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COMPARATIVE BIOEQUIVALENCE STUDIES OF ANTIDIABETIC DRUGS FOR OPTIMIZED DIABETES TREATMENT

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Abstract:

Diabetes mellitus is a chronic metabolic disorder affecting millions of people worldwide. The development and evaluation of effective anti-diabetic drugs are crucial for managing this disease. Bioequivalence studies play a significant role in assessing the comparability between different drug formulations, ensuring their safety and efficacy. These studies aim to determine the extent to which different drug formulations exhibit similar bioavailability and therapeutic effects. The selection of the rat model for these investigations is justified by its anatomical and physiological similarities to humans, making it a valuable tool for preclinical assessments. Various anti-diabetic drug classes, including biguanides, sulfonylureas, thiazolidinediones, and dipeptidyl peptidase-4 (DPP-4) inhibitors, have been investigated in rat bioequivalence studies. Pharmacokinetic parameters, such as maximum plasma concentration (Cmax), time to reach Cmax (Tmax), and area under the plasma concentration-time curve (AUC), are commonly measured to assess drug absorption, distribution, metabolism, and elimination.

Keywords: Anti Diabetic, Bioequalence Studies, Examine, Glimepiride, Glybenclamide, Metformin, Branded And Government Drug.

Introduction:

Diabetes

According to the World Health Organization (WHO), diabetes mellitus (DM) is described as a diverse metabolic disorder exhibiting a shared characteristic of persistent high blood glucose levels, accompanied by disruptions in the metabolism of carbohydrates, fats, and proteins.

CHEMICALS

Alloxan was procured from Sd-Fine Chem. Limited, India. Standard drugs Glibenclamide, Glimepiride, Metformin were procured from Government supplied medicines and open marketed supply. All the other chemicals used were of analytical grade.

5.4.1.2. INSTRUMENTS

Electronic balance, Glucometer (Dr.Morepen®Gluco-one)

5.4.1.3. EXPERIMENTAL ANIMALS:

- The study utilized experimental animals of both genders weighing between 250-350g. Prior to conducting the study, the research protocol underwent thorough review and approval by the institutional animal ethics committee of Santhiram College of Pharmacy (Approval number: 1519/PO/a/11/CPCSEA). The study adhered to the guidelines outlined by the Indian National Science Academy for the appropriate use and care of experimental animals in research.
- The animals were procured from Invivo Bio Sciences, located in Bangalore. They were housed in polyacrylic cages measuring 38x23x10 cm. The animals were kept in a controlled environment, including an air-conditioned room, under standard laboratory conditions. The lighting conditions followed a natural light and dark cycle, with approximately 14 hours of light and 10 hours of darkness. The humidity was maintained at 60±5%, and the ambient temperature was kept at 25±2°C. All experiments were conducted between 9:00 am and 4:00 pm.
- During the acclimatization period, the animals had unrestricted access to a standard diet and water ad libitum. They were allowed to adjust to the laboratory environment for one week before the commencement of the experiments. The commercial pellet diet provided to the animals contained 22% protein, 4% fat, 4% fiber, 36% carbohydrates, and 10% ash (w/w).

5.4.1.4. Preparation of drug and mode of administration:

Glimepiride, Glibenclamide, Metformin were formulated as aqueous suspension with 1% CMC (Carboxy methyl cellulose) as suspending agent. The suspensions of these all drugs were administered orally consecutively for 7 days. The suspension was freshly prepared immediately before use and the Animals.

ANTIDIABETIC STUDY

In the present study, diabetes was induced by single intraperitoneal injection of alloxan (150mg/kg) (katsumata *et al*., 1992). The alloxan was freshly prepared by dissolving 150 mg of alloxan in 1ml of normal saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia.

72 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of > 200 mg/dl were included in groups II-VIII.

The rats were divided into 8 groups consisting of six rats in each group, the animals treated for 7 days.

