

## Effect of Organic Trivalent Chromium in the Form of Yeast on Diabetic Rats

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**Abstract:** The biomass of Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* contained Cr<sup>3+</sup>, Zn<sup>2+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup> in amounts higher than those in the original one when grown in the presence of 50 µg Cr<sup>3+</sup>/ml, while, the biomass of original strain contained Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup> and K<sup>+</sup> in amounts higher than the adapted strain grown in the same conditions. When the two strains grown on Cr<sup>3+</sup> free medium, the Cr<sup>3+</sup>-adapted strain gives high amounts of Cr<sup>3+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Mg<sup>2+</sup> and Mn<sup>2+</sup> compared to the original one. The results indicate a quantitative increase in the amino acid compositions of both the original and the Cr<sup>3+</sup>-adapted strains when grown in the presence of 50µg Cr<sup>3+</sup>/ml, but the Cr<sup>3+</sup>-adapted strain had more amounts of amino acids than the original one, except glutamic acid which was very high. Also, the Cr<sup>3+</sup>-adapted strain had more of most amino acids than the original one when the two strains grown in the absence of Cr<sup>3+</sup>. These amino acids in addition to the biopolymers of yeast cell wall which combined with Cr<sup>3+</sup> to consist chromium yeast (organic form) like glucose tolerance factor (GTF). For determination of therapeutic effect of organic chromium on the hyperglycemic rats, the rats were divided into four groups each of it received one does (1 ml) daily of a different concentration of Cr-yeast biomass solutions via oral dropping for 60 days. The results showed high significant decreases in glucose (from 457 to 196 and 180 mg/dL), significant decreases in total cholesterol (from 184 to 164 and 157 mg/dl) and triglycerides (from 430 to 395 and 390 mg/dL) of serum of diabetic rats administrated with 1.0 ml of non-adapted yeast biomass solution (700 mg dried biomass dissolved in 50 ml of citrate phosphate buffer solution contains 140 µg Cr<sup>3+</sup>) or with 1.0 ml of Cr<sup>3+</sup>-rich adapted yeast biomass (700 mg dried biomass dissolved in 50 ml of citrate phosphate buffer solution contains 428.4 µg Cr<sup>3+</sup>), respectively.

**Key words:** Chromium, *Saccharomyces bulardii*, diabetic rats and adaptation

### INTRODUCTION

Chromium was known for many years to be an element causing allergic reactions and having neurotoxic and carcinogenic effects. These effects can be observed especially in the case of hexavalent chromium. Only a little more than four decades ago trivalent chromium has been known as an essential element with relation to glyceride and lipid metabolism. And only during several last years this chromium function has been revealed on a molecular level. After absorption in the gastrointestinal tract, chromium is most likely transported to cells bound to the plasma protein transferrin. Insulin initiates chromium transport into the cells where it is bound to the oligopeptide apochromodulin. This oligopeptide combined with four chromium (III) atoms forms chromodulin, which is important for amplifying the insulin signaling effect. After binding to insulin-activated receptor, chromodulin increases tyrosine kinase activity by one order. This enzyme forms a part of intracellular portion of insulin receptor (Racek, 2003). As known

inorganic chromium compounds are poorly absorbed in man<sup>[23]</sup>. Organic chromium such as that found in brewer's yeast, is much better absorbed than inorganic chromium<sup>[7]</sup>.

Trivalent chromium complexes with nicotine acid, picolinic acid, yeast and amino acids, is known as glucose tolerant factor (GTF)<sup>[22]</sup>. In 1992, Simonoff *et al.* published finding related to the isolation of GTF from brewer's yeast. They tested the GTF activity of two different of yeasts, an untreated control and chromium yeast created via incubation with chromium, on rat adipocytes. In addition to the interest in GTF, other researchers have looked at the interaction between Cr<sup>3+</sup> and the insulin receptor. One of the first attempts to investigate the specific effect of Cr<sup>3+</sup> on the insulin-receptor complex intemalization was reported by Evans and Bowman<sup>[11]</sup>. They looked at the action of various Cr-containing compounds on the intemalization rate of insulin in rat muscle cells and observed that it markedly increased in the presence of a Cr picolinate complex. This response was accompanied by an increase in glucose intake.

