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HYPOGLYCEMIC EFFECT OF 2-HYDROXYCHALCONE ON HIGH FRUCTOSE FED DIABETIC RAT

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ABSTRACT

The objective of the project work was to study the effect of Hypoglycemic and Hypolipidemic activity of 2- hydroxychalcone on high fructose diet induced insulin resistance in male Wister Albino rats. Rats were rendered insulin resistant by feeding 66% (w/w) fructose and 1.1% (v/w) coconut oil mixed with normal pellet diet (NPD) for 3 weeks. Insulin resistance high fructose diabetic rats receiving hydroxychalcone intraperitoneally (i.p.) at the dose of 25mg/kg body weight daily for 7 consecutive days. At the end of the study blood glucose, serum insulin, glycosylated haemoglobin was estimated. The tissue was used for the assay of enzyme activity of Hexokinase, Glycogen, Glu-6-phosphatase and fru-1, 6 bis phosphatase were also estimated. Fructose diet with 2-hydroxychalcone significantly reduced the blood glucose, insulin, glu-6-phosphatase, fru-1, 6 bis phosphatase and significantly increase the hexokinase and glycogen activity. The flavonoid present in the cinnamon 2-hydroxychalcone were found to exhibit a significant Hypoglycemic activity in fructose fed insulin resistant rats.

INTRODUCTION: Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or alternatively when the body cannot effectively use the insulin it produces hyperglycemia or raised blood sugar, is a common effect of uncontrolled diabetes and overtime leads to serious damage to many of the body's systems, especially the nerves and blood vessels [1]. According to World Health Organization projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 [2].

Insulin resistance is defined as diminished tissue response to the hormone at one or more sites in the complex pathway of its actions. The condition presents profound alteration in the metabolic pathways regulated by insulin. Defects in glucose uptake, hexokinase activity have been reported in the insulin resistant states [3]. High dosage of fructose in the diet has been documented to induce insulin resistance accompanied by deleterious metabolic consequences including hyperinsulinaemia, hyperglycemia, glucose intolerance, hypertriglyceridemia and hypertension [4, 5]. The fructose – fed rat is therefore used as an animal model of insulin resistance and is considered to parallel multiple metabolic syndrome observed in humans [6].

Chalcones are precursor compound for flavonoids and biosynthesized in plants and can also be synthesized in laboratory. The flavonoid hydroxychalcone has reputed to possess medicinal properties like antioxidant [7], antihepatotoxic, antimalarial [8], cytotoxic activity

towards leukemia cell. The potential role of hydroxychalcone on insulin action has been shown in several in-vitro studies [9]. It enhances the glucose uptake, phosphorylation of insulin receptor in 3T3-L1 adipocytes. These studies suggest that hydroxychalcone could act as insulin mimetic [10].

In view of the above, it is of importance to generate scientific data to ascertain the effects of hydroxychalcone in-vivo in the insulin-resistant state and to study dose-response of hydroxychalcone on certain variables related to glucose metabolism. We used rats fed a high fructose diet as a model of insulin resistance and determined the level of glucose, insulin, glycated haemoglobin and the activities of glucose metabolizing enzymes of HFD-fed rats treated with hydroxychalcone.

MATERIALS AND METHODS:

Drugs and Chemicals used: The standard drug pioglitazone, fructose and commercially prepared hydroxychalcone were purchased from sigma Aldrich private limited Bangalore. Triglyceride wet reagent diagnostic kits were the products of crescent diagnostic kits. Dimethyl sulfoxide was purchased from Loba chemie, Bombay, India and it is used to dissolve hydroxychalcone.

Animals: Rats used in this experiment were highly inbred male Wister Albino rats from laboratory (APCAS). The rats weighed 200g were used. The animals were housed in spacious caged under hygienic conditions (12hr light 12hr dark cycle at room temperature) and maintained on commercial pellet diet containing protein-21%, lipid-5%, nitrogen, free extract 55% and provided with metabolically energy at 3600 kcal/kg and also enriched by vitamins and minerals. It was supplied by the "Hindustan Lever Limited" Mumbai marked under the trade name "Gold Mohur feeds" water was provided. The rats were kept in animal house for ten days before starting the experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) with ref no.: APCAS/IAEC/2011/09

Preparation of hydroxychalcone: Hydroxychalcone was dissolved in dimethylsulfoxide (DMSO) – normal saline. The final concentration of DMSO in normal saline did not exceed 0.5%. The animals in experimental groups were administered with Chalcones intraperitoneally (i.p.) at the dose of 25mg/kg body weight daily for 7 consecutive days.

