ± 0.6	15.9 ± 0.0	15.9 ± 0.8	15.2 ± 0.8
	MCHC (g/dL)	27.4 ± 1.3	28.3 ± 0.1	27.3 ± 0.1	27.5 ± 0.5	27.2 ± 0.0	27.0 ± 0.3

Data are expressed as mean ± SEM, $n = 10$.

Table 6
Biochemistry studies of male rats in sub-acute toxicity studies.

	Albumin (g/dL)	ALP (IU/L)	T Bilirubin (mg/dL)	D Bilirubin (mg/dL)	T. Cholesterol (mmol/L)	Creatinine (mg/dL)	Glucose (mmol/L)	T Protein (g/dL)	AST (IU/L)	ALT (IU/L)	TGL (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Honey	3.26 ± 0.12	245 ± 12.35	0.32 ± 0.02	0.14 ± 0.05	88.60 ± 7.44	0.48 ± 0.04	103.00 ± 8.31	5.74 ± 0.33	161.80 ± 15.07	76.00 ± 7.87	41.20 ± 13.95	40.08 ± 4.64	1.41 ± 0.32
80 mg/kg	3.26 ± 0.12	245 ± 12.35	0.32 ± 0.02	0.14 ± 0.05	88.60 ± 7.44	0.48 ± 0.04	103.00 ± 8.31	5.74 ± 0.33	161.80 ± 15.07	76.00 ± 7.87	41.20 ± 13.95	40.08 ± 4.64	1.41 ± 0.32
320 mg/kg	3.59 ± 0.16	242.80 ± 17.99	0.26 ± 0.05	0.13 ± 0.04	80.20 ± 9.78	0.47 ± 0.04	100.20 ± 9.01	5.48 ± 0.25	147.80 ± 11.30	73.80 ± 9.68	43.20 ± 3.27	41.38 ± 4.26	1.23 ± 0.48
1280 mg/kg	3.60 ± 0.23	268 ± 15.23	0.35 ± 0.08	0.14 ± 0.04	86.80 ± 7.60	0.46 ± 0.04	103.20 ± 11.99	5.42 ± 0.32	140.20 ± 9.88	81.20 ± 8.07	44.00 ± 16.73	42.94 ± 2.92	1.19 ± 0.24
Satellite-Honey	3.31 ± 0.15	240 ± 10.34	0.31 ± 0.04	0.16 ± 0.03	87.60 ± 7.23	0.46 ± 0.05	101.80 ± 8.98	5.69 ± 0.37	156.60 ± 12.78	75.80 ± 5.54	42.20 ± 14.13	40.20 ± 5.37	1.40 ± 0.24
Satellite-1280 mg/kg	3.51 ± 0.27	270 ± 12.75	0.34 ± 0.06	0.14 ± 0.04	85.20 ± 6.83	0.47 ± 0.04	100.40 ± 15.21	5.35 ± 0.11	145.20 ± 11.19	78.40 ± 7.02	44.40 ± 16.59	42.70 ± 3.18	1.19 ± 0.25

The above data was expressed as mean ± SEM, with normality $n = 10$.

disease control (318.75 mg/dL) and similar to that observed in Metformin standard group (175 mg/dL). Thus, even low dosage of AB was effective in reducing the blood glucose level, which was comparable to that of Metformin standard group as compared to control group and satellite group [20].

3.2.7. Effect of AB on weekly body weight of STZ-induced hyperglycemic rats

The changes in the body weight of animals in various groups were compared at the end of the study on day 28 (Table 8). The normal control animals presented an increase in the body weight, whereas the disease control animals showed significant decrease in the body weight, which confirmed hyperglycemia and glucosuria. Treatment with 40, 80 and 160 mg/kg b.w. AB and Metformin standard (100 mg/kg b.w.) substantially prevented decrease in body weight, when compared with that observed in disease and normal controls. While the body weight of all the hyperglycemic rats was similar at the start of the experiment, AB treatment significantly increased the body weight, when compared with that observed in the untreated group.

3.2.8. Effect of AB on relative weight of vital organs of STZ-induced hyperglycemic rats

The relative weights of vital organs, including liver and kidneys (right and left) of STZ-induced diabetic rats after 28 days of AB treatment (40, 80 and 160 mg/kg b.w. AB) were determined. Following 28 days of AB treatment, the kidney weight of diabetic rats was found to be slightly higher, when compared with that of normo-glycemic rats, similar to that reported in previous studies [29]. In contrast, as slight decrease in the liver weight of treated rats was noted, when compared with that of control rats. Nevertheless, no significant changes in the weight of vital organs were observed in the AB-treated groups, when compared with those in the control (Table 8).