Table -1: Treatment Schedule

S.NO	Group	No. Of Animals	Treatment	Treatment period (days)
1.	Group-I	6	1% CMC (10ml/kg. P.O)	7
2.	Group-II	6	Alloxam (150mg/kg i.p)	7
3.	Group-III	6	Alloxam (150mg/kg i.p) + Glimepiride(0.1mg/kg)-govt	7
4.	Group-IV	6	Alloxam(150mg/kgi.p)+	7

			Glibenclamide(10mg//kg)-govt	
5.	Group-V	6	Alloxam (150mg/kgi.p)+ Metformin(100mg/kg)-govt	7
6.	Group-VI	6	Alloxam (150mg/kg i.p) +Glimepiride(0.1mg/kg)-branded	7
7.	Group-VII	6	Alloxam (150mg/kg i.p)+ Glibenclamide(10mg/kg)-branded	7
8.	Group-VIII	6	Alloxam (150mg/kg i.p)+ Metformin(100mg/kg)	7

PARAMETERS EVALUATED

5.4.3.1. BODY WEIGHT:

Weight of each rat was recorded on day 0 and 7 day of study and at termination to calculate relative organ weights. From the data, the groups mean body weights and percent body weight gain were calculated.

5.4.3.2. RELATIVE ORGAN WEIGHT:

After dosing period of 7^{th} days autopsies on all the animals are performed and relative Organ weights of internal body organs are recorded.

5.4.3.3. BLOOD GLUCOSE LEVEL

- Before test, please clean hands with soap, water and also use an alcoholic cotton cloth to clean finger and then dry well.
- By using the iancet rupture the vessel on tail tip of animal and place drop of blood on glucometer strip.
- Touch the blood drop to the sample channel at the end of the strip.
- When you hear the beeper, you can start the test. After 5 seconds, the result will appear.

5.4.4. STATISTICAL ANALYSIS

The results are expressed as means ± SEM. Statistical analysis was performed by Two-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. Statistical significance set accordingly.

BIO-EQUALENCE STUDY:

In this three parameters include:

1. Body weight
2. Blood glucose level
3. Relative organ weight

1. Body weight:

- Animals treated with alloxan showed a significant decrease in body weight in Group-II compare with Group-I.($P<0.001$).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) did not showed any significant changes or abnormal changes in body weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) did not showed any significant changes or abnormal changes in body weight in Group-IV and Group-VII.
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) did not showed any significant changes or abnormal changes in body weight in Group-V and Group-VIII.
- The results showed in the tale no.1 and Graph no.1, 2.
- Above three tested drugs of two different brands were showing a similar response on body weight.

S.No	**Groups**	**Treatment**	**Body weight (mean + SEM)**	
			0 day	**7th day**
1	Groups I	1% vehicle, 1%CMC (10mg/kg)	320.0 ± 6.83	319 ± 6.88
2	Groups II	Alloxon (150mg/kg) – (IP)	313.3 ± 6.14 -ns	163.3 ± 6.791 -###
3	Groups III	Glimipiride (Govt) + alloxon – (O.P)	321.7 ± 6.54 -ns	337.5 ± 3.594 -***
4	Groups IV	Glibenclamide (Govt) +alloxon– (O.P)	305.6 ± 3.416 -ns	331.7 ± 5.426 -***
5	Groups V	Metformin (Govt) + alloxon– (O.P)	300.0 ± 4.47 -ns	330.0 ± 4.472-***
6	Groups VI	Glimipiride (brd) + alloxon– (O.P)	298.3 ± 4.77 -ns	321.7 ± 8.333-***
7	Groups VII	Glibenclamide (brd) +alloxon– (O.P)	340.0 ± 5.16 -*	365.0 ± 7.63-***
8	Groups VIII	Metformin (brd) + alloxon– (O.P)	340.0 ± 8.16 -*	313.3 ± 8.02-***

Table no.1: Body weight

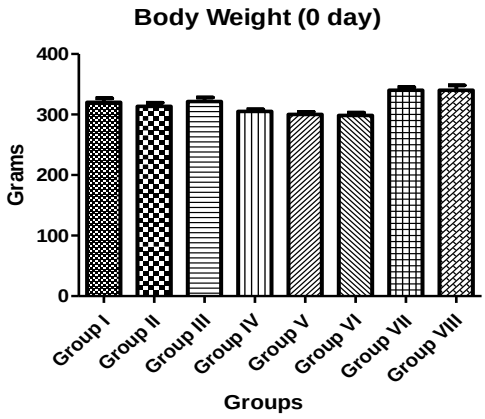


Fig.no.1 Body weight (0 day)

