

## Chalcones: Differential effects on glycogen contents of liver, brain and spinal cord in rats

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### Abstract

Chalcones are precursor compounds for flavonoids biosynthesis in plants, and they can also be synthesized in laboratory. Chalcones possess a broad spectrum of biological activities including antioxidative, antibacterial, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic and immunosuppressive. Aldose reductase (AR) enzyme inhibitors have been proposed to prevent or ameliorate long term diabetic complications. Chalcones showed good level of inhibitory activity towards bovine lens aldose reductase (AR) and have been shown the promising compound for the prevention or treatment of diabetic complications. Although a broad range of biological activities of chalcones have been reported, the mode of action of chalcones on glycogen is not yet elucidated. Chalcones were injected intraperitoneally at the dose of 25mg/kg for seven days. On the seventh day, one hour after the last dose rats were killed by cervical decapitation and their liver, brain and spinal cords were dissected out. Our results showed that out the eight tested chalcones, four chalcones significantly inhibited ( $P > 0.001$ ) the liver glycogen. There are no significant effects of chalcones on brain and spinal cord glycogen contents. In conclusion, chalcones exert their antidiabetic effect by decreasing only the liver glycogen content. By comparing the glycogen inhibitory activity structural elements of chalcones responsible for glycogen inhibition could be identified.

**Keywords:** Chalcones; glycogen content; liver; brain; spinal cord; rat.

### Introduction

Glycogen stores in the brain are small relative to the liver especially muscle. Nevertheless, brain glycogen turns over rapidly (Pentreath and Kai kai, 1982; Swanson *et al.*, 1989) and contributes significantly to normal brain energy metabolism (Swanson, 1992; Wender *et al.*, 2000). Brain glycogen is located entirely in astrocytes (Koizumi, 1974) which are distributed throughout the brain but are more concentrated in fiber bundle and white matter (Savchenko *et al.*, 2000; Sloano *et al.*, 2001). In the mammalian liver, glycogen is present in the form of rosettes (Luft, 1956). Glycogen is present in central nervous system (CNS) although at much lower concentrations than liver or skeletal muscle with the commonly accepted ratio of liver/ skeletal muscle/ brain: 100:1:1 (Nelson *et al.*, 1968). The concentration of the glycogen in the liver can vary according to the nutritional state of the animal. In the liver of fasted rats or mice, this concentration is about 1-5 mg/g of wet wt and increases rapidly at the rate of about 10 mg (g wet wt)<sup>-1</sup>h<sup>-1</sup> (Cori, 1926). The accepted role of glycogen is that of a carbohydrate reserve utilized when glucose falls below need.

However, there is a rapid continuous breakdown and synthesis of glycogen (17 $\mu$  mol/kg/min) (Watanabe and Passonneau, 1973). The enzyme systems that synthesize and catabolize glycogen in other tissues are also found in brain, but their kinetic properties and modes of regulation appear to differ.

Aldose reductase (Alditol: NADP<sup>+</sup> oxidoreductase, E.C. 1.1.1.21, ALR2) is the first enzyme of the polyol pathway, glucose flux through this pathway, during diabetes, has been linked to the development of long-term diabetic complications. Thus, ALR2 inhibitors (ARIs) have been developed as potential agents to prevent or delay the onset of diabetic complications (Raskin and Rosenstock, 1987). There is strong evidence to show that diabetes is associated with increased oxidative stress (Mohamed, 1999; Yue, 2003).

Flavonoids with insulin-trigerring and/or insulin like properties have been extracted from plants (Hii and Howell, 1985). Chalcones considered as precursors of flavonoids and isoflavonoids are abundant in edible plants, and have been shown to display a diverse array of pharmacological activities (Dimmock *et al.*,

1999). Several hydroxylated and methoxylated chalcones showed good level of inhibitory activity towards bovine lens aldose reductase (AR) (Saveri, 1990) and have been shown the promising compound for the prevention or treatment of diabetic complications (Lim *et al.*, 2001). In recent report 3-nitro- 2'-benzyloxychalcone showed potent insulin-stimulated glucose uptake in a concentration dependent manner in 3T3-L1 adipocytes in a cell based glucose uptake screening assay (Rothman *et al.*, 1995).