3.2.9. Effect of AB on serum lipid profile of STZ-induced hyperglycemic rats

The effect of AB on the serum lipid profile, including cholesterol and TGL levels, was assayed (Table 8). The serum cholesterol and TG levels were found to be elevated in the disease group, when compared with those in the control. In the AB-treated group, considerable reduction in the serum cholesterol and TG levels was noted after 28 days of treatment. Lipid dysfunction is an associated complication of diabetes in rats and the findings of this study revealed that AB could reduce the total cholesterol and TGL levels and was effective in treating hyperglycemia.

3.2.10. Histopathological effects of AB on STZ-induced hyperglycemic rats

Histopathological observation of endocrine pancreas, liver and kidneys of AB-treated rats showed no significant morphological changes, when compared with those observed in the normal control and disease control. The tissue morphology of endocrine pancreas of both disease control and AB-treated groups was similar, presenting diffused and moderate islet cell atrophy/degeneration, whereas that of liver of control and AB-treated groups exhibited granuloma; multifocal, random, minimal necrosis; hepatocellular, multifocal, random, minimal infiltrates and mononuclear, periportal, diffuse, minimal cells. The kidneys of both the control and AB-treated groups showed vacuolar degeneration; tubular, cortical, bilateral, multifocal, moderate pigment accumulation and interstitial, multifocal, minimal cells. These results clearly demonstrated that AB had no significant toxicity on STZ-induced hyperglycemic rats (Fig. 3).

Subsequently, histopathological analysis of endocrine pancreas of the experimental rats was performed. In the control group, the islets showed no significant pathological changes and were of normal size and evenly distributed throughout the pancreas. In the STZ-induced hyperglycemic groups, marked atrophy of islets was noted with respect to both size as well as number of islets. In the case of Metformin standard group, the islets presented moderate atrophy, whereas in the AB-treated groups, the islets showed only minimal to moderate atrophy and exhibited improvement in the number and size of islets (Fig. 3).

The acute oral toxicity of AB was investigated according to OECD-425 guidelines. Female rats aged 8—12 weeks were orally administered with a single dose of AB (maximum dose of 2000 mg/kg b.w.) dispersed in honey water (at a ratio of 2:3), and the mortality, weekly body weight, daily feed intake, clinical parameters, and gross pathology were observed during the study period of 14 days. The results revealed no significant toxic pathological effects following AB treatment. The maximal tolerated dose of AB was >2000 mg/kg b.w., and the mortality rate was 0%. Besides, no visible signs of toxicity were observed, the gross pathology was agonal in nature, and all the animals survived at the end of the acute toxicity study.

Sub-acute toxicity investigation was performed as per the OECD-407 guidelines by orally administering 80, 320 and 1280 mg/kg b.w. AB dispersed in honey water (at a ratio of 2:3) to Wistar rats
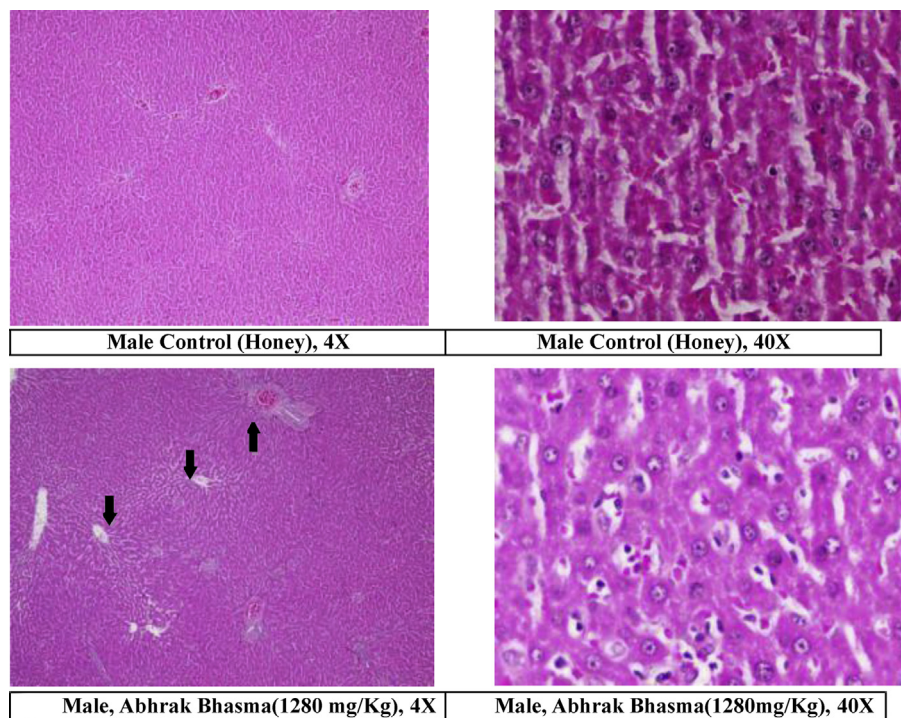
Table 7
Biochemistry studies of female rats in sub-acute toxicity studies.

