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Comparative Ranking of Metformin, *Syzygium cumini* Mother Tincture and a Ayurvedic Test Drug Preparation Using STZ Induced Type II Diabetes in Rats by Linear Regression

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ABSTRACT

Diabetes mellitus type II is an illness characterized by a disruption in the body's normal glucose homeostasis, affects people worldwide and disrupts their everyday routines. Sorting out the various pharmacological treatments for type- II diabetes mellitus is an immediate need (Boruah, Chakraborty and Dash, 2017; Liang et al., 2017). With little adverse effects and extra protective impact on the several essential organs afflicted by Type- II diabetes mellitus, the basic principle of antidiabetic treatment is short -term and long -term lowering of HbA1c level with minimum side effects and additional protective action on the different vital organs that are affected in the condition of the Type II diabetes mellitus (WHO, 2011). Medication from several medical systems, such as allopathy, ayurveda and homoeopathy, has a well-deserved reputation for helping metabolic diseases like diabetes mellitus, but which drug we select for the therapeutic purposes to treat diabetes is a bias. Using a high fat-diet and STZ caused type II diabetes experimental model, this study aimed to rank the anti-diabetic efficacy of test medications from various medical systems as the Met, Ayu and Hom test groups. In albino wistar rats, type II diabetes mellitus was induced by providing them a high fat-diet for 28 days before they were given STZ. After that, till three months test drug given at six dosage levels orally. Various parameters, including HbA1c, fasting blood-glucose, postprandial two hour blood-glucose level, fasting blood insulin, HOMA-IR, insulin sensitivity index and body weight were on different days of the experiment, namely on days 0, 28, 31, 76, and 121.

The study found that varied doses of Met, Ayu and Hom test drugs significantly reduced the HbA1c level ($p < 0.001$). All test groups treated with STZ+HFD, used in this study were diabetic at all six dose levels as shown by HOMA-IR, and insulin sensitivity index levels found in this study. Met, Ayu and Hom test drugs significantly reduced the fasting glucose level ($p < 0.05$) and postprandial two hour blood-glucose level ($p < 0.05$ significantly). Based on statistical analysis, the following pharmacological treatment rankings were determined for antidiabetic parameters in STZ+HFD diabetic rats: Comb, Ayu, Una, Ext, and Hom.

When looking at the effectiveness ranking of the various drugs tested in this study, determination of EC50 values by the linear regression analysis revealed that on the basis of fasting blood-glucose values and 2h-BG

values fasting blood insulin and HbA1c level, Met was found to be the most effective, followed by Ayu and Hom. Based on the study results, the order of medication therapies for type II diabetes mellitus in pancreatic cells is as follows: Met > Ayu > Hom. Various combinations of these drugs can be further evaluated in such a manner for selection of a better one, with HbA1c level lowering capacity with minimum side effects and additional protective action on the different vital organs of a diabetic patient.

Keywords: Allopathy, ayurveda, homeopathy, STZ, FBIN, HFD, HOMA-IR, ISI, Metformin, EC_{50} Value.

INTRODUCTION

The basic principle of this work is that the antidiabetic test drugs must decrease the glycated hemoglobin HbA1C value (as % of the hemoglobin for the life of the RBC, all antidiabetic drugs currently available in the market are decreasing its value maximum 2%) and must provide additional protective benefits, specially to the vital organs of the body, like low side-effect and maximum organ protection (UpToDate, 2023).

This dissertation work was undertaken to develop the novel anti-diabetic combinations by developing a equivalence ranking of antidiabetic drugs from different systems of medicine (allopathic, ayurvedic and homeopathic system of medicine) by estimating blood HbA1c, FBG, 2h-BG, FBIn, HOMA-IR & ISI levels and body weight in type- II diabetes induced by STZ in rats for 121 days. The results was prepared in the format of equivalence ranking of all tested drug preparations using the various statistical analysis methods (Boruah, Chakraborty and Dash, 2017; Liang et al., 2017).

Till date none of the reported ayurvedic medicine have such effectiveness that it effectively down the blood-glucose levels in comparison to oral hypoglycemic drugs. So comparison of oral hypoglycaemic drugs on the basis of their safety profiling and various therapeutic actions without inclusion of the mechanism of action or independent from the restrictions of similar the mechanism of action, is the current need of the day. So our aim is to find out the antihyperglycemic drug alternatives. Since none research articles & texts have given this combination prepared and used in this study (WHO, 2011), hence present study has novelty.

The present study evaluated the anti-diabetic potential and equivalence ranking of marketed drugs (most prescribing allopathic, ayurvedic and homeopathic test drugs) used in this study from different systems of medicine, for their blood-glucose level normal homeostasis establishment and also to develop novel antidiabetic combinations. Also reduction in medicine cost & effectiveness for antidiabetic action required for a diabetic patient can be achieved using such studies. This study further more commercialize the anti-diabetic evaluation methods, compare to the conventional testing procedures, for development of a cost effective of anti-diabetic drugs. Method used in this study can be used, to evaluate and correlate medicinally used plants and marketed drugs, their dosage form, content uniformity and pharmacological activity.

The proposed hypothesis of the anti-diabetic new combination preparation will further toxicologically, physiochemically and pharmacologically evaluated for its anti-diabetic action. For these novel optimized combinations, pharmaceutical dosages forms can be developed as per the FDA requirements. Thus, the developed combination will be an ideal alternative for the existing hypoglycemic formulations in the market with an assured and authenticated formula to minimizing risk factors associated with type II diabetes.

Developed novel antidiabetic combinations will provide alternative therapy for diabetes treatment, based on antidiabetic ranking or score of drugs used in this study. This study will provide and pave the way of easy and low cost evaluation and development of of particular anti-diabetic drug. This study will provide easy evaluation of a particular antidiabetic drug. Calculated values of each & every antidiabetic drug used in this study on our experimental conditions will helps the researchers for further work on diabetes using streptozotocin induced diabetic rat model.

MATERIALS AND METHODS

A. INDEPENDENT VARIABLES (X) OF THE STUDY

Test drugs

Test drugs 1. Metformin (Met), 2. *Syzygium cumini* Mother Tincture (Hom) and 3. "Ayu" test drug (Combination preparation of Asphaltum Pdr., *Mangifera Indica* seed, *Momordica charantia* seed, *Gymnema Sylvestre* leaf, *Syzygium cumini* seed. and CMC) were procured from the Bilwal medchem research Pvt. Ltd. Sikar, India. There is no standard group, all drugs are standard for each other (Control & drug groups). Toxicity investigation of selected drugs is not needed in this study, because all of the drugs used in this study are currently available in market and their toxicity study is already done.

Dose of the test drugs

Six dosage (D1, D2, D3, D4, D5 and D6, n=6) for each test drug - Metformin, Ayu and Hom were used (as) as MetD1 - 6.25 mg/kg, MetD2 - 12.5 mg/kg, MetD3 - 25 mg/kg, MetD4 - 50 mg/kg, MetD5 - 100 mg/kg, MetD6 - 200 mg/kg, AyuD1 - 9.37 mg/kg, AyuD2 - 18.75 mg/kg, AyuD3 - 37.5 mg/kg, AyuD4 - 75 mg/kg, AyuD5 - 150 mg/kg, AyuD6 - 300 mg/kg, HomD1 - 3.125 mg/kg, HomD2 - 6.25 mg/kg, HomD3 - 12.5 mg/kg, HomD4 - 25 mg/kg, HomD5 - 50 mg/kg and HomD6 -100 mg/kg.