ALR<sub>2</sub> inhibitors have been shown to prevent or delay significantly diabetic complications (Raskin and Rosenstock, 1987) and chalcones display significant inhibitory activity towards this enzyme. Thus, the aim of the present study was to determine the effects of chalcones on glycogen contents of liver, brain and spinal cord.

## Materials and Methods

### Animals

Adult male albino rats weighing 200-250 gram were used for the study. The animals were procured from the central animal house facility at Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. The rats were group housed in polypropylene cages (38×23×10 cm) under standard laboratory conditions. They were allowed free excess of dry rat diet and tap water *ad libitum*. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

### Materials

Chalcones used in the study were prepared by the previously described methods: 2',4',4'-trihydroxychalcone (Saveri *et al.*, 1998); 2',4',3,4'-tetrahydroxychalcone (Sogawa *et al.*, 1993); 2',2'-dihydroxychalcone (Hsieh *et al.*, 1998); 2'-hydroxy-3,4-dimethoxychalcone (Hsieh *et al.*, 2000); 4',4'-dichlorochalcone, and 4'-chloro,4-methoxychalcone (Davey and Gwilt, 1957); 1,3-bis (4-chlorophenyl) -3- (carboxymethylthio) propan-1-one, and 1-(4-chlorophenyl) -3- (4-methoxyphenyl) -3- (carboxymethylthio) propan-1-one (Levai, 1991). Chemical structures of chalcones used in this study were given in Figure 1. The identification of chalcones was assessed by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and IR spectroscopic data.

### Methods

Rats were divided into nine groups of six animals each. Chalcones were 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. The animals of control group received a similar volume of dimethylsulfoxide-normal saline. On the seventh day, after one hour of the last dose animals were sacrificed by cervical dislocation. Their brains, spinal cord, and liver were removed quickly, placed on the petridish over ice, and rinsed with ice-cold physiological saline solution. The brain, spinal cord and liver were weighed and then subjected for the assay of glycogen estimation.

### Glycogen estimation

Glycogen content was estimated colorimetrically by the method described by Hassid and Abraham (1957). The tissue (approx 1gm) was placed in a pre-weighed centrifuge tube containing 3 ml of 30% KOH. After the weight of the tissue had been recorded, the tubes were placed in a boiling water bath for about 20-30 min. When the tissue was dissolved, the glycogen was precipitated by the addition of 5 ml of 95% ethanol. The precipitate obtained was dissolved in 1 ml of distilled water and again precipitated with 95% ethanol and centrifuged at 3000 rpm for 10 min. The glycogen precipitate was then dissolved in distilled water, and this solution was used to estimate the quantity of glycogen. To 0.1 ml of aliquot, 5 ml of anthrone reagent was added and mixed by swirling the tube. The tubes were heated for 10 min in boiling water, followed by cooling, and the absorbance was recorded at 590 nm. The readings were compared with that of standard glycogen.

### Statistical analysis

The data was represented as mean ± SEM. The statistical analysis of the results was carried out with a SPSS 11.0 program and based on an analysis of variance (ANOVA) followed by post hoc test (Tukey). Differences were considered as significant for p < 0.05 or less.

## Results

### *Effects of chalcones on the glycogen content*

Table 1 depicts the values of glycogen in the brain, spinal cord and liver after intraperitoneal administration of variably substituted chalcones at the dose of 25mg/kg body weight for 7 days. The results showed that chalcones caused no significant alterations of glycogen content in brain and spinal cord as compared to vehicle treated group.

ANOVA followed by Tukey post hoc test showed that intraperitoneal administration of chalcones at the dose of 25mg/kg body weight significantly reduced the glycogen content in liver [ $F_{(4, 25)} = 15.337$ ,  $p < 0.001$ ] as compared to vehicle treated control group.

## Discussion

Results of in vitro studies in diabetic and in non-diabetic rats have suggested a hypoglycemic/insulin-like effect of certain individual flavonoid-containing seed extracts (Ahmed *et al.*, 2000; Anila and Vijayalakshmi, 2000). Both citrus fruits and apples are rich dietary sources of chalcones and dihydrochalcones, and these compounds could even make a greater contribution to the total daily intake of natural phenolics than the more extensively studied flavonoids (Tomas-Barberan and Clifford, 2000). It is a largely unresolved question at present, whether a significantly enhanced intake as to be expected from dietary food supplements or certain functional foods, is consistent with beneficial effects on human health or whether adverse effects are also to be taken into account.

