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EFFECTS OF SOME ANTIDIABETIC, ANTIHYPERTENSIVE, ANTIHYPERLIPIDEMIC AND ANTIPSYCHOTIC DRUGS ON INDIRECT BILIRUBIN LEVELS OF RABBITS (PART 1)

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ABSTRACT

This paper is a part of experimental studies done on chronic effects of different combination of drugs which are commonly used to treat hypertension, diabetes, hyperlipidemia and psychosis. In this study the effects of some commonly used drugs were observed on the serum level of unconjugated bilirubin. Male and female albino rabbits were divided into 9 groups of 7 animals in each. Treatments were given as: Group I (Distilled water 2 ml /kg), Group II (Risperidone 0.17 mg/kg), Group III (Olanzapine 0.17 mg/kg), Group IV (Metformin 22 mg/kg), Group V (Pioglitazone 0.5 mg/kg), Group VI (Losartan 0.7 mg/kg), Group VII (Candesartan 0.3 mg/kg), Group VIII Atorvastatin 0.35 mg/kg) and Group IX (Simvastatin 0.35 mg/kg). After 60 days of drug administration the serum indirect bilirubin level was determined. It was found that Simvastatin increased whereas Metformin decreased the level of indirect bilirubin significantly ($P<0.05$).

KEYWORDS: Diabetes, Bilirubin, Metformin, Simvastatin, indirect bilirubin.

INTRODUCTION

Antihypertensives such as Angiotensin receptor blockers (ARBs) are the drugs that acts on angiotensin and inhibit the physiological response produced by this hormone. Angiotensin as

a part of RAAS (renin angiotensin aldosterone system) family is responsible to maintain the blood pressure and helps kidney in regulating its glomerular filteration rate (GFR). (Hirohama et al., 2017) Angiotensin is formed by a series of steps in which Angiotensinogen first convert into angiotensin 1 and then angiotensin 2. The enzyme involved in the second step is angiotensin converting enzyme. Once angiotensin 2 is formed it acts on its receptor for which it has high affinity. Many drugs target these steps to elicit their pharmacological actions. (Verdecchia et al., 2008) There are two types of angiotensin receptors AT1 and AT2 and produce its action by Gq coupled mechanism. The ARBs competitively inhibit the binding of angiotensin to its receptor, these drugs can be used to treat hypertension, cardiac diseases and renal problems with less effect on GFR. (Zhang et al., 2017). They can be used for the treatment of many diseases like hypertension, myocardial infarction and congestive cardiac failure and as per new research it can also be used for inflammatory conditions like arthritis and atherosclerosis. (Hamilton., 2018).

Antilipemic drugs such as statins act on 3-hydroxy-3-methylglutaryl (HMG)-CoA. It is an enzyme that is involved in the synthesis of cholesterol by forming an important intermediate compound i.e. mevalonate. In a person who has high levels of cholesterol in body, the HMG CoA reductase inhibitors are prescribed to lower down the cholesterol level and to prevent from various health problems such as atherosclerosis, cardiac over load and several other conditions (Leone et al., 2018). Antilipemic drugs inhibit the action of HMG CoA reductase by binding to this enzyme, thus acting as competitive inhibitor for the substrate and decreasing the cholesterol synthesis (Istvan., 2001).

Oral antidiabetic agents are most commonly used to treat type 2 diabetes Mellitus, which is an insulin independent diabetes and in which the high level of glucose is due to the insulin resistance and oral therapy is thus required to bring the glucose level to normal. These drugs are divided into different classes such as thiazolidinedione, sulphonyl ureas, biguanides and α-glucosidase inhibitors. (Krentz & Bailey., 2012).

Metformin acts by improving the uptake of glucose and the sensitivity of insulin. It can treat several metabolic disorders with lesser side effects and a very good ability to decrease the hyperglycemic crisis over other oral antidiabetics by variety of mechanisms in liver and intestine to affect the process of gluconeogenesis (Rena et al., 2017). Metformin can increase the activity of tyrosine kinase, it is the receptor through which insulin acts. Metformin acts as antioxidant, it increases the activity of glucose 6 phosphate dehydrogenases. It increases

transport of type 4 glucose transporter (GLUT4) across the plasma membrane and increases glycogenesis. Polycystic ovary syndrome which is associated with hyperinsulinemia and type 2 diabetes can also be treated by Metformin. (Carmina., 2014).

