International Journal of Applied Ayurved Research ISSN: 2347- 6362

EXPERIMENTAL EVALUATION OF ANTI DIABETIC ACTIVITY OF SWARNAMAKSHIKA BHASMA

[1]Joshi B.B. [2]Agnihotri Pradeep [3]Joshi Anitha [4]Arun

[1]Principal & Head,Department of R.S.& B.K.,AMV,Hubballi. R.G.U.H.S University, Bengaluru, India.

[2]Reader, Department of R.S.& B.K. AMV, Hubballi. R.G.U.H.S University,Bengaluru,, India.

[3]Reader, Department of R.S.& B.K. AMV, Hubballi. R.G.U.H.S University,Bengaluru,, India.

[4]Research Scholar,Department of R.S.& B.K, AMV, Hubballi. R.G.U H.S University, Bengaluru, India.

ABSTRACT :

Swarnamakshika identified under *Maharasa* Varga is considered as *Chalcopyrite Mineral* which contains iron (Fe), Copper (Cu) and Sulphur(S) as major components along with other trace elements of therapeutic importance. *Swarnamakshika* possess *Rasayanagrya, Dusadhya Rogahara, Sakalamayaghna* and *Mehahara* properties. Present research work has been taken to evaluate anti-diabetic activity of *Swarnamakshika Bhasma (SMB)* on Albino rats.Five groups viz Normal Control, Diabetic Control, Glibenclamide (GLB, 10mg/kg, p.o.) *SMB* (0.45mg/0.2kg ,p.o) and *SMB+Honey* (0.9mg/0.2kg ,p.o) containing 6 rats in each group are subjected for Acute (i.e. single day) and chronic (15days) anti-diabetic study on Alloxan (ALX) induced diabetic rats and compared statically by Anova Test. Blood Glucose level of the drug was evaluated by Oral Glucose tolerance test (1stDay), and Fasting Blood Glucose Estimation (1,3,5,7,9,11,13,15-Days) for evaluation of Anti- Diabetic activity. Results showed that *SMB* and *SMB+HONEY* possess significant anti-hyperglycemic activity. Histo-pathological Studies showed that SMB has regenerated islets of langerhanns, and relatively increased granulated and normal β-cells of pancreas.

Key words: Anti-Diabetic Activity, Histo-pathological Study, *Swarnamakshika Bhasma*, Serum Glucose Level.

INTRODUCTION: *Rasashastra* deals with variety of *Metals* and *Minerals* which play an important role in *Ayurveda Therapeutics*. *Swarnamakshika* is one such mineral of *Maharasa Varga* used by Vaidyas since *Samhita Period* as it has wide range of therapeutic activity. For scientific revalidation of data and to generate facts and figures of effect of *SMB* on Diabetes present research work was planned. Diabetes Mellitus is a metabolic disorder caused by complex interactions of genetics, environmental factors and life style choices characterized by Polydipsia, Polyuria, polyphagia, Hyperglycemia, Glycosurea, and Hyperlipidemia.Several new molecules are being developed with new progressive researches in contemporary medical System. But none of these are free from untoward effects in the body, therefore it was felt that Swarnamakshika Bhasma can be proved as an effective Anti- Diabetic drug as per the literatures of *Rasashastra.*

Preparation Of *Swarnamakshika Bhasma:*

SMB was prepared as per the reference of *Rasa Ratna Samuchaya* at PG DEPT of *Rasashasra&B.K*, Ayurveda

Mahavidyalaya, Hubballi .Bhasma siddhi lakshanas obtained after ten *Gaja Putas*.Then *SMB* was subjected for different analytical procedures to ensure the quality of the *Bhasma.*

After assessed by all these analytical parameters, genuine sample of *SMB* was taken for experimental study for evaluation of Anti- Diabetic activity on Albino rats.

ANTI-DIABETIC STUDY:

Study was performed under two phases viz Single-dose one-day study and Multiple-dose fifteen-day study to evaluate short term and long term Anti-Diabetic effect of test drug on Alloxan induced Albino rats in two different doses

Male Wistar rats weighing 150–200 gm were used for the present study. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, India EG.No.112/1999/CPCSEA).

MATERIALS:

Drugs: Test drug- *Swarnamakshika Bhasma*, Standard drug- Glibenclamide

Associated drug- *Honey*, Diabetic inducing drug- Alloxan Monohydrate.

Animals Used: Wistar strain Albino Rats.

Equipments: Animal cage, Day night cycle chamber, weighing balance.