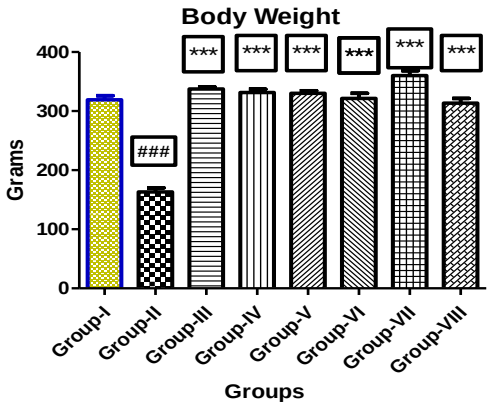


Fig.no.2 Body weight(after treatment)

2. Blood glucose level:

- Animals treated with alloxan showed a significant increase in blood glucose level in all group rats except Group-I after 72hrs of alloxan treatment when compared to Group-II (P<0.001).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicine) showed a significant decrease in elevated blood glucose level compare to Group-II (P<0.001).
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) showed a significant decrease in elevated blood glucose level compare to Group-II (P<0.001).
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) showed a significant decrease in elevated blood glucose level compare to Group-II (P<0.001).
- The results showed in the tale no.2 and Graph no.3, 4
- Above three tested drugs of two different brands showed a similar response on blood glucose level.

Table no.2 blood glucose level

S.No	Groups	Treatment	Alloxon (mean ± SEM)	
			0 day (after 72 hrs of alloxon treatment)	7th day (after treatment of doses)
1	Groups I	1% vehicle, 1%CMC (10mg/kg)	129.3 ± 38.31	129.3 ± 38.31
2	Groups II	Alloxon (150mg/kg) – (IP)	383.0 ± 30.75 -###	343.7 ± 38.09 -###
3	Groups III	Glimipiride (Govt) + alloxon – (O.P)	226.5 ± 6.02 -***	127.3 ± 6.10 -***
4	Groups IV	Glibenclamide (Govt) +alloxon– (O.P)	231.2 ± 7.55 -***	112.3 ± 8.27 -***

5	Groups V	Metformin (Govt) + alloxon–(O.P)	227.7± 7.33 -***	138.8 ± 4.22 -***
6	Groups VI	Glimipiride (brd) + alloxon–(O.P)	241.5± 5.03-***	125.2 ± 6.69 -***
7	Groups VII	Glibenclamide (brd) + alloxon–(O.P)	227.0± 10.38 -***	141.2 ± 16.66 -***
8	Groups VIII	Metformin (brd) + alloxon–(O.P)	229.2± 5.97 -***	123.8 ± 8.21 -***

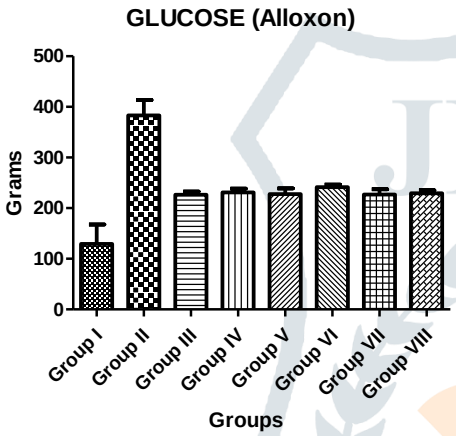


Fig.no.3 Blood glucose level (0 day)

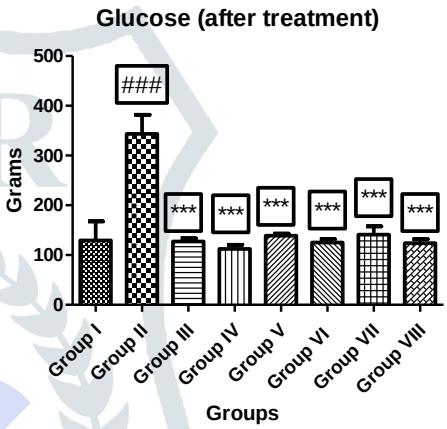


Fig.no.4 Blood glucose(after treatment)

3. Relative organ weight:

(i) Liver:

- Animals treated with alloxan showed a significant decrease in Liver weight in Group-II compare with Group-I.(P<0.001).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) showed a significant decrease in Liver weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) showed a significant decrease in Liver weight in Group-IV and Group-VII.
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) showed a significant decrease in Liver weight in Group-V and Group-VIII.
- The results showed in the tale no.3 and Graph no.5.

(ii) Kidney:

- Animals treated with alloxan showed a significant decrease in Kidney weight in Group-II compare with Group-I.(P<0.001).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) showed a significant decrease in Kidney weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) showed a significant decrease in Kidney weight in Group-IV and Group-VII.

- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) showed a significant decrease in Kidney weight in Group-V and Group-VIII.
- The results showed in the tale no.3 and Graph no..6

(iii) Pancreas:

- Animals treated with alloxan showed a significant decrease in Pancres weight in Group-II compare with Group-I.($P<0.001$).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) showed a significant decrease in Pancreas weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) showed a significant decrease in Pancreas weight in Group-IV and Group-VII.
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) showed a significant decrease in Pancreas weight in Group-V and Group-VIII.
- The results showed in the tale no.3 and Graph no..6

(iv) Heart:

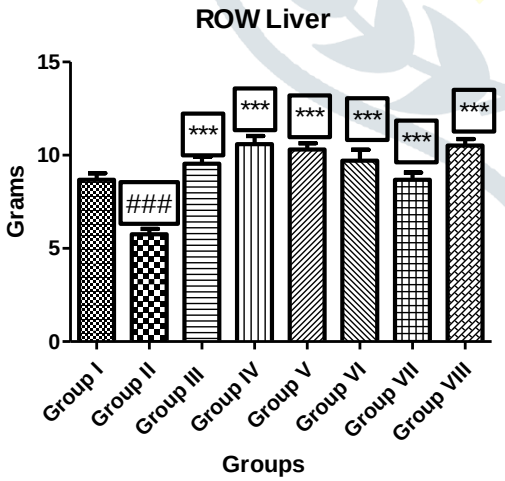
- Animals treated with alloxan showed a significant decrease in Heart weight in Group-II compare with Group-I.($P<0.001$).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) showed a significant decrease in Heart weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) showed a significant decrease in Heart weight in Group-IV and Group-VII.
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) showed a significant decrease in Heat weight in Group-V and Group-VIII.
- The results showed in the tale no.3 and Graph no.7

(v) Brain:

- Animals treated with alloxan didnot showed any significant changes in Brain weight in Group-II compare with Group-I.($P<0.001$).
- Two different brands of Glimepiride received rats (Group-III received a government supplied medicines and Group-VI received a open marketed medicines) didnot showed any significant changes or abnormal changes in brain weight in Group-III and Group-VI.
- Two different brands of Glibenclamide received rats (Group-IV received a government supplied medicines and Group-VII received a open marketed medicines) didnot showed any significant changes or abnormal changes in Brain weight in Group-IV and Group-VII.
- Two different brands of Metformin received rats (Group-V received a government supplied medicines and Group-VIII received a open marketed medicines) did not showed any significant changes or abnormal changes in Brain weight in Group-V and Group-VIII.
- The results showed in the tale no.3 and Graph no.8
- Above three tested drugs of two different brands showed a similar response on Organ weight (Liver, Kidney, Pancreas, Heart, Brain).

Table no.3: Relative organ weight

S.No	Groups	Treatment	Relative organ weight				
			Liver	Pancreas	Brain	Heart	Kidney
1	Group I	Normal	8.67 ± 0.35	0.66 ± 0.09	2.06 ± 0.07	1.16 ± 0.03	2.23 ± 0.21
2	Group II	control	5.75 ± 0.28 -###	0.02 ± 0.02 -###	2.01 ± 0.05 – ns	0.69 ± 0.03 -###	1.37 ± 0.04 -###
3	Group III	Glimipiride (Govt)	9.54 ± 0.37 -***	0.71 ± 0.05 -***	2.03 ± 0.05 – ns	0.27 ± 0.02 -***	2.14 ± 0.13 -**
4	Group IV	Glibenclamide (Govt)	10.59 ± 0.44 -***	0.75 ± 0.05 -***	2.17 ± 0.03 -ns	1.46 ± 0.11 -***	2.213 ± 0.06 -***
5	Group V	Metformin (Govt)	10.31 ± 0.33 -***	0.71 ± 0.05 -***	1.99 ± 0.05 -ns	1.24 ± 0.05 -**	2.03 ± 0.09 -*
6	Group VI	Glimipiride (brd)	9.70 ± 0.58 -***	0.67 ± 0.04 -***	1.85 ± 0.10 -ns	1.08 ± 0.06 -***	2.33 ± 0.14 -***
7	Group VII	Glibenclamide (brd)	8.68 ± 0.38 -***	0.73 ± 0.07 -***	1.86 ± 0.06 -ns	1.24 ± 1.12 -***	1.79 ± 0.04 -ns
8	Group VIII	Metformin (brd)	10.52 ± 0.34 -***	0.74 ± 0.04 -***	2.04 ± 0.17 -ns	1.42 ± 0.04 -***	1.07 ± 0.12 -**