The of test drug preparations suspension dosage forms were prepared using 0.5% sodium carboxy methyl cellulose for oral route of drug administration of test drugs. These dosage were selected on the basis of the therapeutic dose, which was taken as D6 dose and for the rest of the below dosage, amount of dose reduced in geometric progression (half of the previous high dose, all dosage were within the range of the therapeutic window), for calculation of the effective concentration of the test drug in 50% of the test animal population (EC_{50} value of a test drug, n=6, linear regression) for a particular drug, on our experimental conditions. Half maximal effective concentration EC_{50} value of a test drug of a test drug is a unique characteristic for a drug, to compare the with the other drug, not having similar mechanism of action (Arivazhahan, 2022; B. Srinivasan & Lloyd, 2024).

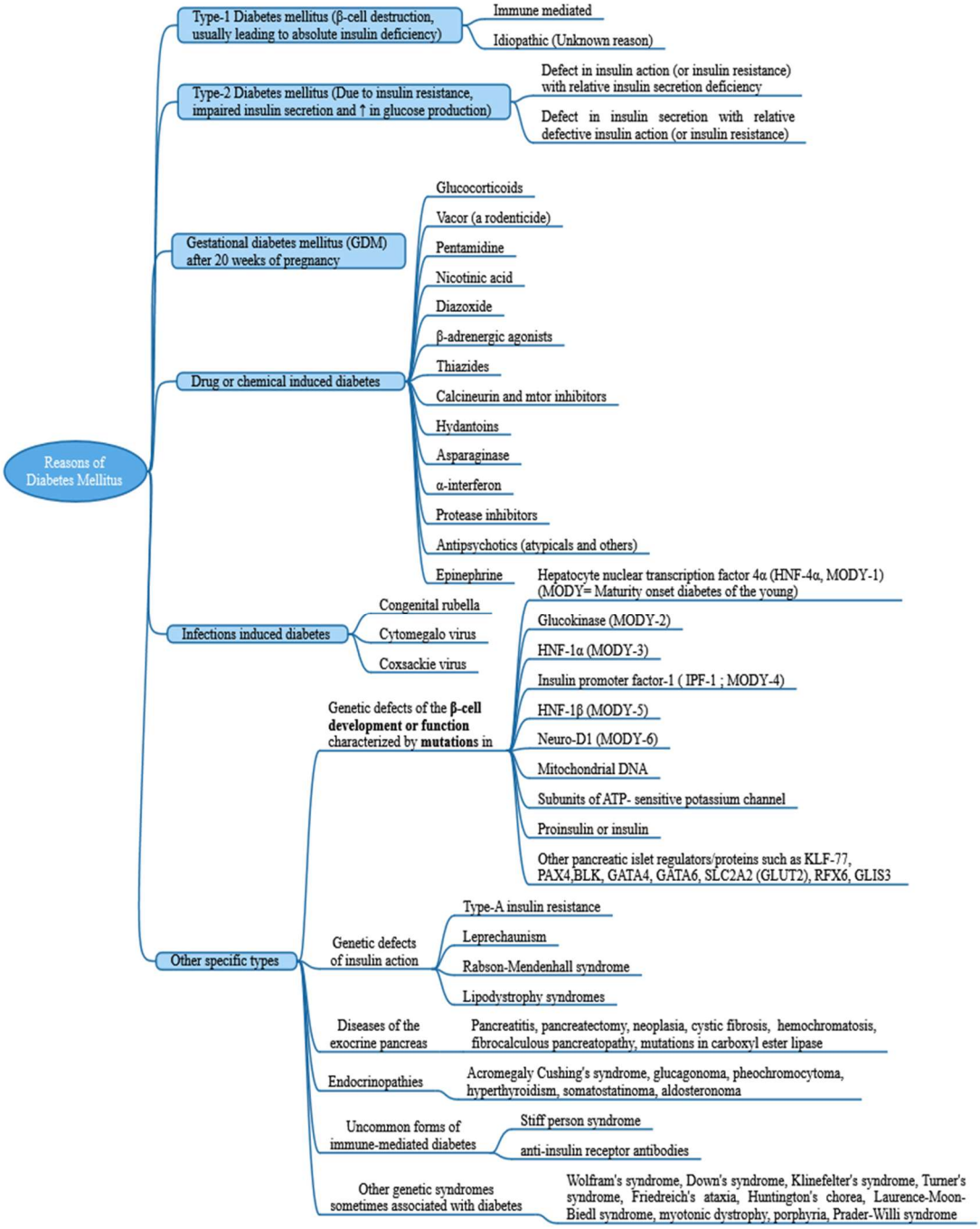

Figure-1: About reported various reasons of the diabetes mellitus in human.

Timings of the study

All data were collected and evaluated for the 121 day period as groups of data for day 0, 28, 31, 76, 121. Daily oral 6 dosage of a test drug preparations treatment at an interval of 24 hours for 90 Days (from Day 31-121) were given to each test animal as per the study design. Duration of Type II Diabetes induction period was 3 Days (Day 28 – day 30) with STZ 35 mg/kg i.p. injection (Goyal et al., 2016) to each animal of HFD+STZ control group. Blood samples collected 2 times pre-prandial and two hour post-prandial (2 hour after a glucose

challenge) were performed on Day 1, Day 28, Day 31, Day 76 and Day 121. Body weight were measured on the each these days. Total duration of study and treatments was 120 days.

B. CONSTANT VARIABLES OF THE STUDY

Animals related variables like Animal strain, feed and water supply to animals, STZ toxicant and test drugs routes of administration, blood collection (for HbA_{1C}, FBG, 2h-BG) etc. were kept constant during the period of the study. Instrumental related variables and experiment environmental conditions related constant variables like temperature, humidity, animal house facilities etc. were kept constant during the period of the study. Variables related to the persons involved in the experimental data collection, test drug preparation, animal handling etc. were also kept constant during the period of the study.

Chemicals and glasswares

Streptozotocin was purchased from Merck, India. Diagnostic kit were purchased from the Transasia Biomedicals Ltd., India. Rat insulin ELISA assay kit was purchased from Merck, India. All other chemicals and solvents were analytical grade. All chemicals used in this study will be purchased from the Sigma Aldrich pvt. Ltd. and HIMEDIA laboratories.

All glassware's used in this study will be procured from the Department of Pharmacology, Maharishi Arvind College of Pharmacy, Ambabari circle, Ambabari, Jaipur, Rajasthan and Bilwal Medchem and Research Laboratory Pvt. Ltd., Sikar.