Glass wares: Glass Beakers, Test tubes, Stirrer, Measuring jar, 18" needle & Disposable Syringe.

Chemicals and Reagents: Normal Saline, 10% Formalin, Bouin Holland solution, Chloroform, Paraffin wax.

METHOD: The suspensions of *SMB* (TEST-1) and *SMB with Honey* (TEST-2) were prepared by using distilled water and administered orally to experimental animals. Fasting Blood glucose level was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was introduced by a single i.p. injection of 120mg/kg Alloxan monohydrate. After 48 hours, the hyperglycemic rats (Glucose level >200 mg/dl) were separated and divided into 5 different groups comprising of 6 rats each for anti- diabetic study.The treatment was started from the same day except normal control and diabetic control groups for a period of 15 days. . Blood glucose levels were estimated in both groups by using a glucose oxidase-peroxidase reactive strips and a Glucometer (S D Check Gold Blood Glucose Meter, Standard Diagnostics, and Korea).

Experimental design for Single-dose one-day study

The experimental rats were divided into five groups of six each and treated as follows

Group-1- Normal control – Normal food and Distilled water (10ml/kg)

Group-2- Diabetic control - Alloxan monohydrate (120mg/kg)

Group-3-Alloxan monohydrate+GLB (10mg/kg, p.o.)

Group-4-AlloxanMonohydrate+*SMB* (0.45mg/ 0.2kg, p.o.)

Group-5-AlloxanMonohydrate+*SMB*+*Honey* (0.9 mg/0.2 kg, p.o.)

Blood samples were collected at 0, 1, 2 and 4 h after drugs administration [single-dose one-day study.

Experimental design for Multiple-dose fifteen-day study:

The experimental rats were divided into five groups of six each and treated as follows

Group-1- Normal control – Normal food and Distilled water (10ml/kg)

Group-2- Diabetic control - Alloxan monohydrate (120mg/kg)

Group-3-Alloxan monohydrate+GLB (10mg/kg, p.o.)

Group-4-AlloxanMonohydrate+*SMB* (0.45mg/ 0.2kg, p.o.)

Group-5-AlloxanMonohydrate+*SMB*+*Honey* (0.9 mg/0.2 kg, p.o.)

Fating Blood samples were collected at 1, 3,5,7,9,11,13 and 15 days after drug administration (Multiple-dose fifteen-day study).

RESULTS:

Organoleptic characters: *SMB* was smooth, tasteless odourless bright red in colour.

Physical test: PH-7.32 ± 0.10, Total Ash-3.52%, Acid insoluble ash: 27.36%, water soluble ash: 2.97%, loss on drying at 110^0c: 0.21%, Loss on ignition at 1000^0 c**:** 1.7%.

Chemical tests: Estimation of sulphur: 12.16 %, Estimation of copper: 19.5%, Estimation of iron: 31.08%.

Solubility: Water-3.5 %w/w, Nitric acid-80 % w/w, Hydrochloric acid-92 % w/w.

Particle size analysis by lazer diffraction method: 4.46 µm (Mean particle size**).**

X-ray diffraction study: Compound identified as, raw *Swarnamakshika* - $cufes_2$,

Compound identified as, *Shodhita Swarnamakshika* - $cufes_2$

Compound identified as *SMB* -$cufes_2$ & fe_2o_3

Stastical evaluation of the data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way Anova followed by Turkey's post-test using Graph Pad Prism version 5.0, USA.

TABLE NO. 1 EFFECT of Five Groups ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (SINGLE-DOSE ONE-DAY STUDY)

Group	0 Minutes	30 Minutes	60 Minutes	120 Minutes
Normal Control	117±7.37	134.7±5.5	126±6.5	113.3±2.8
Diabetic Control	550.3±4.61	600±0	593.3±5.7	590±2.08
GLB	570.7± 9.09	591±8.54	600±0	531.3±8.08
SMB	575.5±5	595±4.35	480±3.4	381.7±4.3
SMB + HONEY	591.3±10.2	600±0	567.3±2.3	502±6.42

1. Diabetic control compared to GLB (P > 0.05)

2. Diabetic control compared to SMB (P > 0.05)

3. Diabetic control compared to SMB+HONEY (P > 0.05)

TABLE NO.2 EFFECT OF Five Groups ON SG LEVELS IN ALX-INDUCED DIABETIC RATS MULTIPLE-DOSE FIFTEEN-DAY STUDY

Groups	1st Day	3erd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
Normal Control	122.5+1.72	129+7.7	114.9+3.7	124+5.2	119.3+10	118.8+17.9	105+2.6	123+12