Similarly, Pioglitazone also can decrease the resistance to insulin and improves the insulin sensitivity, it can also improve the function of β-cell. The overall effect of these drugs is the improvement in hyperglycemic condition and prevent the worsening of diabetes. (Kahn et al., 2006). It belongs to the class of Glitazones and acts as an agonist to those nuclear receptors that are involved in gene transcription of glucokinase (GK), glucose transporter 4 (GLUT4), lipoprotein lipase and fatty acyl- CoA synthase. All these are involved in the metabolism of lipid, carbohydrates and fats. It promotes the action of insulin in tissues of liver, skeletal muscles, and fatty tissues. The utilization of glucose in periphery is increased while the production in liver is decrease by it (Mahadik et al., 2013) It produces its action by lipin 1, which is a protein involved in synthesis of triacylglycerol (TAG), in growth and maturation of adipocytes, use and management of glucose and fatty acids stores that are present in tissues and as a co-activator for the modulation and transcription of genes that are responsible for oxidation of fats. (Finck et al., 2006).

Antipsychotic drugs are used to treat different mental disorder by acting on various neurotransmitters that are released in brain. These antipsychotics are divided into two main classes that are classical (first generation) and atypical (second generation). The first-generation antipsychotics are also called typical antipsychotics for example Chlorpromazine and Haloperidol. First generation drugs act on dopamine receptor D_2 as antagonist, and found to have more side effects like impairment of cognitive function so now a day their use has been decreased and replaced by atypical antipsychotics. (Lieberman., 2005).

Second generation antipsychotics or atypical antipsychotics like olanzapine and risperidone, have a different mechanism of action as compared to the first generation. They act on different receptors such as adrenergic, 5HT receptor and have a little action on dopamine receptor. These agents have less side effects and better therapeutic profile as compared to typical antipsychotics so now a days they are widely used to treat different psychiatric conditions such as schizophrenia, epilepsy etc. (Buksh et al., 2014).

Bilirubin is formed when heme is broken down and biliverdin is reduced. It is pigment of bile that is released in plasma and go to liver to be conjugated. (Ruinan & Zheng., 2014). If the

concentration of bilirubin is increased, then it may lead to a disorder called jaundice. The word jaundice is derived from a Latin word "galbinus" which means yellow green coloration this coloration occurs due to the abnormal functioning of liver that leads to hyperbilirubinemia. (Kathpalia & Joseph., 2015).

Bilirubin is further divided into two forms i.e. conjugated and unconjugated bilirubin. The conjugated bilirubin is also called as direct bilirubin. It is that bilirubin which is taken up by liver and is conjugated to form a bilirubin diglucuronide which is soluble in water and is excreted out from body in the form of bile while the unconjugated bilirubin which is also called as indirect bilirubin is not soluble in water, but soluble in lipids because it is not conjugated with glucuronic acid. To convert into water soluble form, it must go to liver, it remains in blood in association with different proteins that are found in plasma (Szabo.,2014). The level of both the conjugated and unconjugated form should remain in the limit so that the total bilirubin level do not exceed. If the level increases, it indicates any hepatic disorder (Boland etal., 2014).

The unconjugated bilirubin level may increase due to different reasons. The first reason might be the excessive breakdown of heme i.e. hemolysis then the liver capacity to conjugate is not sufficient for this excessive production of bilirubin. The other reason might be the loss of liver's ability to conjugate. It may be due to any reason in which the enzyme bilirubin-UGT(Uridine Diphosphate-Glucuronosyltransferase) which is required for conjugation is not available. It may also be due to any congenital disease like Crigler-najjar syndrome and Gilbert syndrome (Chaouch.,2014). The increase in level of unconjugated bilirubin can also result due to any problem in which erythropoiesis is not efficient. Different other conditions can also contribute to the increased level of indirect bilirubin such as in case of surgery, any heart disease, improper clearance rate, direct bilirubin's hydrolysis by enzyme beta-glucuronidase and returning into blood from enterohepatic circulation. Different hormones such as thyroid hormone and ethynyl estradiol can also increase this hydrolysis, different hepatic diseases such as hepatitis, cirrhosis and some drugs such as antibiotics and probenecid can also increase unconjugated bilirubin level (Merchant., 2014).