Experimental Animals

For this randomized-controlled experimental study male albino wistar rats (140–200 g, 6 – 7 week old) were purchased from Bilwal medchem research Pvt. Ltd. Sikar, India. The animals were kept in the animal house facility at 25ºC ± 1ºC, relative humidity of 45% - 55% humidity with a 12 hour dark/light cycle throughout the duration of the study. Animals were fed with a regular nutritional diet with a proper diet plan as per the CCSEA feed standards (Ahmed et al., 2017) and provided with free accessibility to purified water ad libitum. The animals were acclimatized to the environment for 7 days before initiation of the experiment, and standard laboratory feed and water were given. Ethical approval of the this study was taken from the institutional animal ethical committee (IAEC) of the Bilwal Medchem and Research Laboratory Pvt. Ltd., Sikar, Rajasthan, India, with Approval Number BMRL/AD/CPCSEA/IAEC/2018 /2/II was in accordance with the guidelines of the committee for the control and supervision of experiments on animals (CCSEA), department of animal welfare, government of India. After completion of study animals are submitted to the animal house for rehabilitation. Marking of rats for the identification purposes were marked with picric acid, in each group as H, B, T, HB, BT and HT for the head, back, tail, head-back, back-tail and head-tail of albino wistar rats, respectively.

High fat diet (HFD)

The composition of regular fed was of 5% fat, 53% carbohydrate and 23% protein, with total calorific value 25 kJ/kg, while the composition of high-fat diet consisting of 58% fat, 17% carbohydrate, and 25% protein as a percentage of total kcal. The high fat diet was prepared in laboratory as per the procedure described by Srinivasan et al. (2005) and Kulkarni and Garud (2016). High fat diet were given to the animals of HFD, HFD+STZ, all test drug groups, for the whole duration of the study period mentioned.

Type II diabetes mellitus induction

Type II diabetic patients may have a decreased secretion and decreased action of insulin. Therefore, investigators have to use such animal models with decreased secretion and decreased action of insulin. A HFD with low-amount of STZ, closely mimics the natural parameters of the disease. Rats and mice fed with a high energy diet (HED; 58% fat, 25% protein and 17% carbohydrate) caused insulin resistance. Further administration of STZ at a low dose (thirty five mg/kg) induced type II diabetes in rats with minimal toxicity to other organs.

Type II diabetes was induced after 16 hour fasting period using low dose of STZ (35 mg/kg, i.p., in a volume of 1 ml/kg body weight, dissolved in 0.01 M sodium citrate buffer pH 4.5) after 4 weeks of dietary modification with HFD. On the day 31, the animals with fasting blood glucose level greater than 200 mg/dl were considered diabetic and selected for the further study (De MagalhÃes et al., 2019; Pournaghi et al., 2012; Rehman et al., 2023; K. Srinivasan et al., 2005). The effect of STZ depends on its bioavailability and the route of STZ administration in rats, is a determinant for the amount of diabetes induction.

Rat express GLUT2 that specifically uptakes the STZ into beta cells and cause damage to pancreatic islets, ↑ inflammation which leads to the of beta cell activity loss, which further cause deficiency of insulin and high blood glucose level that resembles human type 1 diabetes, but with low dose STZ and HFD, cause insulin resistance, that resembles human type II diabetes mellitus. In male rats STZ produce more diabetogenic action than females (Goyal et al., 2016; Kasper et al., 2015)

Confirmatory assessment of the diabetes

On the day 31, the animals with fasting blood glucose level greater than 200 mg/dl were considered diabetic and selected for the further study (De MagalhÃes et al., 2019; Pournaghi et al., 2012; Rehman et al., 2023; K. Srinivasan et al., 2005)

Self-recovery assessment

Self-recovery was also findout for the rats with FBG less than 150 mg/dl, after the achieved hyper-glycemia (FBG >150 mg/dl) by STZ induction (Fajarwati et al., 2023).

Test drug Treatment schedule

The albino wistar rats were divided into the groups (n=6) as CMC control group, High fat-diet control group (HFD), STZ+HFD control group and groups for each six dosage of each test drug as MetD1, MetD2, MetD3, MetD4, MetD5, MetD6, AyuD1, AyuD2, AyuD3, AyuD4, AyuD5, AyuD6, HomD1, HomD2, HomD3, HomD4, HomD5 and HomD6 groups. CMC control group were recieved 0.5% sodium carboxy methyl cellulose (0.5% CMC) orally whearas HFD group rats were given standardized high fat-diet (58% fat, 17% carbohydrate, and 25% protein as a percentage of total kcal) from day 1 to day 121 and not received any treatment throughout the study. Rest of the all animals received HFD (from day 1 to day 121) + STZ (on day 28) 35 mg/kg ip., 1 ml/kg body weight (Goyal et al., 2016) and received oral test drug treatments (from day 31 to day 121) and named as Met, Ayu, and Hom, in six different dosage as D1, D2, D3, D4, D5 and D6 at the dose mentioned previously, to determine glucose lowering potential and their equivalence ranking by estimating blood HbA1c, FBG, 2h-BG, FBIn, HOMA-IR, ISI levels and body weight in type- II diabetes induced by STZ in rats for 121 days. On day 01-28 only body weight estimations were performed on each animal and subsequently on day 28- Blood estimation (HbA1c, FBG, 2h-BG, Body weight), on day 31, day 76, day 121 - Blood estimation (HbA1c, FBG, 2h-BG, Body weight, Insulin level, HOMA-IR value and ISI) were performed. For determination of equivalence

ranking with the help of calculate EC_{50} values from the % values HbA1c, FBG, 2h-BG, FBIn, HOMA-IR, ISI levels and body weight, and generating a linear regression line between log dose of test drug Vs % responses, so that all drug doses could be equalize and could compare to find out best antidiabetic drug, out of the available antidiabetic drug option, also from different disciplines of the systems of medicine (Boruah et al., 2017; Goyal et al., 2016; Guven et al., 2006; Liang et al., 2017; Price & Thomas, 2006; Singhal et al., 2014).

C. DEPENDENT VARIABLES (Y) OF THE STUDY (PARAMETERS)

Glycated Hemoglobin (HbA_{1c}, on the day 31, 76 and 121, at each 45 days interval, as % of the total hemoglobin during the life cycle of rats RBC), fasting blood glucose (FBG, on the day 28, 31, 76 and 121), two hour postprandial blood glucose (2h-BG, on the day 28, 31, 76 and 121), fasting blood insulin (FBIn, on the day 121), Homeostatic Model Assessment-Insulin Resistance (HOMA-IR, on the day 121), insulin sensitivity index (ISI, on the day 121), body weight (Bd.Wt., on the day 0, 28, 31, 76 and 121) and EC_{50} values (for each test parameter) are individual parameters that evaluated during the mentioned period (Aba & Asuzu, 2018),(De Tata et al., 1996).

1. Fasting blood glucose (FBG, pre-prandial) show efficiency of insulin. 24 h fasting is suggested before measuring blood glucose level on 28, 31, 76 &61th day.

2. Two hour blood glucose (2h-BG) after a glucose challenge is a best diabetes indicator. Calculated of EC_{50} value of a particular test drug was for in above specified laboratory conditions only.