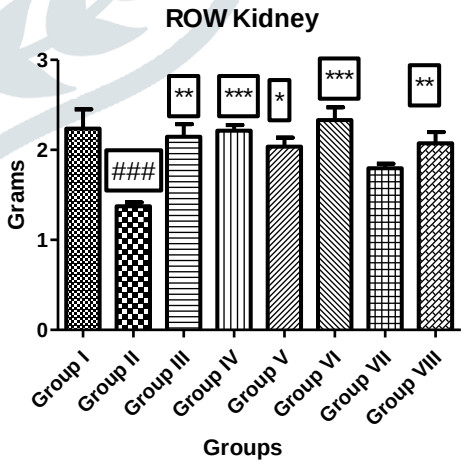


Fig.no.5 organ weight of Liver **Fig.no.6 organ weight of Kidney**

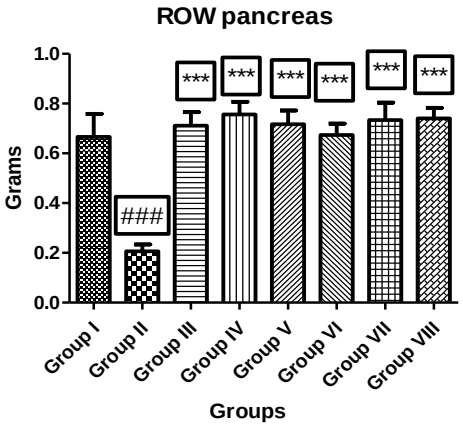


Fig.no.7 organ weight of Pancreas

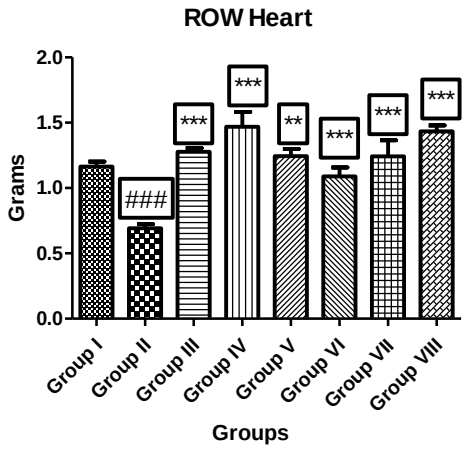


Fig.no.8 organ weight of Heart

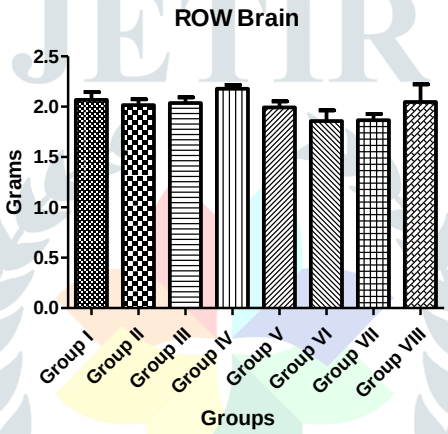


Fig.no.8 organ weight of Brain.

6.4. Discussion:

We showed that evaluated physico chemical characteristics of antidiabetic drugs like Glimepirede, Glibenclamiode and metformin can represent the weight varaition, hardness, thickness, friability ,content uniformity test, disintigration , dissolution, and UV spectroscopy method. The similarity factors comparing with the open market drugs and government supplied medicines. all the brands are open market drugs used were within shelf life at the time of study these open market drugs and government drugs of antidiabetic drugs like Glimepiride, Glibenclamide, and metformin obtained from different retail prices pharmacy out lets with in a logos were subjected to a few pharmacopeial test in order to assay their biopharmaceutical equivalence the assessment gives upto equal from the mean value.

The results of different antidiabetic drugs complies bioequivalence

Conclusion:

We were taken the antidiabetic drugs like glimepiride, glibenclamide, metformin both government supplied medicines and open marked drugs together with the originator have been subjected to Evaluation test, analysis according to the monograph of Indian Pharmacopoeia (I.P) and also performed bioequalence study. The results have shown that all the tested brands satisfied the I.P, requirements in terms of identification, assay and dissolution. The conclusion was Government medicines and open marketed medicines both are equalent in activity and quality.

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