In the present study, we used rat liver, brain and spinal cord as an easily accessible model system to measure the inhibitory potential of chalcones on glycogen content. Comparing effects of chalcones on the glycogen content allowed concluding on structural elements responsible for effective glycogen inhibition. Presence of hydroxy and methoxy groups seems to be a mandatory element for potential inhibition of glycogen content, but this inhibition is observed only in liver and not in brain and spinal cord. The order of inhibition of glycogen content in liver is as follows:

ISL > BUT > DHC > HDMC

Compound ISL (isoliquiritigenin; 2',4',4'-trihydroxychalcone) and BUT (butein; 2', 4', 3, 4-tetrahydroxychalcone) with three and four hydroxy groups in positions 2 and 4 on both rings (A and B) are found to be more potent

inhibitor than compound DHC (2',2'-dihydroxychalcone) having only two hydroxy groups. Presence of two additional methoxy groups with hydroxy group as in HDMC may suffice to achieve effective glycogen inhibition. Presence of chloro groups as in DCC and CMC showed no activity. According to Severi *et al* the introduction of thioglycolic group in the chalconic structure gave an increase in the inhibitory activity against ALR2 (aldose reductase enzyme). However, in our study introduction of thioglycolic group in the chloro substituted chalcones (CCP and CMCP) has no influence on the glycogen inhibitory activity. Thus, altogether, these data shows that for an efficient inhibition of glycogen presence of hydroxy groups in the 'A' ring or methoxy groups in 'the' B ring is responsible for inhibition.

Our study indicated that ISL caused a significant reduction of glycogen content in the liver (37.17%), without affecting the brain and spinal cord glycogen values. Recently it has been published that isoliquiritigenin found in licorice roots increases glycogenolysis in rat hepatocyte perfusion (Abdollahi *et al.*, 2003). This is consistent with our study that isoliquiritigenin (ISL) increased the glycogen depletion in the liver of rats. The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose as glycogen and release of glucose formed by glycogenolysis and/or gluconeogenesis (Nordlie *et al.*, 1999). There is also evidence that c-AMP increasing agents exert a glycogenolytic effect by maintaining glycogen phosphorylase in a phosphorylated state (Bollen, 1998). PDE inhibitors can increase serum glucose levels (Gilfrich and Dieterich, 1991) Isoliquiritigenin is a PDE 3 inhibitor with significant cardiac inotropic and vasodilatory effect which were attributed to an increase in c-AMP levels (Abdollahi, 2003; Kusano, 1991; Wegener and Nawarh, 1997). Isoliquiritigenin was found to decrease sorbitol levels in blood cells, the sciatic nerve and the lens of the diabetic rats and thus could have implications in diabetic neuropathy (Severi *et al.*, 1998). There is also evidence that the antidiabetic drug metformin, which acts through inhibition of hepatic gluconeogenesis, is produces concurrent antioxidant effects that are most beneficial in treatment of diabetes (Cosic *et al.*, 2001). Isoliquiritigenin has been reported to have the property to scavenge free radicals and have the antioxidative capacity (Vaya *et al.*, 1997; Haraguchi *et al.*, 1998).

The disturbance of gluconeogenesis and glycogenolysis could have significant influences on glucose metabolism and pathogenesis of diabetes (Atefi *et al.*, 2004; Abdollahi *et al.*, 2004). Therefore, it could be concluded that isoliquiritigenin stimulates glycogenolysis and thus depletes hepatic glycogen storage which in turn needs to be compensated by blood glucose because hepatic gluconeogenesis pathway is occluded and this may be a mechanism for antidiabetic effect of isoliquiritigenin and seems to be in relation with its antioxidant properties. Since the hyperaggregability of platelets has been implicated in the pathogenesis of diabetic complications and isoliquiritigenin showed inhibitory effect on platelet aggregation, comparable to that of aspirin (Twata *et al.*, 1992). Thus, the dual or triple effects of isoliquiritigenin i.e having aldose reductase inhibition, antioxidant and platelet aggregation inhibition may offer a unique process for maintaining the glucose by increasing the glycogenolysis rate.