MATERIALS AND METHODS

The study was carried out after the approval from Board of advanced studies and research, University of Karachi. All experimental procedures were in accordance with the ethics of research using animals.

Male and female healthy rabbits of 1.2 to 1.5 kg body weight were purchased from a local supplier. They were kept for a conditioning period of a week in the animal house, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. The animals were maintained on standard feed and water ad libitum, at an ambient temperature between 22-25 °C, with a 12 h light and dark cycle. Animals were randomly divided into 9 groups with 7 rabbits in each group.

All group of animals received drugs orally. Group I served as control and was given 2ml/kg distilled water. Other groups were administered drugs as described below:

Group II	Risperidone	0.17 mg/kg
Group III	Olanzapine	0.17 mg/kg
Group IV	Metformin	22 mg/kg
Group V	Pioglitazone	0.5 mg/kg
Group VI	Losartan	0.7 mg/kg
Group VII	Candesartan	0.3 mg/kg
Group VIII	Atorvastatin	0.35 mg/kg
Group IX	Simvastatin	0.35 mg/kg

Duration of treatment was 60 days.

Laboratory Assay

The blood sample was collected on day 60, the sample was collected in plain tubes from heart and kept for 6 hours and then centrifuged in order to get clear serum. The concentration of indirect bilirubin in serum was measured with the help of kit method (by taking the difference of total bilirubin and direct bilirubin).

Statistical Analysis

Data was analyzed by SPSS version 13.0 and t-test was used for independent samples test.

$P \leq 0.05$ was considered significant and $P \leq 0.01$ was considered highly significant

RESULTS AND DISCUSSION

Results indicate that amongst the drugs which were tested in this study only metformin and simvastatin had significant effect on indirect or unconjugated bilirubin (refer table; figure 1).

Bilirubin is a heme metabolism product which may have some beneficial effects on various organs like heart and coronary vessels. It also possess antioxidant and anti-inflammatory

properties (Mao et al., 2018). When the concentration of bilirubin increases so much that the liver enzyme bilirubin UGT that converts bilirubin into conjugated form and excrete it from body cannot cope up with this increase, then it may lead to increased level of unconjugated bilirubin or indirect bilirubin in serum (VanWagner & Green 2015).

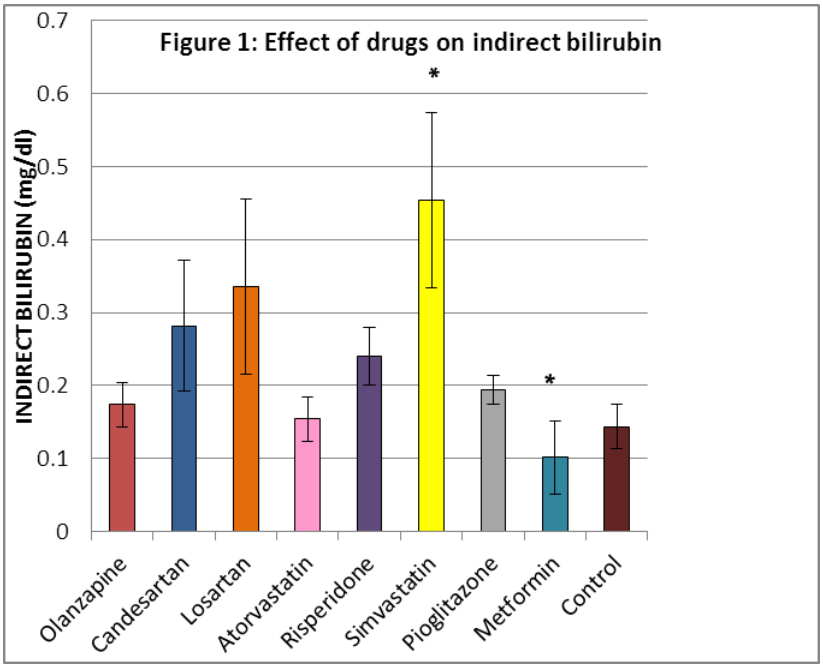
The increase in unconjugated bilirubin by simvastatin is also seen in other studies where it has been linked to the modulation of hepatobiliary transport of bilirubin, multidrug resistance associated proteins and decrease uptake of bilirubin (Szabo et al., 2014).