Chromium supplementation in people with chromium deficiency can improve glucose tolerance and some lipid metabolism parameters. Chromium is necessary for proper insulin function and blood glucose regulation because it helps facilitate the uptake of glucose for energy production. It also known to increase the efficacy of insulin, therapy reducing the amounts the body needs to produce (Racek, 2003). During continuous fermentation protocol for enhanced production of organically bound chromium yeast, Demirici and Pometto<sup>[9]</sup> obtained an adapted strain (C11-1) that produced higher biomass than the wild type but significantly lowered chromium incorporation. Also Saad<sup>[17]</sup> obtain a chromium-adapted strain of *Saccharomyces bulardii* by subculturing (5 transfers, 15 days) on 50 mg Cr<sup>3+</sup>/ml as Cr Cl<sub>3</sub> containing medium. The biomass containing Cr<sup>3+</sup> was less than the non adapted one when grown on Cr<sup>3+</sup> free medium, but it contains Cr<sup>3+</sup> higher than that in the original one when grown in the presence of 10mg Cr<sup>3+</sup>/ml. Bahijiri *et al.*<sup>[5]</sup> found that a higher percentage of subjects responded positively to Brewer's yeast chromium, which was retained more by the body, with effects on fructosamine, triglycerides and HDL-cholesterol maintained in some subjects when placebo followed it and the mean of urinary chromium remaining was significantly higher than zero time mean. Arkuszewska *et al.*<sup>[3]</sup> studied the effect of Cr<sup>3+</sup> in the form of yeast preparation on productive results of broiler fattening. They found that Cr<sup>3+</sup> positively affected the effectiveness of broiler chicken fattening and did not affect the chromium level in the muscle tissue. Also Vinson and Bose (1984) found that the chromium rich yeast improved blood glucose, lowered serum lipids and decreased risk of coronary heart diseases. Therefore, the dosage of chromium yeast can be given daily by oral route is 100 mg containing 218 mg of chromium for a period of six months. The present study aims at investigating the effect of organic trivalent chromium in the form of yeast on diabetic rats.

## MATERIAL AND METHODS

**Yeast Strain and Cultivation:** Medical yeast (*Saccharomyces bulardii*) was transferred from a capsule produced by an international pharmaceutical Co. on YPD/agar medium containing (g/L): yeast extract, 3.0; peptone, 10.0; glucose 20.0 and agar, 20 and maintained by subculturing on the same medium and stored at 4°C.

**Adaptation of *Saccharomyces bulardii* to Cr<sup>3+</sup> Culture Conditions:** Saad<sup>[17]</sup> in a previous work found that the half maximal growth inhibition of original strain occurred at 50 µg Cr<sup>3+</sup>/ml, after that, the strain whose growth was inhibited to 50%, by Cr<sup>3+</sup> was adapted to

Cr<sup>3+</sup> by subculturing (5 transfers) on 50 µg Cr<sup>3+</sup>/ml containing medium for 15 days. The strain was grown with shaking for 24 h. at 30°C±2 on liquid medium consists of (g/L): glucose, 20.0 and peptone, 10.0 (pH, 5) not amended or amended with 50 mg/ml of Cr<sup>3+</sup> as Cr Cl<sub>3</sub>.6H<sub>2</sub>O.

**Harvesting Cells:** Yeast cells were harvested by centrifugation at 6000 g for 10 min. washed three times with cold distilled water and stored frozen until used.

### Preparation of Yeast Solutions:

**Solution No. 1:** contains, 7.0 gm wet wt. (equal 0.7 gm dry wt.) of Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* which previously grown on 50µg Cr<sup>3+</sup>/ml containing medium, dissolved in 50ml of citrate phosphate buffer solution (0.1 M; pH, 4.5).

**Solution No. 2:** contains, 7.0 gm of non-adapted *Saccharomyces bulardii* (original) dissolved in 50 ml of citrate phosphate buffer solution (0.1 M; pH, 4.5) contains 50 µg/ml Cr<sup>3+</sup>.

**Solution No. 3:** contains, 7.0 gm of Cr<sup>3+</sup>-adapted strain grown on a medium not amended with Cr<sup>3+</sup>, dissolved in citrate phosphate buffer solution (0.1 M; pH, 4.5).

**Solution No. 4:** contains, 7.0 gm of non-adapted strain which previously grown on 50 µg Cr<sup>3+</sup>/ml containing medium dissolved in 50 ml citrate phosphate buffer solution (0.1 M; pH, 4.5).