Compound BUT (butein; 2', 4', 3, 4-tetrahydroxychalcone) also caused a significant reduction of glycogen content in the liver (31.60%), but caused no alterations in the brain and spinal cord glycogen values. It has been reported that increase of c-AMP formation is elicited by butein (Sheu-Meei, 1995); c-AMP also affects storage of glycogen (Larner, 1968) which is hydrolysed or reduced with the rise in blood sugar level (Vane, 1962). Butein showed strong lens AR inhibitory activity. It also showed strong activity in transition metal chelation and free radical scavenging activity and the most promising compound for the treatment of diabetic complications (Lim, 2001). Recent human studies have demonstrated that ACE inhibition improves glucose disposal rate and that the effect may be primarily due to increased muscle glucose uptake (MGU). Butein inhibits contraction-stimulated glucose transport by inhibiting the glucose transporter function (David *et al.*, 2005). In insulin-resistant conditions, ACE inhibitors can also enhance whole-body glucose disposal and glucose transport activity in skeletal muscle (Henriksen *et al.*, 1999). Butein significantly inhibited the ACE activities in a dose-dependent manner (Chen, 1992). Tumor necrosis factor- $\alpha$  is implicated in induction of insulin resistance seen in type-2 diabetes (Ruan and Lodish, 2003). Butein suppressed the nuclear factor (NF) $\kappa$ B activation and also the expression of COX (Pandey, 2007). Butein

(BUT) is an effective metal ion chelator and a powerful-chain breaking antioxidant in LDL systems (Cheng, 1998). In addition, butein inhibits the platelet aggregation response (Jeon, 2006). Butein also has properties like aldose reductase inhibition, antioxidant and platelet aggregation inhibition. Thus, it might be concluded that butein increases the glycogenolysis rate by depleting the hepatic glycogen and the resulted higher level of glucose is compensated by increased rate of glucose uptake by muscles in order to balance the blood glucose level.

Compounds DHC (2',2-dihydroxychalcone) and HDMC (2'-hydroxy-3,4-dimethoxy-chalcone) also caused a significant reduction of glycogen content in the liver (23.72% and 21.29%), but caused no alterations in the brain and spinal cord glycogen values.  $\alpha$ -Glucosidase plays a physiologically important role for the digestion process of dietary carbohydrates to suppress postprandial hypoglycaemia (Matsura, 2002). The inhibitors of  $\alpha$ -glucosidase are effective for delaying glucose absorption, which makes them potent drugs to control glucose levels in blood (Matsui, 1996). In addition,  $\alpha$ -glucosidase inhibitors are used to ameliorate insulin-resistance (Stumvoll *et al.*, 2005) and lower blood glucose concentration.  $\alpha$ -glucosidase inhibitors are widely used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (Hasegawa *et al.*, 1998). Ansari *et al.*, 2005 showed that hydroxy and methoxy-substituted chalcones are potent inhibitors of  $\alpha$ -glucosidase. Also PPAR $\gamma$  agonists improve insulin-resistance by opposing the effect of TNF- $\alpha$  in adipocytes (Moller, 2000). Moreover, hydroxy and methoxy chalcones have more potent or similar PPAR-agonistic activity compared with troglitazone (insulin sensitizing CTZP drug) (Jung 2006). Therefore, it may be concluded that these chalcones increase the glycogenolysis rate but delay the absorption of glucose to control the glucose levels in blood.

### Conclusion

Chalcones, which are potent inhibitors of aldose reductase, caused an increase in glycogenolysis rate by decreasing the liver glycogen content. It is interesting to note that chalcones showed no activity against brain and spinal cord glycogen content.



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**Table 1 and Figure 1 follow.....**

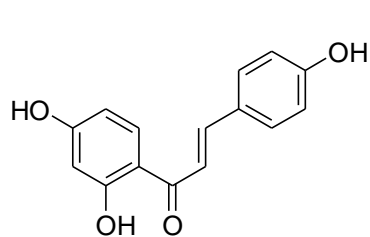
**Table 1** Effects of chalcones on glycogen content. Data are expressed as means  $\pm$  SEM.