High fructose diet induced diabetic rat model: After acclimatization, rats were made insulin resistant by feeding 66% (w/w) fructose and 1.1% (v/w) coconut oil mixed with normal pellet diet (NPD) for 3 weeks. The high fructose diets (HFD) were prepared fresh daily and mixed with respective extract and fed to rat ad libitum.

Experimental Design: The experimental rats were divided into 5 groups of six each and treated as follows:

- Group 1: Six albino rats are maintained in normal condition
- Group 2: High fructose diet for 3 weeks
- Group 3: Normal control rats received only hydroxychalcone (25mg/kg body weight i.p.) for 7 days
- Group 4: HFD-fed rats treated with hydroxychalcone (25mg/kg body weight i.p.) for 7 days
- Group 5: HFD-fed rats treated with pioglitazone (0.05% (w/w)/day, po)

Estimation of Biochemical parameters: After the treatment period, the rats were sacrificed and blood was drawn from ventricles and serum separated for various biochemical parameters like blood glucose, insulin and glycosylated haemoglobin.

Enzymatic Activity Assay: On the final day of experiment, liver, kidney and adipose tissue was collected was excised from each animal; the tissue was washed with ice cold saline and homogenized in Tris HCl buffer pH 7.5. The tissue was used for the assay of Hexokinase, Glucose-6-PO_4 ase, fructose 1, 6 bis phosphatase and Glycogen.

Statistical Analysis: The statistical significance was assessed using one-way Analysis of Variance (ANOVA) using SPSS 12.0 version (spss, cary, Nc, USA) followed by Bonferroni's multiple comparison test (BMCT). The values are expressed as mean ± SD and $p<0.05$ was considered significant.

RESULTS: The results of the levels of plasma glucose, insulin and glycated heamoglobin in circulation are shown in **Table 1**. There was a significant elevation in glucose, insulin and glycated haemoglobin at the 30th day of fructose feeding. On treatment with 2-hydroxychalcone reduced the levels of glucose, insulin and glycated haemoglobin to near normal values. **Table 2I and Fig 1, 2** shows the activity of hexokinase and the level of glycogen in the liver and skeletal muscle. The values were lower in fructose-fed rats as compared to normal rats. Treatment with 2-hydroxychalcone significantly increased the activity of hexokinase and glycogen content.

The activities of glucose-6-phosphatase and fructose-1, 6 bisphosphatase in liver, kidney and skeletal muscle are presented in **Table 3 and fig 3, 4**. In fructose fed rats significant increase were observed. Treatment with hydroxychalcone prevented the increase.

TABLE 1: Levels of glucose, insulin and glycosylated heamoglobin in plasma of control and experimental animals

Groups	Glucose (mg/dl)	Insulin (µ/ml)	Glycosylated Hb (mg/g Hb)
Normal	85.95±0.88	31.29±1.03	0.86±0.09
HFD (Diabetic)	164.8±3.66	92.25±1.03	3.92±0.17
HCC Alone	87.66±1.26	34.74±0.38	1.10±0.22
Diabetic (HCC 25mg/kg)	92.95±5.4*	36.2±3.5*	1.46±0.51*
Diabetic pioglitazone (0.05% (w/w)/day)	86.46±1.09*	33.55±0.39*	1.00±0.24*

HFD- high fructose diet HCC- hydroxychalcone, the values are expressed as mean ± SD. Statistical significance test was done by ANOVA followed by BMCT method. n=6. a-Group II is compared with Group I, b- Groups III, IV, V are compared with group II. *P<0.05

TABLE 2: HEXOKINASE ACTIVITY AND GLYCOGEN CONTENT IN LIVER, KIDNEY AND SKELETAL MUSCLE OF CONTROL AND EXPERIMENTAL ANIMALS

Parameter	normal	HFD	HCC Alone	Diabetic (HCC 25mg/kg)	Diabetic pioglitazone
LIVER					
Hexokinase*	0.23 ± 0.02	0.18 ± 0.01	0.20 ± 0.02	0.21 ± 0.1*	0.22 ± 0.25*
Glycogen#	42.76 ± 3.45	30.58 ± 2.56	38.12 ± 0.36	37.25 ± .6*	40.00 ± 1.26*
KIDNEY					
Hexokinase*	8.40 ± 0.32	5.10 ± 0.23	7.92 ± 3.35	8.00 ± 3.5*	8.25 ± 3.64*
SKELETAL MUSCLE					
Hexokinase*	4.41 ± 0.32	2.36 ± 0.21	3.95 ± 0.34	3.91 ± 0.4*	4.00 ± 0.58*
Glycogen#	8.85 ± 0.44	3.21 ± 0.24	8.54 ± 1.24	8.00 ± 2.6*	8.62 ± 0.44*