According to the result of this study metformin decreased indirect bilirubin($P \leq 0.05$). According to previous studies metformin interferes with the membrane stability of red blood cells and can cause hemolysis, this can ultimately lead to increased production of bilirubin (kirkiz et al., 2013). The mild hyperbilirubinemia is associated with lesser risk of cardiovascular diseases and diabetes (Vitek, 2012). Therefore the decrease in unconjugated bilirubin by metformin noted in this study requires further investigation. Some important enzymes involved in bilirubin metabolism can be focused for futher study such as heme oxygenase, uridine glucuronosyl transferase and biliverdin reductase.

Table 1: Effect of drugs on indirect bilirubin.

Drugs	Indirect Bilirubin (mg/dL)
Olanzapine	0.17±0.03
Candesartan	0.28 ±0.09
Losartan	0.34 ±0.12
Atorvastatin	0.15 ±0.03
Risperidone	0.24 ±0.04
Simvastatin	0.45 ±0.12*
Pioglitazone	0.19 ±0.02
Metformin	0.10 ±0.05*
Control	0.14 ±0.07

*Values expressed as Mean±S.E.M; Number of animals (n) =7; * P≤0.05 significant as compared to control*



REFERENCES

1. Boland BS, Dong MH, Bettencourt R,Barrett-Connor E and Loomba R. Association of Serum Bilirubin with Aging and Mortality. Clinical and experimental Hepatology, 2014; 4(1): 1-7.
2. Buksh A, Ali Z, Tayyab M, Gohar Z, Amjad S, Abidi SSR, and Safdar N. Comparative Evaluation of Side Effects of Typical and Atypical Antipsychotics in Psychotic Patients at Punjab Institute of Mental Health, Lahore, Pakistan. Advances in Pharmaceuticals and Ethnomedicines, 2014; 2(1): 14-17.
3. Carmina E. Polycystic ovary syndrome: metabolic consequences and long-term management. Scandinavian Journal of Clinical and Laboratory Investigation, 2014; 74(244): 23-26.
4. Chaouch L, Darragi I, Barkaoui E, Ammar SB, Chaouachi D, Boudrigua I and Tunisia T. Clinical variability among patients with Gilbert syndrome. Journal of Molecular Biomarkers & Diagnosis, 2014; 5(6).
5. Flink BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, Lawrence JC, and Kelly DP. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metabolism, 2006; 4(3): 199-210.
6. Hamilton K, Dunning L, Ferrell WR, Lockhart JC, and MacKenzie A. Endothelium derived contraction in a model of Rheumatoid Arthritis is mediated via angiotensin II type 1 receptors. Vascular Pharmacology, 2018; 100: 51-57.