Groups	Glycogen content (mg/g tissue)		
	Liver	Brain	Spinal cord
Control (6)	51.3533 $\pm$ 2.94	1.8970 $\pm$ 0.02	0.7953 $\pm$ 0.02
ISL (6)	32.2627 $\pm$ 0.88 <sup>abc</sup>	1.9050 $\pm$ 0.08	0.8033 $\pm$ 0.02
BUT (6)	35.1220 $\pm$ 2.01 <sup>abc</sup>	1.9133 $\pm$ 0.03	0.7833 $\pm$ 0.04
DHC (6)	39.1705 $\pm$ 0.09 <sup>ab</sup>	1.9005 $\pm$ 0.02	0.7900 $\pm$ 0.04
HDMC (6)	41.2540 $\pm$ 1.75 <sup>a</sup>	1.9243 $\pm$ 0.06	0.7700 $\pm$ 0.04
DCC (6)	48.0687 $\pm$ 1.71	1.8482 $\pm$ 0.03	0.7637 $\pm$ 0.02
DCCP (6)	49.9385 $\pm$ 1.72	1.8833 $\pm$ 0.05	0.7865 $\pm$ 0.06
CMC (6)	49.9778 $\pm$ 3.03	1.8035 $\pm$ 0.05	0.7575 $\pm$ 0.03
CMCP (6)	49.5712 $\pm$ 2.97	1.8750 $\pm$ 0.03	0.7772 $\pm$ 0.05

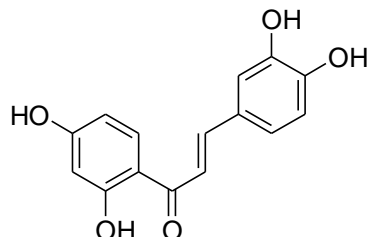
Figures in parenthesis indicate number of rats. <sup>a-c</sup> Indicate statistical significance in comparison to vehicle treated control group at P<0.05, P<0.01, P<0.001, respectively.



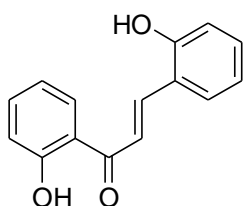
Figure 1 Chemical structures of the chalcones used in the study



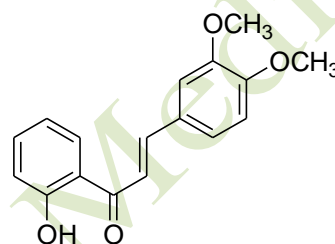
2',4',4'- Trihydroxychalcone  
Isoliquiritigenin, ISL



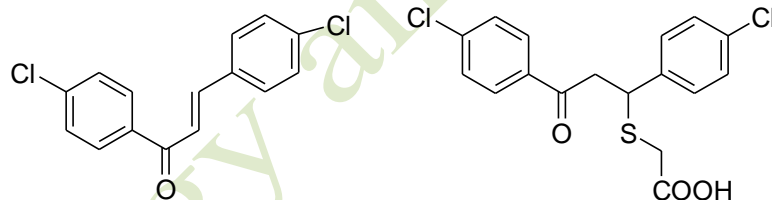
2',4',3,4'- Tetrahydroxychalcone  
Butein, BUT



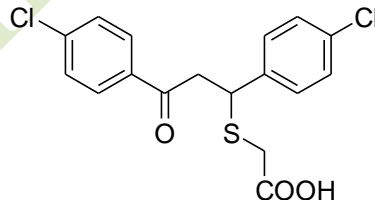
2',2'- Dihydroxychalcone  
DHC



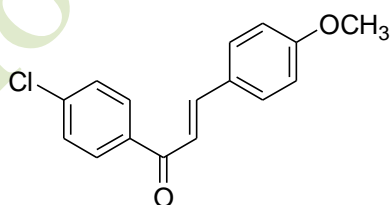
2'- Hydroxy-3,4'- Dimethoxychalcone  
HDMC



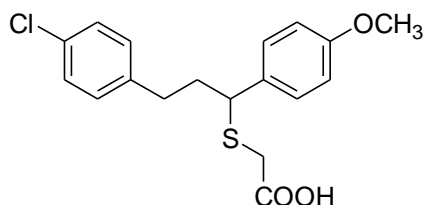
4',4'- Dichlorochalcone  
DCC



1,3- bis (4- chlorophenyl) - 3- (carboxy-  
methyl - thio) prop- an- 1- one, DCCP



4'- Chloro- 4- methoxy-  
chalcone, CMC



1- (4- Chlorophenyl)- 3 (4- methoxy-  
phenyl)- 3- (carboxymethylthio) prop-  
an-1- one, CMCP