3. Glycated hemoglobin evaluation

Glycohemoglobin was determined in freshly collected whole blood samples obtained at the day 31, day 76 & day 121 of the study using HPLC method on automated glycohemoglobin analyzer as per the manufacturer's protocol (Tosoh Bioscience's, India). HLC-723GX automated glycohemoglobin analyzer is an automated Ion exchange High-Performance Liquid Chromatography, for separation of the haemoglobin fractions by use of a negatively charged column and positively charged buffers that compete with the different haemoglobins to bind to the column (= cation exchange). For the HbA_{1C} level determination 5-10 µl blood required. (George et al., 2012).

4. Fasting blood Insulin

Fasting blood insulin levels for different test drug groups were assayed by an ELISA kit following the manufacturer's protocol on day 121 of the study.

5. Homeostatic Model Assessment - Insulin Resistance (HOMA-IR)

HOMA-IR of were calculated using the formula HOMA-IR = (mmol/l glucose X mIU/L insulin)/22.5 of different test drugs (Oza & Kulkarni, 2018).

6. Insulin Sensitivity Index assessment

Insulin sensitivity index was calculated using the formula ISI = Ln (1/FBIn X FBG) (Oza & Kulkarni, 2018).

7. EC_{50} value of test drugs(George et al., 2012)

For the quantification of the anti-diabetic potential of the test drugs in a comparative manner, antidiabetic

ranking of test drugs as EC_{50} values were calculated. EC_{50} values were calculated by plotting regression lines between six log dosage of test drugs vs percent responses of each mentioned parameter form the day 121. Different test drugs (Met, Ayu and Hom) with six different geometrically progressed dosage (as D1, D2, D3, D4, D5 and D6 at the dose, mentioned previously) and percentage response data of a particular parameter (HbA_{1C}, FPG, 2h-PG, FBIn and Bd.Wt.) in our experimental conditions were evaluated using the graphpad prism software version 9 for Windows.

Statistical Analysis

All data are expressed as mean SD. Results were statistically evaluated by using GraphPad Prism ver. 9.00 for Windows. Significant differences between the experimental groups were assessed by ANOVA (analysis of variance) test followed by bonferroni's multiple comparison test, p-value less than 0.05 were considered to be significant.

Results

Results of glycated Hemoglobin (HbA_{1c}, on the day 31, 76 and 121, at each 45 days interval, as % of the total hemoglobin during the life cycle of rats RBC) shows p-value <0.001 for CMC control vs. HFD+STZ control groups and HFD control vs. HFD+STZ control groups which clearly states that hyperglycemia was induced. In the ordinary one-way ANOVA test followed by bonferroni's multiple comparison test, p-value for HFD+STZ Control vs. dose-1 of all 6 test drugs was non-significant and for HFD+STZ Control vs. dose-6 of all test drugs group <0.001 was found. Similarly, for MetD6 (***), AyuD6 (*) and HomD1 (ns) was found. Homeopathic preparation mother tincture of *Syzygium cumini* was found ineffective in respect to the HbA_{1c} levelMetD1 vs. MetD6 (****) was found.

Results of the study demonstrate that Met, Ayu and Hom test drug treatments reduces the HbA1c FBG, 2h-BG, FBIn levels ($p < 0.001$ significantly).

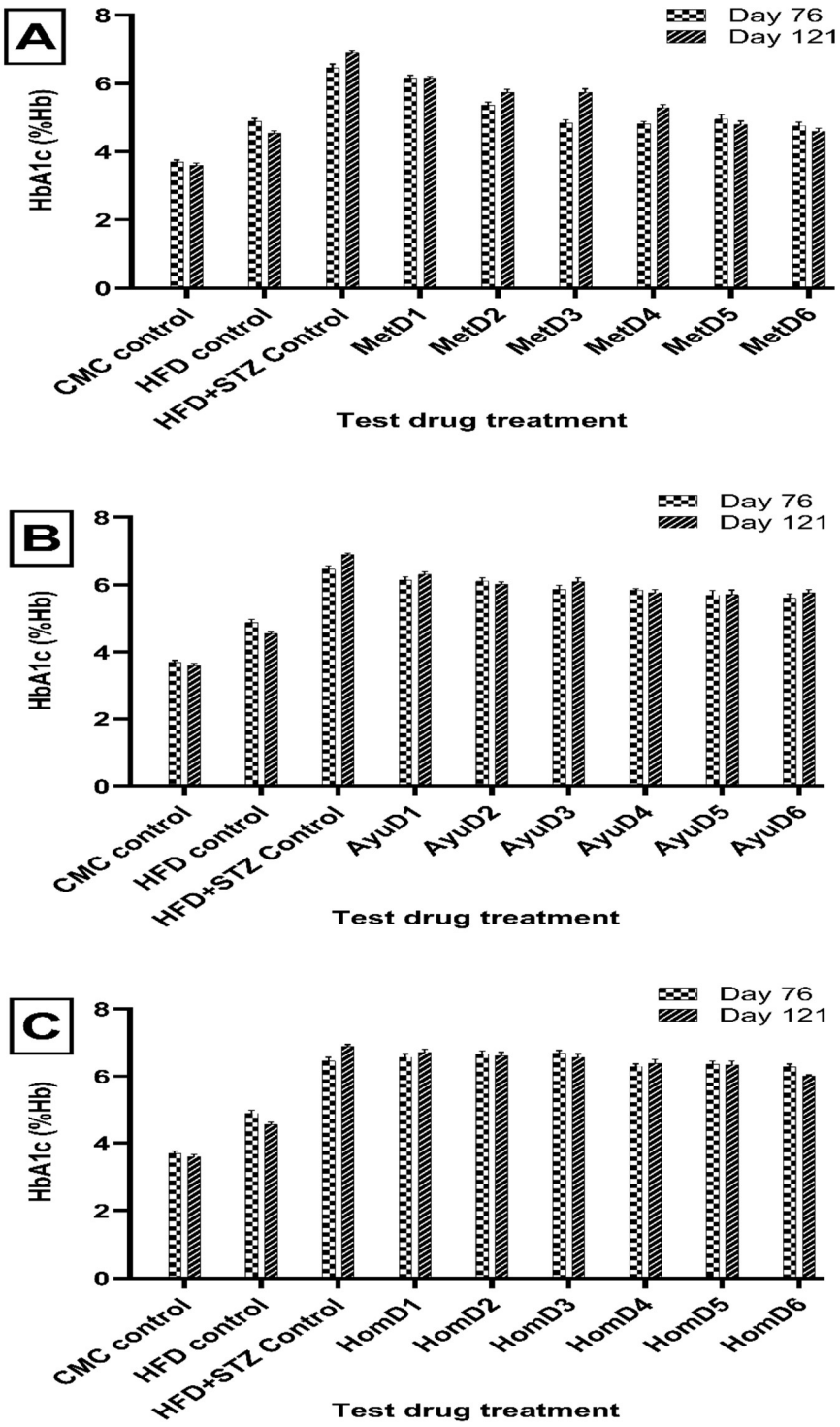

Figure-2: On the day 76 and 121, effect of six different doses of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on HbA1c level (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats (n=6) in comparison to

controls.