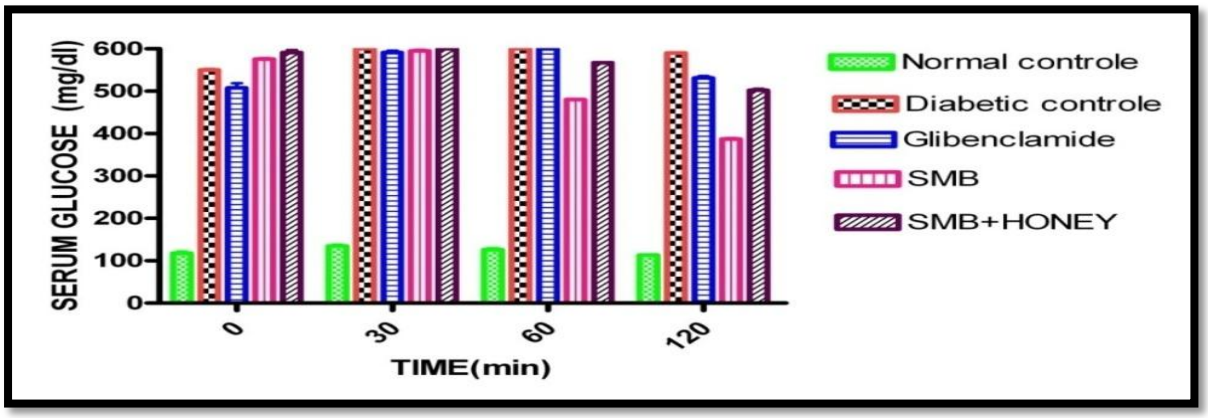
Diabetic Control	585+10	596+2.0	597.3+3.4	564.3+1.7	589.8+0.5	597+4.76	559.5+3.2	531+7.6
Glibenclamide,	418+8.1	581+14.4	434.3+13.9	468.3+13	507+5.7	425.5+10	458.8+6.7	375.5+7.1
SMB	596+4.1	449.3+13.2	146+7.1	113.5+10	136.3+9.9	111.3+1.1	115+17	123+5.2
SMB +Honey	597+3.46	592+3.4	107+10.9	598.5+3	201.5+3.8	173.8+10	138.3+12	135+10

1. Diabetic control compared to GLB (P > 0.05)

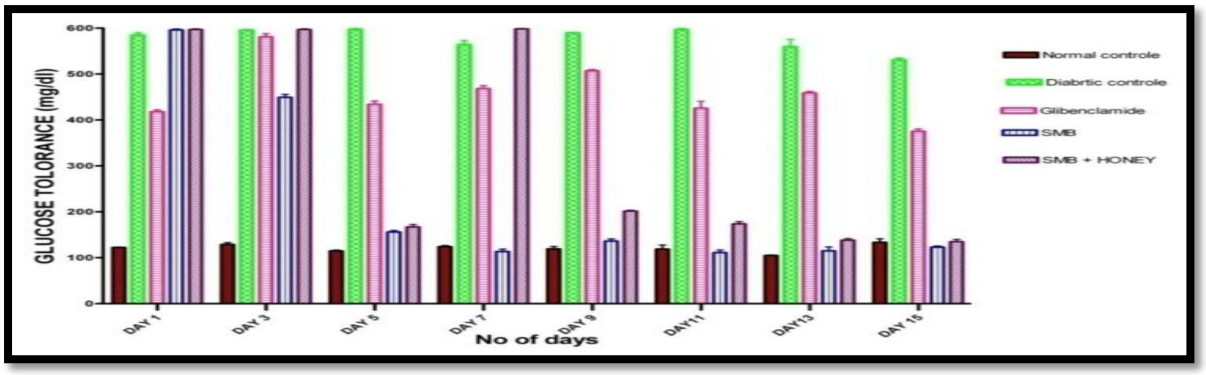
2. Diabetic control compared to SMB (P < 0.001)

3. Diabetic control compared to SMB+HONEY (P < 0.01)

GRAPH NO.1 SHOWING THE EFFECT OF *SMB* AND *SMB+HONEY* ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (SINGLE-DOSE ONE-DAY STUDY)



GRAPH NO.2 SHOWING THE EFFECT OF *SMB* AND *SMB+HONEY* ON SG LEVELS IN ALX-INDUCED DIABETICRATS(MULTIPLE-DOSE FIFTEEN-DAY STUDY)



Histo-pathological Examination: The whole pancreas, kidney and liver from each animal were removed after sacrificing the animals, collected and preserved in 10% formalin solution. The samples were submitted to Hubli Diagnostic Lab Pvt Ltd. (Hubli, Karnataka) India for Histo-pathological examination.