	Albumin (g/dL)	ALP (IU/L)	T Bilirubin (mg/dL)	D Bilirubin (mg/dL)	T. Cholesterol (mmol/L)	Creatinine (mg/dL)	Glucose (mmol/L)	T Protein (g/dL)	AST (IU/L)	ALT (IU/L)	TGL (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Honey	3.39 ± 0.11	227.60 ± 23.92	0.36 ± 0.09	0.13 ± 0.03	77.20 ± 10.71	0.49 ± 0.03	98.40 ± 6.66	5.32 ± 0.14	137.20 ± 10.11	67.20 ± 7.40	30.60 ± 5.94	43.62 ± 5.70	1.28 ± 0.39
80 mg/kg	3.28 ± 0.17	218.00 ± 23.91	0.41 ± 0.06	0.15 ± 0.06	77.40 ± 8.53	0.50 ± 0.02	86.80 ± 7.46	5.34 ± 0.26	147.60 ± 8.02	71.20 ± 7.92	29.40 ± 4.10	43.54 ± 3.99	1.65 ± 0.63
320 mg/kg	3.58 ± 0.29	230.60 ± 25.77	0.33 ± 0.14	0.12 ± 0.03	96.80 ± 9.42	0.49 ± 0.03	87.60 ± 41.35	5.62 ± 0.30	141.80 ± 15.19	68.80 ± 13.48	47.20 ± 9.26	37.54 ± 3.41	1.61 ± 0.34
1280 mg/kg	3.50 ± 0.24	215. ± 46.83	0.33 ± 0.03	0.12 ± 0.03	85.60 ± 9.07	0.50 ± 0.01	80.40 ± 37.45	5.59 ± 0.28	162.00 ± 15.07	73.60 ± 10.60	48.20 ± 9.52	40.94 ± 3.49	1.84 ± 0.3
Satellite-Honey	3.45 ± 0.16	230.80 ± 27.56	0.35 ± 0.07	0.12 ± 0.03	80.60 ± 16.46	0.49 ± 0.04	97.20 ± 6.14	5.34 ± 0.09	142.80 ± 7.92	66.60 ± 7.70	31.00 ± 4.53	44.14 ± 6.68	1.32 ± 0.39
Satellite-1280 mg/kg	3.47 ± 0.29	200 ± 22.47	0.32 ± 0.04	0.14 ± 0.03	84.40 ± 7.80	0.50 ± 0.02	96.00 ± 5.87	5.51 ± 0.31	161.40 ± 17.24	75.20 ± 11.41	48.80 ± 8.47	41.38 ± 3.19	1.86 ± 0.27

The above data was expressed as mean ± SEM, with normality $n = 10$.

Fig. 2. Liver section treated *Abhrak Bhasma* in rats. Represent Liver section (Hematoxylin and eosin staining) 4X & 40X of Control and 1280mg/kg b.w treated Abhrak Bhasma in Rats.

for 28 days. The results presented no clinical difference in weekly body weight and organ weight changes during the study period. It must be noted that hematological system is highly sensitive to toxic materials and could reveal the physiological changes in animals or human beings [29]. In the present study, the hematological parameters, including RBC and WBC, of AB-treated rats were not affected even at a high dose of AB (1280 mg/kg b.w. AB), indicating that AB is non-toxic and safe while in circulation. Furthermore, biochemical studies revealed that the levels of total bilirubin, direct bilirubin, and creatinine were not significantly altered ($P > 0.05$) among AB-treated groups and control. However, a considerable increase in serum liver enzymes (AST, ALT, and ALP), albumin, total cholesterol, glucose, total protein, TGL, urea, and uric acid ($P < 0.05$)

in rats administered with 80, 320, and 1280 mg/kg b.w. AB was observed. Moreover, histopathological studies showed improved size and number of islets in AB-treated rats, when compared with those in normal control and disease control, suggesting minimal to moderate atrophy of islet cells. Besides, AB treatment produced no relative changes in the levels of glucose, creatinine, AST, and ALP, implying that AB did not affect liver and kidney tissues.