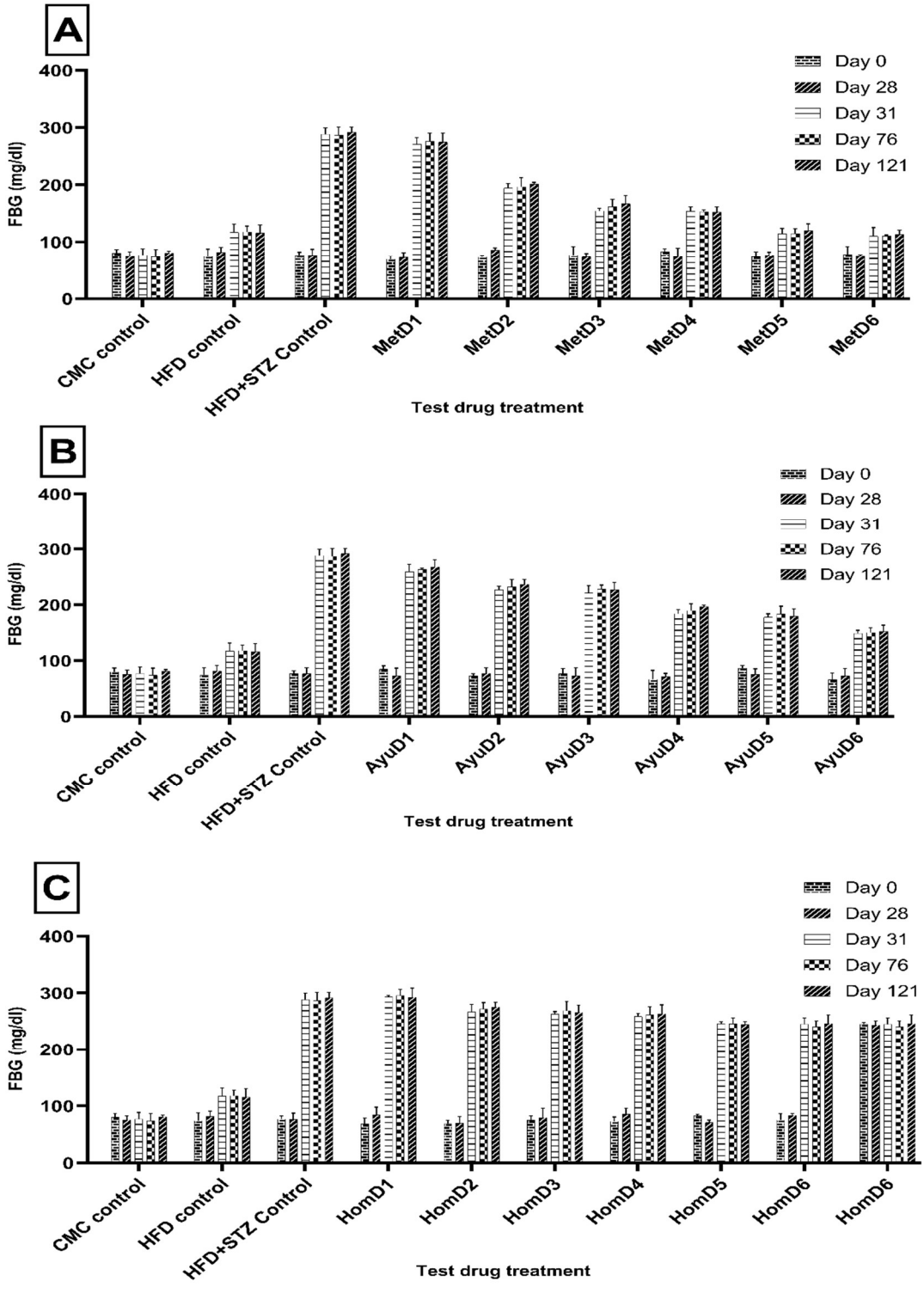
A
Day 0
Day 28
Day 31
Day 76
Day 121
FBG (mg/dl)
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
MetD1
MetD2
MetD3
MetD4
MetD5
MetD6
Test drug treatment
B
Day 0
Day 28
Day 31
Day 76
Day 121
FBG (mg/dl)
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
AyuD1
AyuD2
AyuD3
AyuD4
AyuD5
AyuD6
Test drug treatment
C
Day 0
Day 28
Day 31
Day 76
Day 121
FBG (mg/dl)
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
HomD1
HomD2
HomD3
HomD4
HomD5
HomD6
HomD6
Test drug treatment

Figure-3: On the day 0, 28, 31, 76 and 121, effect of six different doses of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on fasting blood-glucose (FBG) level (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats (n=6) in comparison to controls.