Values are mean ± SD of six rats from each group. Treatment of animals and comparisons are as given in Table 1 *p<0.05. *µmoles of glucose phosphorylated/h/mg protein. # mg of glucose/g tissue

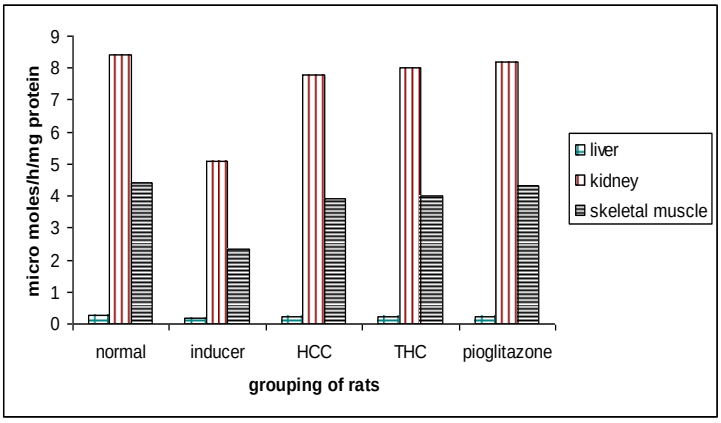

FIG. 1: GRAPHICAL REPRESENTATION OF HEXOKINASE ACTIVITY IN LIVER, KIDNEY, SKELETAL MUSCLE IN DIFFERENT GROUPS OF EXPERIMENTAL RATS

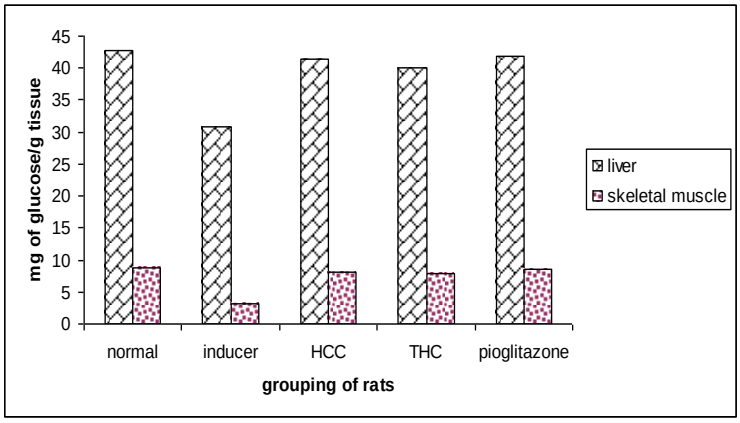

FIG. 2: GRAPHICAL REPRESENTATION OF GLYCOGEN ACTIVITY IN LIVER, SKELETAL MUSCLE IN DIFFERENT GROUPS OF EXPERIMENTAL RATS

TABLE 3: ACTIVITIES OF G-6PASE AND FRU-1,6PASE IN LIVER, KIDNEY AND SKELETAL MUSCLE OF CONTROL AND EXPERIMENTAL ANIMALS

Parameter	normal	HFD	HCC Alone	Diabetic (HCC 25mg/kg)	Diabetic pioglitazone
LIVER					
Glu6pase*	4.11 ± 0.23	6.24 ± 0.46	4.23 ± 0.38	4.5 ± 0.58*	4.20 ± 3.35*
Fru1,6 pase#	3.96 ± 0.26	11.79 ± 0.8	4.21 ± 0.38	4.41 ± 0.7*	4.00 ± 0.24*
KIDNEY					
Glu6pase*	3.81 ± 0.28	4.40 ± 0.34	4.00 ± 0.27	4.11 ± 3.2*	3.9 ± 0.21*
Fru1,6 pase#	4.22 ± 0.37	12.88 ± 0.81	5.12 ± 2.75	5.15 ± 0.1*	4.31 ± 0.28*
SKELETAL MUSCLE					
Glu6pase*	4.97 ± 0.37	5.79 ± 0.44	4.82 ± 1.14	4.91 ± 2.2*	4.81 ± 1.15*
Fru1,6 pase#	4.09 ± 0.37	11.08 ± 0.73	5.13 ± 2.22	5.16 ± 0.1*	4.31 ± 0.38*

Values are mean ± SD of six rats from each group. Treatment of animals and comparisons are a given in table 1. *p<0.05. * µg of p_i liberated/min/mg protein. # mIU/mg protein