**Preparation of the Experimental Rats for Hyperglycemia:** Streptozotocin (STZ) as a diabetogenic agent in a dose of 65 mg/Kg body weight was dissolved in 0.1 M citrate phosphate buffer solution, pH 4.5 according to Bell and Hye<sup>[6]</sup>.

**Experimental Design:** 50 male albino rats were divided into five groups, each contain 10 rats. The first group was injected with 1 ml of 0.1 M citrate phosphate buffer (pH 4.5) and used as a control group and the other 4 groups were injected intraperitoneally with a single dose of the prepared STZ solution and used as diabetic experimental groups. After 72 hs., blood samples were drawing from retroorbital venous plexus of each rat. STZ treated rats having blood glucose more than 300 mg/dL were excluded the study.

The dosage (1.0 ml) of a prepared yeast solution was given daily by oral route that contained or not contained different concentrations of Cr<sup>3+</sup> in the form of yeast for a period of 60 days. The experimental rats were fed on a commercial diets obtained from Animal Department at NRC.

**Determination of Some Heavy Metals:** The dried biomass was subjected to wet digestion according to the method of Simmons<sup>[18]</sup>.

**Determination of Amino Acids in Yeast Biomass:** Amino acids of the cells were determined using amino acid analyzer, LC3000 Eppendorf/Germany after hydrolysis of cells with 6 N HCl in sealed tubes for 12 h at 110°C where the flow rate was 0.2 ml/min., pressure of buffer from 0 to 50 bar, pressure of reagent was from 0-150 bar and reaction temp. was 123°C.

**Determination of Blood Glucose, Cholesterol and Triglycerides:** The blood glucose and cholesterol concentrations were determined according to the methods of Trinder<sup>[20,21]</sup>. Triglycerides were determined according to Wahlefeld<sup>[24]</sup>.

### RESULTS AND DISCUSSIONS

**Some Metal Ions Content in Cr<sup>3+</sup>-Adapted and Original *Saccharomyces bulardii* biomass:** The contents of some metal ions in the whole biomass of Cr<sup>3+</sup> adapted and original *Saccharomyces bulardii* were compared (Table 1). The biomass of Cr<sup>3+</sup>-adapted strain contained Cr<sup>3+</sup>, Zn<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> in amounts higher than those in the original one when grown in the presence of 50 µg Cr<sup>3+</sup>/ml, while, the biomass of original strain contained Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup> and K<sup>+</sup> in amounts higher than the adapted strain when grown in the same conditions. When the two strains grown on Cr<sup>3+</sup> free medium, the Cr<sup>3+</sup>-adapted strain gives high amounts of Cr<sup>3+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Mg<sup>2+</sup> and Mn<sup>2+</sup> compared to the original one. In contrast the amounts of Zn<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> in case of the original strain were higher than those in the Cr<sup>3+</sup>-adapted strain (Table 1). Saad<sup>[17]</sup> found that the Cr<sup>3+</sup>-adapted *Saccharomyces bulardii*, grown in the presence or in the absence of Cr<sup>3+</sup> contained more protein than the non-adapted one (original) grown under the same conditions. This higher protein content may be one of the reasons for the high accumulation of Cr<sup>3+</sup> Zn, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> in that NH<sub>2</sub> and SH groups of the

proteins complexed these metals. Recently, several metal-binding peptides were expressed in *E. coli* or yeast strains in order to increase their metal resistance or bioaccumulation ability<sup>[14,13,10]</sup>. Therefore, glucose regulation depends on a wide range of vitamins, minerals and other micronutrients<sup>[1]</sup>.

**The effect of Cr<sup>3+</sup> at a concentration of 50µg/ml on the pattern of the amino acids of Cr<sup>3+</sup>-adapted and original *Saccharomyces bulardii* biomass:** As shown in Table (2), the amino acids composition of the dried biomass of original and Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* grown in the presence or in the absence of Cr<sup>3+</sup> were different. The adapted and the original strains have more amounts of most amino acids when grown in the presence of Cr<sup>3+</sup> than these strains grown in the absence of Cr<sup>3+</sup>. On the other hand, the adapted strain grown in the presence of Cr<sup>3+</sup> had more amounts of amino acids than the original strain grown under the same conditions, except glutamic which was very high. Also the adapted strain had more of most amino acids when grown in the absence of Cr<sup>3+</sup> than the original strain grown under the same conditions. The results indicate that quantitative increase in the amino acid compositions of both the original and the Cr<sup>3+</sup>-adapted strains when grown in the presence of 50 µg Cr<sup>3+</sup>/ml. The present study reveals that the original and Cr<sup>3+</sup>-adapted strains have large amount of Cr<sup>3+</sup> in the form of yeast (organic form) when the two strains were grown in the presence of Cr<sup>3+</sup>, consequently more amino acids were increased. Inorganic chromium compounds are poorly absorbed in man<sup>[23]</sup>. Organic chromium such as that found in brewer's yeast, is much better absorbed than inorganic chromium. This form of trivalent chromium, complexed with nicotinic acid and amino acids, is known as glucose tolerance factor (GTF)<sup>[22]</sup>. Davis and Vincent<sup>[8]</sup> recently proposed that Cr increases glucose tolerance through a Cr-dependent oligopeptide that activates insulin receptor tyrosine kinase activity.