The anti-hyperglycemic potential of AB was studied by employing STZ-induced diabetic rat model, and the results confirmed the dose-dependent effect of AB (40, 80, and 160 mg/kg b.w.). While obvious increase in the blood glucose level was noted in disease control and normal control, rats treated with medium concentration of AB showed significantly lower blood glucose level.

Table 8
Weekly body weight, BGL level, lipid profile and organ weight studies of rats in anti-diabetic studies.

Parameter	Normal control	Disease control	STD 100 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Day 0	282.46 ± 17.99	271.55 ± 34.41	271.45 ± 20.42	258.00 ± 21.41	261.93 ± 20.70	266.22 ± 29.83
Day 7	298.24 ± 17.55	245.92 ± 30.03	270.67 ± 27.31	258.30 ± 22.48	250.28 ± 20.51	249.19±31.00
Day 14	306.88 ± 19.13	246.34 ± 29.55	258.96 ± 28.88	262.57 ± 25.80	254.58 ± 15.78	250.68±35.16
Day 21	313.05 ± 16.68	245.77 ± 32.76	262.02 ± 26.12	255.65 ± 32.61	253.91 ± 30.20	242.41±37.06
Day 28	302.17 ± 19.20	229.86 ± 33.73	252.66 ± 29.57	240.97 ± 30.20	244.66 ± 27.33	234.63±40.11
Blood glucose level studies of rats in anti-diabetic studies						
Day 0	84.38 ± 4.96	378.88 ± 130.55	390.25 ± 119.55	409.25 ± 108.97	350.25 ± 60.2	398.88 ± 167.22
Day 7	86.00 ± 5.01	549.00 ± 70.82	458.00 ± 162.21	421.50 ± 104.87	555.63 ± 120.3	586.00 ± 112.24
Day 14	86.38 ± 3.11	542.13 ± 57.98	454.75 ± 138.53	386.38 ± 96.51	468.88 ± 67.27	489.00 ± 69.68
Day 21	83.13 ± 3.56	494.25 ± 78.49	360.88 ± 112.41	376.25 ± 80.07	433.13 ± 59.37	416.38 ± 129.93
Day 28	60.38 ± 7.15	318.75 ± 71.89	175.00 ± 88.33	182.13 ± 56.55	220.63 ± 95.92	267.25 ± 97.2
Lipid Profile studies of rats in anti-diabetic studies						
Parameter	Normal control	Disease control	Metformin 100 mg/kg	Low 40 mg/kg	Medium 80 mg/kg	High 160 mg/kg
TC	84.39±9.21	113.13±4.93	93.9±2.48	93.55±11.65	89.36±9.92	95.65±4.11
TGL	117.62±15.87	166.96±17.39	138.48±20.07	150.25±13.81	155.37±8.57	143.16±10.0
Organ weight studies of rats in anti-diabetic studies						
Liver	9.65 ± 1.25	8.88 ± 1.22	9.17 ± 0.78	8.16 ± 0.53	9.03 ± 0.87	8.17 ± 1.52
Kidney(R)	1.08 ± 0.09	1.17 ± 0.14	1.19 ± 0.06	1.18 ± 0.12	1.23 ± 0.14	1.15 ± 0.21
Kidney(L)	1.20 ± 0.29	1.16 ± 0.09	1.17 ± 0.08	1.18 ± 0.12	1.23 ± 0.12	1.13 ± 0.15