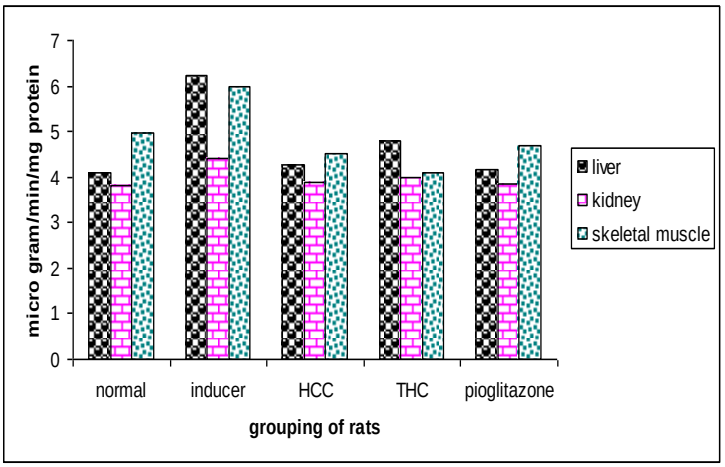

FIG. 3: GRAPHICAL REPRESENTATION OF GLU-6-PHOSPHATASE ACTIVITY IN LIVER, KIDNEY, SKELETAL MUSCLE IN DIFFERENT GROUPS OF EXPERIMENTAL RATS

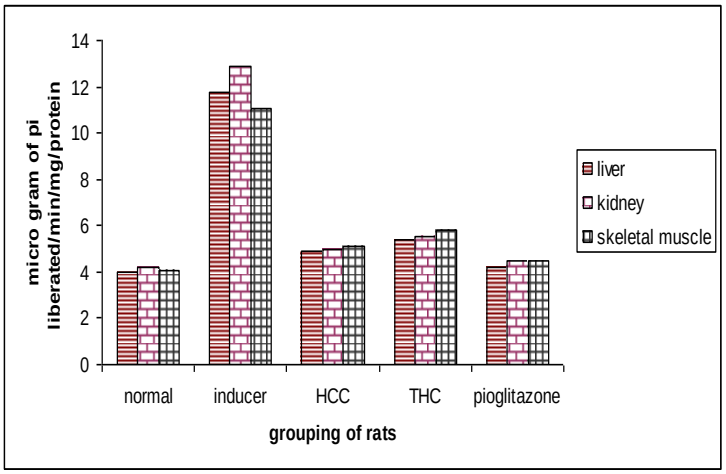

FIG. 4: GRAPHICAL REPRESENTATION OF FRU-1, 6-BISPHOSPHATASE ACTIVITY IN LIVER, KIDNEY, SKELETAL MUSCLE IN DIFFERENT GROUPS OF EXPERIMENTAL RATS

DISCUSSION: Increase in plasma glucose level associated with hyperinsulinaemia suggests impaired insulin action in fructose-fed rats. The degree of insulin resistance was higher in fructose fed rats; insulin resistance in fructose fed rats has been attributed to a low level of insulin-stimulated glucose oxidation due to modifications in the post receptor cascade of insulin action [10]. Increased glycated haemoglobin level is an index of hyperglycaemia level is an index of hyperglycaemia. During hyperglycaemia glucose reacts non-enzymatically with neighbouring protein through the milliard reaction. Haemoglobin is particularly susceptible to glycation among the other proteins [11].

Decreased activities of hexokinase and glycogen indicate impaired glucose oxidation and decreased peripheral glucose utilization [12]. While the increased activities of glu-6-phosphatase and fructose-1, 6-bisphosphatase and decreased glycogen content in fructose-fed rats confirm the gluconeogenic state. Decreased glycogen content in tissues could be due to reduced synthesis or increased breakdown of glucose. Reduction in hepatic glycogen concentration in liver has been reported in fructose-fed rats [13].

Hydroxychalcone administration (25mg/kg body weight) was effective in mitigating the adverse effects of HFD. Glucose tolerance and insulin sensitivity were improved by hydroxychalcone treatment. Hydroxychalcone brought back the activities of glucose metabolizing enzymes, levels of glycogen and protein bound sugars to near normal values when administered along with fructose.

Hydroxychalcone can stimulate autophosphorylation of the insulin receptor and can inhibit protein tyrosine phosphatase-1, which inactivates insulin receptor in the adipocytes. It was suggested that hydroxychalcone could affect protein phosphorylation-dephospho rylation reactions in the intact adipocytes [14].

On treatment of normal rats with hydroxychalcone enhances the glucose utilization in-vivo in a dose-dependent manner and potentiates the insulin stimulated tyrosine phosphorylation of insulin receptor substrate. Hydroxychalcone prevents the development of insulin resistance in HFD rats. They attributed this to the activation of the insulin signaling possible via the nitric oxide pathway in the skeletal muscle [15].