**Treatment of Diabetic Rats by Different Yeast Solutions:** Results in Table (3) show that the glucose, total cholesterol and triglycerides levels in 10 rats

**Table 1:** The contents of some heavy metal ions in *Saccharomyces bulardii* biomass (original and chromium adapted) in the presence or absence of chromium

<i>Saccharomyces bulardii</i>	Metal ion mg/100mg dry biomass									
	Cr <sup>3+</sup>	Fe <sup>2+</sup>	Zn <sup>2+</sup>	Cu <sup>2+</sup>	Ni <sup>2+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Mn <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
Chromium-adapted*	0.0612	0.0838	0.00787	0.00335	0.00168	0.0226	0.1239	0.0034	0.0158	0.1258
Chromium-adapted**	0.0250	0.0911	0.0035	0.01348	0.0016	0.0093	0.1366	0.00438	0.0110	0.1238
Non-adapted (original)**	0.0250	0.0811	0.00453	0.0037	0.00138	0.0111	0.0679	0.0038	0.0134	0.1362
Non-adapted (original)*	0.0200	0.0978	0.00651	0.00418	0.00202	0.01472	0.0971	0.00392	0.01312	0.1327

\* Organism grown on medium amended with 50 µg Cr<sup>3+</sup>/ml

\*\* Organism grown on medium not amended with Cr<sup>3+</sup>

**Table 2:** Amino acids composition of original and Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* in the presence or in the absence of Cr<sup>3+</sup>.

Amino acid	Composition (mg/200µg) of dry biomass			
	A	B	C	D
Aspartic	352.35	445.3	NR	334.12
Threonine	119.34	191.14	NR	143.63
Serine	206.86	310.39	587.51	228.79
Glutamic	440.48	420.98	18.56	329.34
Proline	193.57	161.169	NR	115.52
Glycine	170.24	250.12	489.44	197.2
Alanine	264.36	344.27	715.85	258.9
Cystin	6.08	NR	86.93	NR
Valine	86.11	214.09	377.02	173.3
Methionine	25.71	44.71	134.34	28.57
Isoleucine	58.78	115	285.01	93.87
Leucine	196.08	346.11	807.32	257.67
Tyrosine	65.44	216.42	525.99	159.09
Phenylalanine	107.98	183.97	447.57	137.96
Histidine	10.92	209.87	566.12	147.64
Lysine	160.13	257.28	610.76	181.75
Arginine	18.41	44.87	98.92	37

A: Original *Saccharomyces bulardii* grown on Cr<sup>3+</sup> free medium.  
 B: Original *Saccharomyces bulardii* grown on 50µg Cr<sup>3+</sup>/ml containing medium.  
 C: Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* grown on 50 µg Cr<sup>3+</sup>/ml containing medium.  
 D: Cr<sup>3+</sup>-adapted *Saccharomyces bulardii* grown on Cr<sup>3+</sup> free medium.  
 NR: Indicates that the analysis was not reported.

**Table 3:** Glucose, cholesterol and triglyceride levels in blood of normal and diabetic rats (values are mean and standard error of 7 rat/group).

Component	Normal	Diabetic
Glucose	155±4.9	457±5.2
Total cholesterol	103±2.2	184±1.8
Triglyceride	187±1.6	430±6.4

**Table 4:** Glucose, cholesterol and triglyceride levels in hyperglycemic rats at different days of Cr<sup>3+</sup> yeast solutions treatments (Values are mean and standard error of 7 rat/group).