TABLE NO.3 SHOWING HISTO-PATHOLOGIC CHANGES IN PANCREAS

	NORMAL CONTROL	**DIBETIC CONTROL**	**GLB**	***SMB***	***SMB+HONEY***
ACINI					
Lining epi-thelium	Normal	Hypertrophic	Hypertrophic	**Normal**	**Normal**
Cytoplasma	Normal	Abundant amount of pink granular material(+++)	Moderate amount of pink granu-lar mate-rial(++)	**Normal**	**Normal**
Nuclei	Normal	Normal	Normal	Normal	Normal
ISLETS OF LANGERHANS					
cells	**Normal**	**Absent**	**Decreased**	**Normal ap-pearing cells with regen-erative ac-tivity**	**Normal**
ducts	Normal	Normal	Normal	Normal	Normal
Intestinal connective tissue	Normal	Congestion	Congestion	Congestion	Congestion

TABLE NO.4 SHOWING HISTO-PATHOLOGIC CHANGES IN LIVER

	NORMAL CONTROL	**DIBETIC CONTROL**	**GLB**	***SMB***	***SMB+HONEY***
Architecture	Normal	Normal	Normal	Normal	Normal
<u>Hepatocytes</u> Cord like Ballooning degeneration	Normal	Ballooning degeneration (++)	Ballooning degeneration (++)	Ballooning degeneration (++)	Ballooning de-generation (++)
<u>Portal triads</u> Portal vein Hepatic ar-tery Bile duct	Normal	Portal vein dilated	Portal vein dilated	Normal	Normal
Central view	Normal	dilation	Central vein dilation	Normal	Normal
Kupffer' s cells	Normal	Hyperplasia prominent	Hyperplasia	Hyperplasia	Hyperplasia
Sinusoidal spaces	Normal	Congestion	Congestion	Congestion	Congestion
others	Normal	Fatty Change (++)	Fatty Change (+)	Normal	Normal

TABLE NO.5 SHOWING HISTO-PATHOLOGIC CHANGES IN KIDNEY

	NORMAL CONTROL	DIBETIC CONTROL	GLB	*SMB*	*SMB+HONEY*
Glomerulaus-Capillary tuft	Normal	Severe congestion	Mild congestion	Moderate congestion	Mild congestion
Bowman' capsule	Normal	Normal	Normal	Normal	Normal
Bowman' space	Normal	Reduced	Normal	Normal	Normal
Proximal convoluted tubes	Normal	Hypertrophic abundant pink granular material(+++)	Hypertrophic pink granular material(++)	Mild(+)	Mild(+)
Distal convoluted tubes	Normal	Normal	Normal	Normal	Normal
Henle's loop	Normal	Normal	Normal	Normal	Normal
Collecting tubes	Normal	Normal	Normal	Normal	Normal
Interstial connective tissue	Normal	Congestion (++) Round cell infiltration(+)	Congestion (+)	Congestion (+)	Congestion (+)

DISCUSSION: *SMB* was prepared by giving 10 *Gaja Putas*.45% yield of SMB was obtained. Then *Amrutikarana* was also carried out with *Panchamrita* to enhance the therapeutic merits and to eradicate *shishta doshas. Panchamrita* is having *Snigdha, Mridu, Shlakshna guna and Sheeta veerya* (except *madhu*), helps to remove the *Rukshata and teekshnata of Bhasma*. The total ash 1.6% in *SMB* indicative of the presence of organic matter in the final product which probably imported during *Shodhana* or *Bhavana* procedure. Acid insoluble ash is 1.6%, which indicates the easy absorption of drug. The low acid insoluble acid ash values facilitate absorption in gut. Loss on ignition at 1000^0C: 1.6 % shows organic material in the bhasma. Loss on drying shows the end product contain 0.49% of moisture in *SMB* which is within normal limits. The mean value of total percentage of particles is 4.46 µm, 100% of the particle size is within the range of 0.0-8.83µm. The falling of total range under 0.0 to 8.83 µm indicating fineness of Bhasma. In XRD analysis, only component identified as $CuFeS_2$ peaks.