Increased glycated Hb is particularly susceptible to glycation among the other proteins. The adverse effect of hydroxychalcone increases the glucose uptake by the cells and reduces the glycosylated Haemoglobin [16]. Enhanced uric acid level in HFD rats could be associated with the fructose had a different transporters, the favorable effect of hydroxychalcone may be attributed to their action on fructokinase activity is enhance so depletion of ATP and AMP decreased so, the uric acid level also decreased.

The activity of hexokinase and glycogen is decreased on treated with hydroxychalcone might be stimulate the insulin receptor activity by increasing the concentration of phosphorylated intracellular protein IRS-1 and to which leads to enhanced cellular glucose uptake and hexokinase activity [17]. On the other hand hydroxychalcone significantly decreases the glu-6-phosphatase and fru-1, 6-bisphosphatase due to insulin mimetic effect of hydroxychalcone and significantly reduction in the level of gluconeogenic enzymes [18].

Research findings clearly show that hydroxychalcone triggers the insulin cascade system. Considering the increased intake of fructose as high fructose corn syrup and the high incidence of insulin resistance in the general population. Our finding provides evidence for the therapeutic potential of cinnamon in the treatment of insulin- resistant states.

CONCLUSION: In conclusion, the effect of hydroxychalcone on high fructose diet induced diabetic animals showed significant increase in glucose, insulin and glycosylated Haemoglobin and enzyme activity. Based on the results, hydroxychalcone at the dose of 25mg/kg body weight were effective and exhibit potent antidiabetic and anti oxidant activity.

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REFERENCE:

1. Resources WDD and Diabetes facts available at : http://www.world diabetes day.org
2. Pickup, J.C., Williams, G., 1991. Textbook of diabetes. (Second ed.) Blackwell, London. (Chapter 6).
3. Cline GW, Petersen KF, Krssak M, et al. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen sysnthesis in type 2 diabetes. N Engl J Med 1999; 341:240-6. Comment in: N Engl J Med 1999; 341:248-57.
4. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension 1987; 10:512-6.
5. Thorburn AW, Storlein LH, Jemkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Clin Nutr 1989; 49:1155-63.
6. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988; 37:1595-607.
7. Alan L. Miller, ND, Alternative medicine Review, Volume 1, Number 2, 1996,103-111
8. Nidhi Mishra, Preeti Arora, Brajesh Kumar, Lokesh C. Mishra, Awasthi, European Jouranl of Medicinal chemistry 43 (2008) 1530-1535
9. Chang H J, Ho-M S, In-wook C, Hee-Don C and Hong-Yon C, Effect of wild *ginse(panax ginseng C.A.Meyer*)leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats. *Journal of ethno pharmacology* 2005; 98: 245-250.
10. Cisse A, Nongonierma R B, Sarr M, Mbodj N A and Faye B, Hypoglycaemic and antidiabetic activity of acetonic extract of *vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. *Journal of ethno pharmacology* 2005; 98: 171-175.
11. Catena C, Giacchetti G, Novello M, et al. Cellular mechanisms of insulin resistance in rats with fructose-induced hypertension. Am J Hypertens 2003; 16:973-8. Comment in: Am J Hypertens 2004; 17:382.
12. Thorburn AW, Storlein LH, Jemkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Clin Nutr 1989; 49:1155-63.
13. Rawana S, Clark K, Zhong S, et al. Low dose fructose ingestion during gestation and lactation affects carbohydrate metabolism in rat dams and their offspring. J Nutr 1993; 123:2158-65.
14. Imparl - Radosevich J, Deas S, Polansky MM, et al. Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: implications for cinnamon regulation of insulin signalling. Horm Res 1998; 50:177-82.
15. Qin B, Nagasaki M, Ren M, et al. Cinnamon extract prevents the insulin resistance induced by high-fructose diet. Horm Metab Res 2004; 36:119-25.
16. Day, C., Cauturight, T., Provost, J.and Bailey, C.J. 1990. Hypoglycemic effect of momordica charantia extracts. Planta Med. 56:426-429
17. Qin B., Nagasaki, V., 2003. Cinnamon extract oshida, Y., Sato Y., 2003.Cinnamon extract potentiates in vivo insulin regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Research and clinical practice 62, 139-148
18. Imparl- Radoservich J, Deas S, Polansky MM, Daedke DA, Ingebruten TS, Anderson RA, Graves DJ. Regulation of phosphotyrosine phosphate and insulin receptor kinase by factions and from cinnamon: implications for cinnamon regulation of insulin signaling hormone Res 50: 177-182-1998.

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