Data are expressed as mean ± SEM, $n = 8$, Body weight unit: grams, TC and TGL unit: mg/dL, Organ weight unit: grams. Blood glucose level unit: mg/dl.
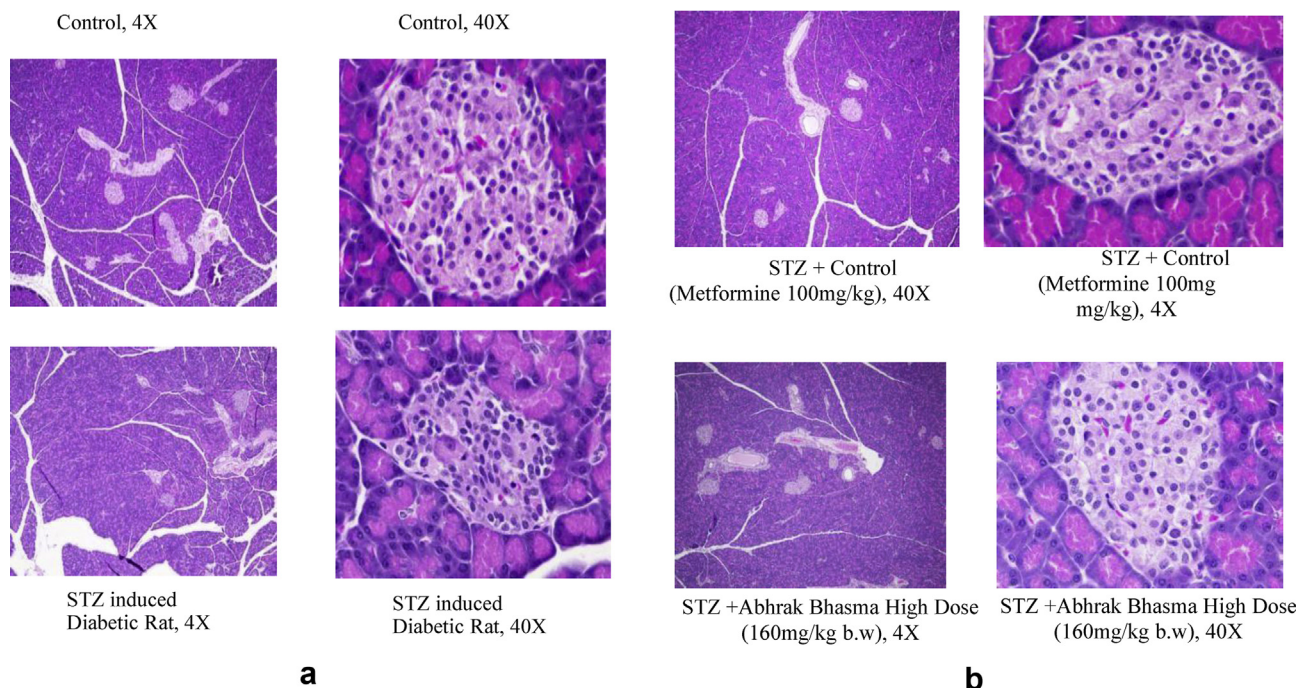
Fig. 3. (a) Pancreases section treated *Abhrak Bhasma* in rats (b) Pancreases section treated *Abhrak Bhasma* in rats. Represent pancreases section (Hematoxylin and eosin staining) 4X & 40X of Control, STZ induced, STZ + STD adn STZ induced 160 mg/kg b.w of *Abhrak Bhasma.*

The body weight of rats both in disease control and normal control was considerably increased, whereas that of rats in AB-treated group was reduced, when compared with the initial weight.

Furthermore, a significant decrease in the blood glucose, total cholesterol, and TG levels was observed in AB-treated diabetic rats. The body weight of control rats presented a significant increase, whereas the body weight of AB-treated rats was lower than that of the disease control and normal control rats. These findings suggest that AB is safe and non-toxic to rats and does not produce any significant toxic effects. AB exhibited beneficial effects as an anti-hyperglycemic agent in diabetic rats by improving various metabolic processes, such as lipid metabolism, with no significant toxicity.

4. Conclusion

The nano-Ayurvedic medicine AB showed improved cell penetration and was effective in the treatment of hyperglycemia, with no significant *in vivo* acute and sub-acute toxicity, even at high dosage (2000 mg/kg b.w.). The acute oral LD_{50} of AB in Wistar rats was >2000 mg/kg b.w and sub-acute studies showed no toxic effects at >1280 mg/kg b.w., along with normal histopathological, biochemical and hematological findings. AB exhibited significant anti-hyperglycemic activity and high cell penetration efficacy in STZ-induced diabetic rats. These results shows AB may induce insulin secretion or protecting pancreatic beta cells, suggesting the potential use of herbo-mineral nanomedicine for the treatment of diabetes.

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Conflict of interest

None.

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