Administration of single dose of GLB,*SMB* and *SMB+Honey* in diabetic rats showed reduction in SG levels at different time intervals compared to base values i.e. at 0 Minutes of the same group. The *SMB* (0.45mg/0.2kg) has shown a significant Anti-Diabetic effect in rats at 120 minutes after oral administration compared to Glibenclamide and *SMB+Honey* (0.9mg/kg) in single dose one day Anti-Diabetic module. But oral administration of *SMB*, *SMB+Honey* and GLB not caused a statistically significant reduction

Long term administration of *SMB* and *SMB+Honey* to diabetic rats for 15 days showed marked fall in SG levels compared to base values i.e. at 1st day. *SMB (*0.45 mg/0.2kg) and *SMB+HONEY* (0.45 mg+0.9mg/0.2kg) showed significant reduction i.e (P<0.001), (P<0.01) respectively. These data suggest that efficacy of both is higher when compared to standard GLB (10mg/kg) (P>0.05). Multiple-dose fifteen-day study revealed that the *SMB* and *SMB+Honey* showed potent anti diabetic activity compared to GLB

Histo-pathological examination showed that *SMB* has the potency to regenerate islets of langerhanns, and relatively increased granulated and normal β-cells. However, the expansion was better with *SMB+Honey* than SMB which possibly regenerated β-cells.

Anti-Diabetic activity of The *SMB* & *SMB+Honey* might be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells along with regeneration of pancreatic β-cell.

CONCLUSION: Swarnamakshika is a *Chalco-pyrite mineral* included under *Maharasa Varga* has got equal importance in both *Dehavada* and *Dhatuvada. SMB* was prepared by giving ten *Gaja Putas* and the nearly 45% of yield obtained.X-RD report of *ashodhita Swarnamakshika* showed compound copper pyrite ($CuFeS_2$) with cubic crystal system, *Shodhita Swarnamakshika* showed compound copper pyrite ($CuFeS_2$) with cubic crystal system, *Swarnamakshika Bhasma* showed compound ferric oxide ($Fe2O_3$) with Rhombohedral crystal system along with compound copper pyrite ($CuFeS_2$) with cubic crystal systemThe mean value of total percentage of *Swarnamakshika bhasma* particles is 4.46 µm, 100% of the particle size is within the range of 0.0-8.83µm.This shows the superiority of Pharmaceutical Procedure. *Swarnamakshika Bhasma* has got considerable Anti- Diabetic activity in single dose one-day study. Whereas in Multiple dose 15 days study *SMB* and *SMB+ Honey* both showed highly significant Anti- Diabetic activity. The P- value of both was P < 0.001, P <0.01 respectively. Histo-pathological studies showed that Swarnamakshika Bhasma has the potential to regenerate islets of langerhans and relatively increases granulated and normal β-cells which proves Anti-Diabetic activity of *SMB.*

REFERENCES:

1.Sri Vagbhatacharya,Dr.Ashok Satpute. Rasa Ratna Samuchaya. Delhi:Chaukamba Sanskrit Pratishthan;2006.P-33,Sloka-77.

2.Sadanad sharma, Kashinada shastri. Rasa Tarangini. Delhi:Motilal Banarasidasa;2004. P-524,Sloka-27.

3.Siddhinandan Mishra, Ayurvedeeya Rasasathra, 14 th edition. Varanasi: chaukhamba orientalia; 2004. P-382

4. Trinsley R. Harrison. Harrisons Principles of internal Medicine. NewYork:Mc Graw-Hill Medical publishing division;2005. P-2152

5. Frank Rutley, C.B.Gribble. Rutleys elements of Mineralology. 27th edition.Delhi:CBS Publishers and Distributors;1991.P-238.

6. Dr.Lohar,Ravindra Singh. Quality control manual for Ayurvedic Siddha and unani medicine. Ghazhiadad: Published by Department of Ayush,Ministry of health and family welfare Pharmacopeial laboratory for Indian medicine; 2008. P-11.

7. Vogel G.H.,Drug discovery and evaluvation-pharmacolagical assey.Second edition.Verlag springer;2002:P-950

8.K.D.Tripathi. Essentials of Medical Pharmacology. New Delhi:Jaypee brothers medical publications;2008. P-254

Corresponding Author: Dr.B.B.Joshi
Principal & Head, Department of R.S.& B.K., AMV, Hubballi. R.G.U H.S University, Bengaluru,, India.