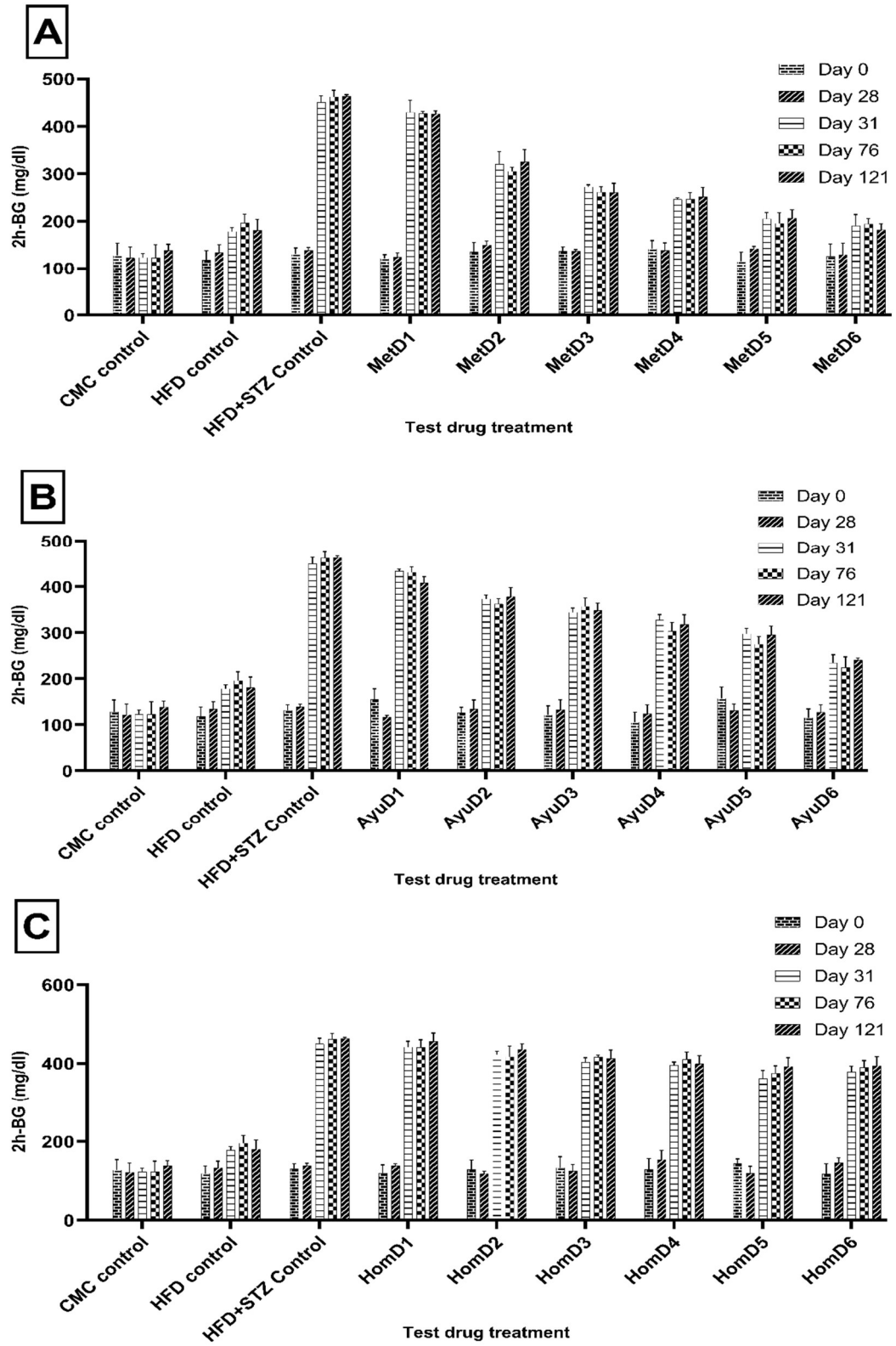

Figure-4: On the day 0, 28, 31, 76 and 121, effect of six different doses of A. metformin (Met), B. aurvedic test

drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on two hour postprandial blood glucose (2h-BG) level (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats (n=6) in comparison to controls.

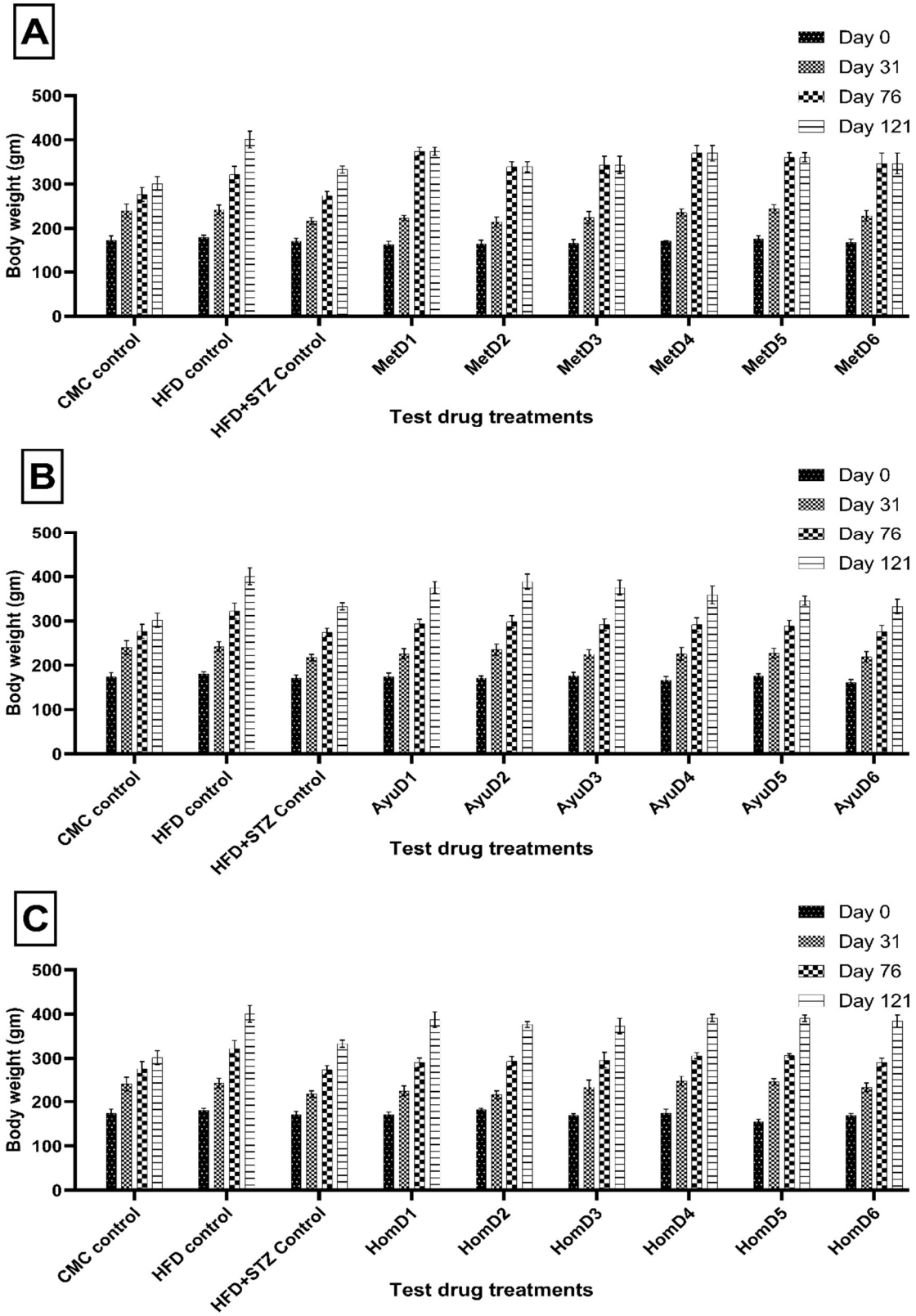
A
Day 0
Day 31
Day 76
Day 121
Body weight (gm)
500
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
MetD1
MetD2
MetD3
MetD4
MetD5
MetD6
Test drug treatments
B
Day 0
Day 31
Day 76
Day 121
Body weight (gm)
500
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
AyuD1
AyuD2
AyuD3
AyuD4
AyuD5
AyuD6
Test drug treatments
C
Day 0
Day 31
Day 76
Day 121
Body weight (gm)
500
400
300
200
100
0
CMC control
HFD control
HFD+STZ Control
HomD1
HomD2
HomD3
HomD4
HomD5
HomD6
Test drug treatments

Figure-5: On the day 0, 28, 31, 76 and 121, effect of six different doses of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on rat body weight (2h-BG) level (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats (n=6) in comparison to controls.