Component	Time in days	Rat groups			
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Glucose	15	410±4.2**	441±6.5*	438±6.5*	396±3.9**
	30	360±3.2**	434±9.9*	427±8.8*	350±2.8**
	45	290±2.2**	430±9.6*	431±9.3*	270±4.1**
	60	196±1.9**	428±8.4*	427±8.9*	180±2.8**
T. cholesterol	15	174±2.6*	184±1.4	180±2.2	178±1.8*
	30	170±3.4**	178±3.4	174±4.9	172±3.9*
	45	166±6.2*	176±3.5	168±8.4	158±9.4*
	60	164±7.1*	174±4.4	170±7.6	157±8.8*
Triglycerides	15	402±8.4*	424±5.4	426±6.2	398±9.6*
	30	398±8.8*	413±8.4	414±5.6	396±9.4*
	45	396±9.4*	410±7.8	411±7.9	392±12.9*
	60	395±9.8*	408±9.2	408±9.6	390±12.6*

\*Significant (P<0.05)  
 \*\* High significant (P < 0.01)

injected with citrate-phosphate buffer solution (0.1 M, pH, 4.50) were 155±4.09, 103±2.2 and 187±1.6 mg/dL, respectively). This group was taken as a control group,

while the other 40 rats injected with STZ have higher glucose, cholesterol and triglycerides levels (457±5.2, 184±1.8 and 430±6.4 mg/dL, respectively) indicated more glucose concentration than 300 mg/dL and these used as diabetic rats, which divided into 4 groups, all groups maintained till experimental periods 60 days. The result in Table (4) show that the 1<sup>st</sup> and 4<sup>th</sup> groups treated with solution No. 1 or solution No. 4 exhibited gradually decreases in glucose, cholesterol and triglycerides levels until reached to the maximal decreased values after 60 days (261, 20 and 35 mg/dL, respectively for group No. 1 and 277, 27 and 40 mg/dL, respectively for group No. 4). While, the 2<sup>nd</sup> and the 3<sup>rd</sup> groups treated with solution No. 2 or solution No. 3 exhibited slightly gradually decreases in glucose, cholesterol and triglycerides levels through the 60 days interval. Generally the highest significant decrease in glucose and significant decrease in the cholesterol and triglycerides levels were occurred in blood of the 1<sup>st</sup> and 4<sup>th</sup> groups treated with solution No. 1 or No. 4, while nonsignificant changes were observed in blood of the 2<sup>nd</sup> and 3<sup>rd</sup> groups treated with solution No.2 or solution No. 3. Rabinovitz<sup>[15]</sup> and Rabinovitz *et al.*,<sup>[16]</sup> found that chromium appears to work by increasing the sensitivity of patients insulin receptors. Arkuzewska *et al.*, (2003) found that Cr<sup>3+</sup> positively affected the effectiveness of broiler chicken fattening and did not affect the chromium level in the muscle tissue. Also, Alan *et al.*,<sup>[1]</sup> found that 33 rats fed a chromium-deficient diet developed hyperglycemia and glycosuria, 34 Monkeys maintained on a low-chromium diet had abnormal glucose metabolism, which was corrected by chromium supplementation. Guan *et al.*,<sup>[12]</sup> found that high Cr-yeast supplementation tended to increase plasma glucose clearance rate by 31.3% and decreased plasma glucose clearance half-life by 25.5% in pigs, corresponding to a smaller 30 min. area of plasma glucose concentration after an i.v. GTT. This study confirmed that high Cr-yeast supplementation increases glucose tolerance in pigs. Improvement in glucose tolerance was found in pigs fed Cr tripicolinate<sup>[2]</sup>.

In conclusion, the results showed significant decreases in glucose, cholesterol and triglycerides of serum of diabetic rats administered with 1.0 ml of Cr<sup>3+</sup> rich adapted *Saccharomyces bulardii* (700 mg dried biomass dissolved in 50 ml of citrate phosphate buffer solution contains 428.4µg Cr<sup>3+</sup>) (solution No. 1) or with 1.0 ml of solution contains non-adapted strain biomass (100 mg dried biomass dissolved in 50 ml for citrate phosphate buffer solution contains 140.0 µg Cr<sup>3+</sup>) (solution No. 4) Cr<sup>3+</sup> considered as essential element for human food and animal feed in a dose of 50-200 µg/day<sup>[2]</sup>. In addition to Cr<sup>3+</sup>, glucose regulation depends on a wide range of vitamins, minerals and other micronutrients<sup>[1]</sup>.

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