Source of support: Nil
Conflict of interest: None Declared

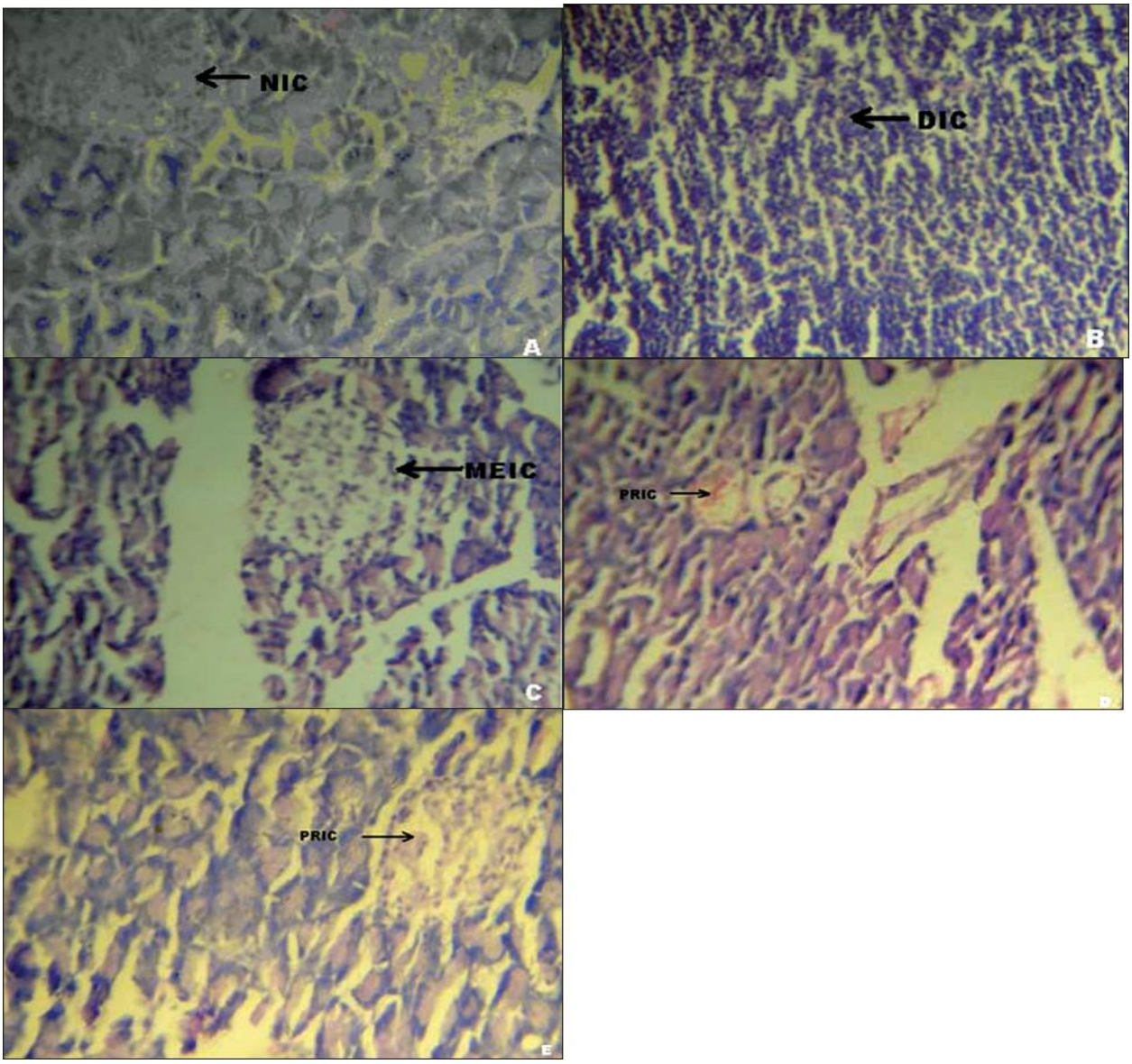


Figure 1: (A) Photomicrographs of normal healthy control group's rat showing normal globules of acini with normal islet cells (NIC), (H&E, x400) (B) : Photomicrographs of diabetic control group's rat showing damaged islet cells (DIC), (H&E, x400) (C) : Photomicrographs of standard (Metformin 0.5 g/mg) treated group's rat showing moderate expansion of islet cells (MEIC), (H&E, x400) (D) : Photomicrographs of ethanolic extract (100 mg/kg) treated group's rat showing partial restoration of islet cells (PRIC), (H&E, x400) (E) : Photomicrographs of ethanolic extract (200 mg/kg) treated group's rat showing partial restoration of islet cells (PRIC), (H&E, x400)