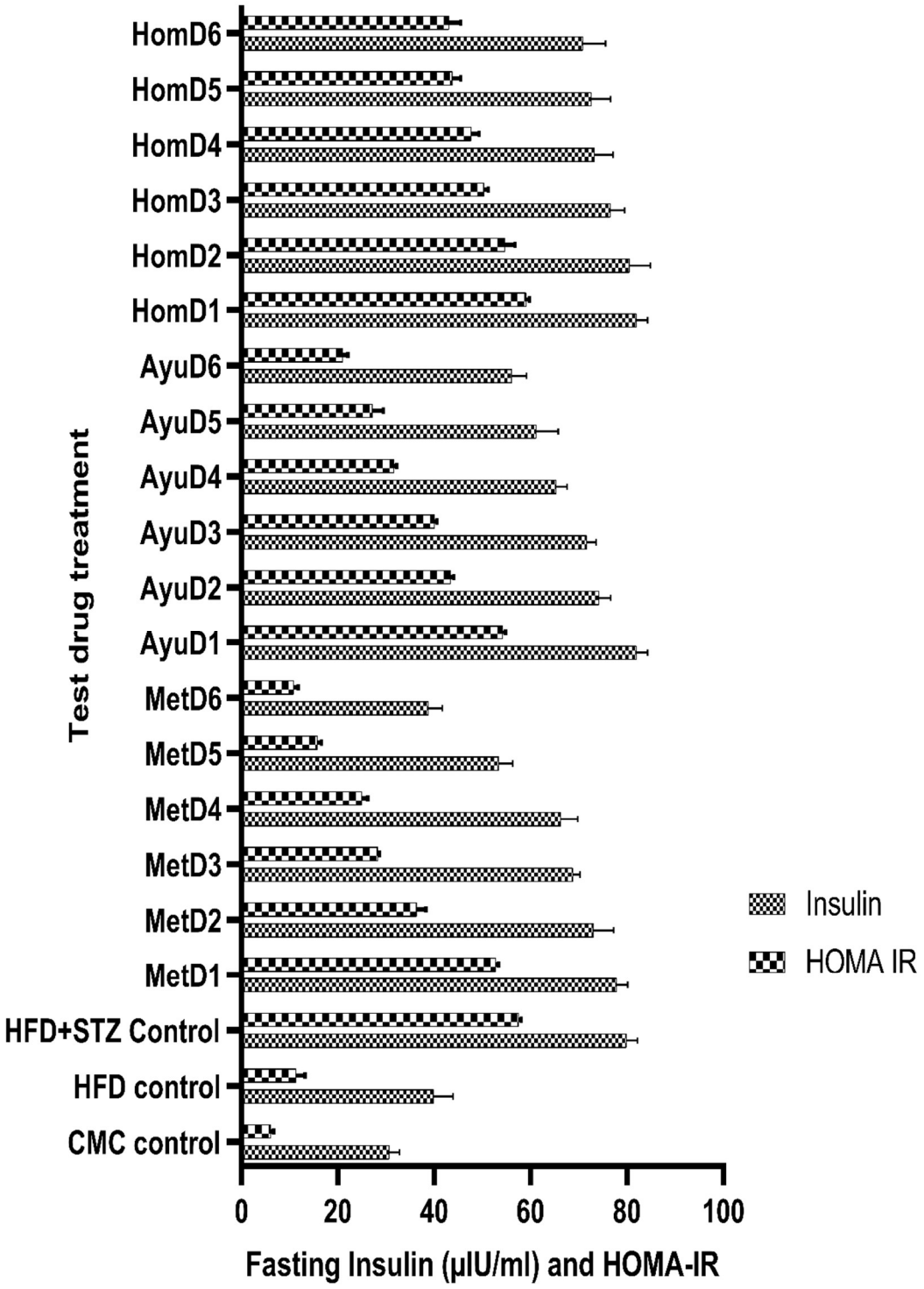

Figure-6: On the day 121, effect of six different doses of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on rat fasting blood insulin (FBIn) level and HOMA-IR values (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats

(n=6) in comparison to controls.

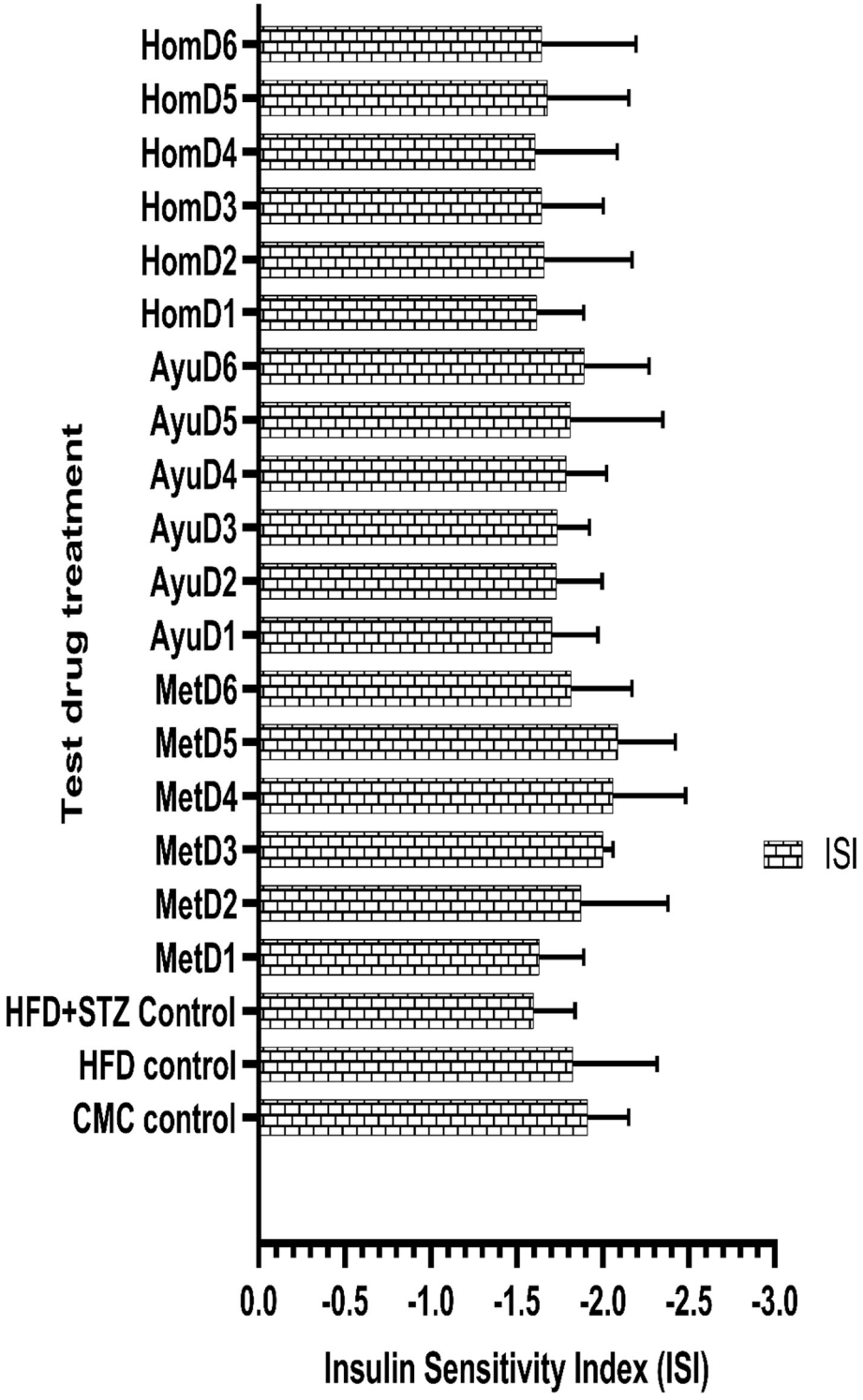

Figure-7: On the day 121, effect of six different doses of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study) on insulin sensitivity index (ISI) values (mean±SD) in the STZ+HFD generated type II hyperglycemic albino wistar rats (n=6) in comparison to controls.