7. Hirohama D, Ayuzawa N, Ueda K, NishimotoM, Kawarazaki W, Watanabe A, Shimosawa T, Marumo T, Shibata S and Fujita T. Aldosterone is assential for Angiotensin II-Induced Upregulation of Pendrin. Journal of the American Society of Nephrology, 2017; 29(1): 57-68.
8. Istvan ES and Deisenhofer J. Structural Mechanism for Statin Inhibition of HMG-CoA Reductase. Science, 2001; 292(5519): 1160-1164.
9. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravtz BG, Lachin JM, O'Neill MC, Zinman B and Viberti G. Glycemic Durability of Rosiglitazone, Metformin, or Glyburide Monotherapy. The New England Journal of Medicines, 2006; 355: 2427-2443.
10. Kathpalia P, and Ahn J, Assessment of Jaundice in the Hospitalized Patient. Clinics in Liver disease, 2015; 19(1): 155-170.
11. Kirkiz S, yarali N, Bilir OA and Tunc B. Metformin-Induced Hemolytic anemia. Medical Principle and Practice, 2013; 23(2): 183-185.
12. Krentz AJ and Bailey CJ, Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs, 2005; 65(3): 385-411.
13. Leone G, Consumi M, Claudi F, Tamasi G, Lamponi S, Donati A, Magnani A, Rossi C and Bonechi C. Development of liposomal formulations to potential natural lovastatin inhibitory activity towards 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase. Journal of Drug Delivery Science and Technology, 2018; 43: 107-112.
14. Libor Vítek˙. The Role of Bilirubin in Diabetes, Metabolic Syndrome, and Cardiovascular Diseases. Front Pharmacol, 2012; 3: 55.
15. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RSE, Davis SM, Davis CE, Lebowitz BD, Severe J and Hsiao JK. Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia. The New England Journal of Medicines, 2005; 353: 1209-1223.
16. Mahadik SR, Lele RD, Mehatalia SD, Deo SS and Parikh V. Regulation of adiponectin secretion in human subcutaneous and omental adipose tissue: effects of pioglitazone and endothelin-1.a pilot study. Journal of association of the physician in India, 2013; 61(4): 244-248.
17. Mao F, Zhu Z, Lu B and Li Y. The Association between serum bilirubin level and electrochemical skin conductance in Chinese patients with type 2 Diabetes. International Journal of Endocrinology, 2018.

18. Marini MA, Mannino GC, Fiorentino TV, Andreozzi F, PErticone F and Sesti G. A polymorphism at IGF1 Locus is associated with anemia. Oncotarget, 2017; 8(20): 32398-32406.
19. Merchant A and Richter H. Jaundice. Common Surgical Diseases, 2015; 213-215. retrieved from http://books.google.com.pk.
20. Rena G, Hardie DG and Pearson ER. The mechanism of action of metformin. Diabetologia, 2017; 60: 1577-1585.
21. Ruinan LU and Zheng XL. Bilirubin Directly Inhibits Proteolytic Cleavage of a Fluorogenic VWF73 Peptide and Plasma Von Willebrand Factor. Blood, 2014; 124(21).
22. Szabo M, Veresa Z, Batai-Konczosa A, Kekesia O, Kisb E, Szabob K, and Jemnitza K. Statins alter the hepatobiliary transport of unconjugated and conjugated bilirubin in sandwich-cultured rat hepatocytes. Toxicol in Vitro, 2014; 28(6): 1136-1143.
23. Unobol M, Yavasoglu I, Acar B, Kadikoylu G and Bolaman Z. The Effect of Losartan on Platelet aggregation and hematological parameters in patients with newly diagnosed hypertension. Meandros Medical and Dental Journal, 2015; 16(3): 91-96.
24. VanWagner LB and Green RM. Evaluating Elevated Bilirubin levels in asymptomatic adults. The journal of American Medical Association, 2015; 313(5): 516-517.
25. Verdecchia P, Angeli F, Mazzotta G, Gentile G and Reboldi G. The renin aangiotensin system in the development of cardiovascular disease: Role of Aliskiren in risk reduction. Vascular Health and Risk Management, 2008; 4(5): 971-981.
26. Vítek L. The Role of Bilirubin in Diabetes, Metabolic Syndrome, and Cardiovascular Diseases. Front Pharmacol, 2012; 3: 55.
27. Zhang H, Won Han G, Batyuk A, Ishchenko A, White KL, Patel N, Sadybekov A, Zamlynny JM, Garcia-Calvo M, Sharma S, Sheth P, Soisson SM, Stevens RC, Katritch V and Cherezov V. Structural basis for selectivity and diversity in angiotensin II receptors. Nature, 2017; 544: 327-332.