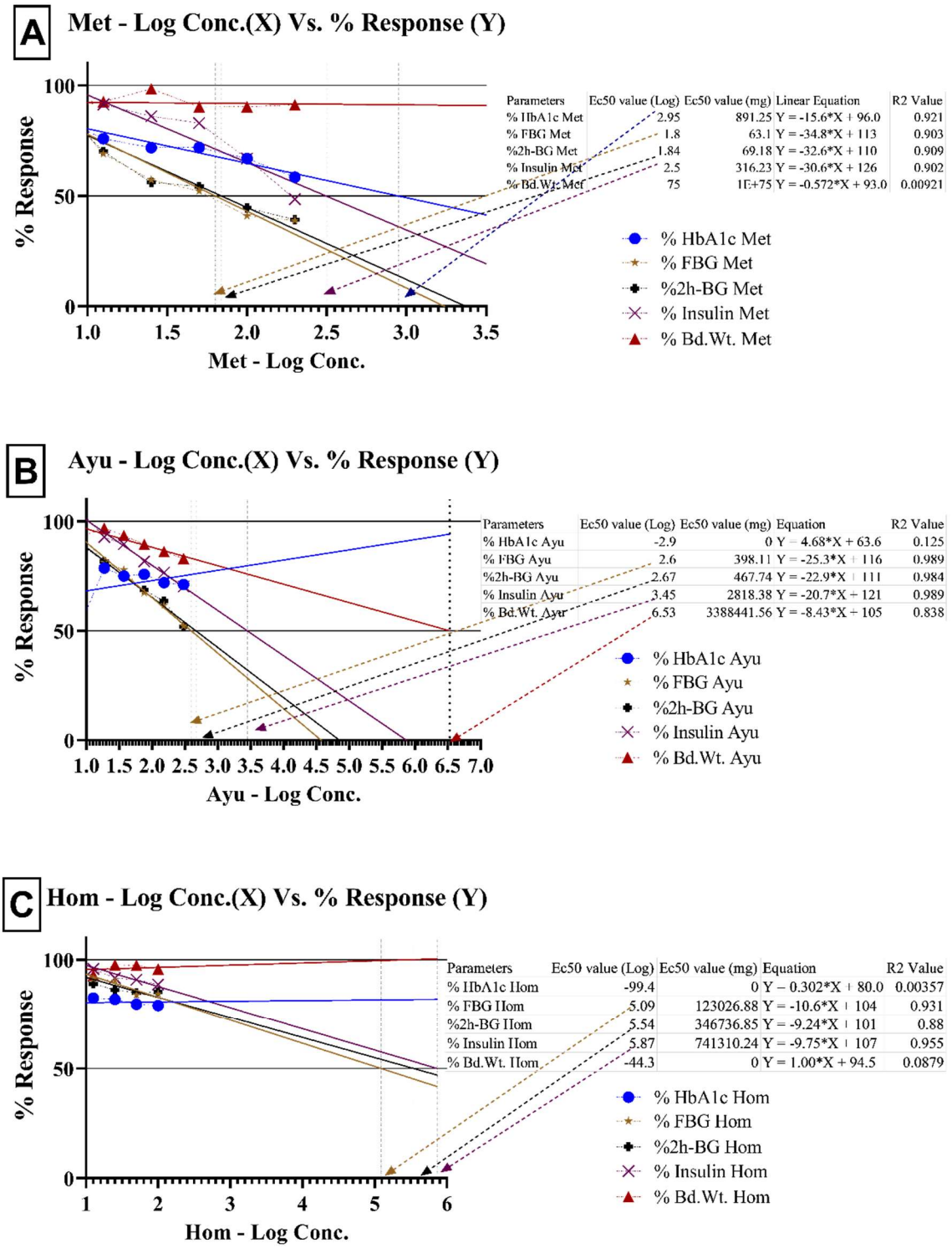

Figure-8: On the day 121, EC50 values (in Log values and mg/kg/day animal body weight) of A. metformin (Met), B. aurvedic test drug preparation (Ayu) and C. homeopathic test drug preparation (Hom) (used in this study), calculated using linear regression analysis of six different doses of each drug, in the STZ+HFD generated

type II hyperglycemic albino wistar rats (n=6) in comparison to controls.

Table-1: Ranking of test drugs (n=6) metformin (Met), aurvedic test drug preparation (Ayu) and homeopathic test drug preparation (Hom) on the basis of calculated EC_{50} values calculated by Log dose vs % FBG, % 2h-BG and % FBIn responses, in log and mg/kg body weight values with linear regression equation and R^2 values.

Ranking of test drugs	Test drug (n=6)	On the basis of %	Calculated Log EC_{50} value	Calculated mg/kg EC_{50} value	Linear Reg. Equation	R^2
1	Met	FBG	1.8	63.1	Y = -34.8*X + 113	0.903
2	Ayu		2.6	398.11	Y = -25.3*X + 116	0.989
3	Hom		5.09	123026.88	Y = -10.6*X + 104	0.931
1	Met	2h-BG	1.84	69.18	Y = -32.6*X + 110	0.909
2	Ayu		2.67	467.74	Y = -22.9*X + 111	0.984
3	Hom		5.54	346736.85	Y = -9.24*X + 101	0.88
1	Met	FBIn	2.5	316.23	Y = -30.6*X + 126	0.902
2	Ayu		3.45	2818.38	Y = -20.7*X + 121	0.989
3	Hom		5.87	741310.24	Y = -9.75*X + 107	0.955

The effectiveness ranking of different test drugs was found as Met > Ayu > Hom on the basis of FBG values, as Met > Ayu > Hom on the basis of 2h-BG values and as Met > Ayu > Hom on the basis of fasting blood insulin.

DISCUSSION

Using log dosage response curves and the EC_{50} value approach for comparing different pharmaceutical systems, this study aimed to demonstrate the hypoglycemic activity of Met, Ayu, and Hom. Based on these findings, doctors can prescribe better and more effective drugs to diabetic patients. We concluded that a combination of drugs is the best option, since it reduces the risk of side effects while protecting vital organs and effectively lowering HbA1c values.

The results suggest that both the Comb and Ayu test medicines have substantial hypoglycemic effects, with the former even outperforming metformin. Further studies on diabetes mellitus should examine the suggested ranking approach, regression analysis, and EC50 values as suitable candidates. In the quest for superior alternatives to the standard treatment methods, this study will serve as a landmark.

CONCLUSION

The efficacy ranking of several test medications was determined to be Met > Ayu > Hom based on FBG values, 2h-BG values, and fasting blood insulin levels.

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CONFLICTS OF INTEREST

There are no conflicts of interest. This study needs further more rigorous anti-diabetic evaluation of various other anti-diabetic parameters.

ABBREVIATIONS

STZ, Streptozotocin; FBIN, fasting blood insulin; HFD, high-fat diet; HOMA, homeostatic model assessment; IR, insulin resistance; ISI, Insulin Sensitivity Index; Met, Metformin; Ayu, Ayurvedic preparation; Hom, Homeopathic preapartion; EC_{50}, Effective concentration in 50 % population.

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