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Research Article

# Accumulation and Hypoglycemic Effect of Chromium-Rich Plants on Animal Physiology

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**ABSTRACT**

The study was designed to investigate chromium (Cr) uptake by plants, hypoglycemic effect of the organic trivalent Cr(3+) on sreptozotocin (STZ)-induced diabetic mouse and the accumulation efficiency of Cr from dietary inorganic-Cr(3+), yeast-Cr, Cr-picolinate and organic-Cr(3+) in rabbit tissues. Radish sprouts was grown in the presence of Cr rich liquid fertilizers (Cr-gluconic-1, Cr-gluconic-2, Cr-picolate, Cr-glutamate and Cr-glycine). In addition, soybean, mung bean and alfalfa were also cultivated using Cr-glycine and Cr-picolinate liquid fertilizer to study the efficiency of Cr(3+) uptake. Sreptozotocin (STZ)-induced diabetic mouse were administrated organic-Cr(3+) for10 days and examined their blood glucose level. On the other hand, rabbits were allowed to take 200 µg/head/day of $CrCl_3.6H_2O$, yeast-Cr, Cr-picolinate or organic-Cr supplemented in diets for 14 days. Body weight, liver and kidney weight, and accumulation of Cr in blood, kidney and liver were examined. Radish sprouts uptake hexavalent Cr (Cr6+) in case of all liquid fertilizer, but the mung bean was grown with Cr-glycine liquid fertilizer without uptake of Cr (6+). Mung bean grown with Cr-glycine liquid fertilizer uptake a higher ($P<0.05$) level of organic-Cr(3+). The dietary organic-Cr significantly ($P<0.05$) reduced blood glucose level in STZ-induced diabetic mouse. The administration of organic-Cr(3+) was safety for rabbit and Cr accumulation was higher ($P<0.05$) in the rabbits fed organic-Cr(3+) diet compared to other Cr supplements. The sequential order of the Cr accumulation in serum, kidney and liver of these rabbit were inorganic-Cr<yeast-Cr<Cr-picolinate<organic-Cr(3+). The study concludes that mung bean plants grown with Cr-Gly liquid fertilizer may uptake non-toxin organic-Cr3+ potentially which have the hypoglycemic effect and may be considered as a safe and excellent element for animal physiology compared to any other Cr supplements.

**Key words:** Accumulation, Hypoglycemic effect, Chromium-rich plants, Animal physiology

## INTRODUCTION

Diabetes mellitus is a leading cause of death and disability worldwide (Lozano *et al*., 2012; Murray *et al*., 2012). Its global prevalence was about 9% in 2015 (WHO, 2014). Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the produced insulin. Insulin is a hormone that regulates blood sugar (WHO, 1999). Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

Glaser and Halpern (1929) discovered brewer's yeast exhibited a potentiating effect on the hypoglycemic action of insulin. In 1958 the potentiating effect was rediscovered when rats fed a Torula yeast-based diet began to show signs of glucose intolerance, which was reversed by a diet of brewers yeast (Murray *et al*., 2012). This discovery led to the isolation of a "glucose tolerance factor" (GTF, Mertz and Schwarz, 1959; Schwartz and Mertz, 1957). Trivalent chromium (Cr3+) was found to be the active component of GTF. The biological activity of chromium (Cr) was found to be contingent on the valence, Cr(3+) being the only biologically active form. There are two types of Cr, one is organic-Cr and another is inorganic-Cr. The bioavailability of inorganic-Cr is <3% while organic-Cr is over 10 times more available (Lyons, 1994). The causes of the low bioavailability of inorganic-Cr are numerous and they are likely to be in connection with the formation of non-soluble Cr oxides, Cr binding to natural chelate-forming compounds in fodders, interference with ion forms of other minerals like, Zn, Fe, V (Borel and Anderson, 1984), also the slow conversion of inorganic-Cr to the bioactive form (Ranhotra and Gelroth, 1986).

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Chromium is widely used supplements are available as Cr chloride, Cr nicotinate, Cr picolinate, high-Cr yeast, and Cr citrate. Chromium chloride in particular appears to have poor bioavailability (Anderson et al., 1997). However, given the limited data on Cr absorption in humans, it is not clear which forms are best to take. Chromium (3+), such as picolinate (Cr-pic) is currently a very popular nutritional supplement; however, its safety has recently been questioned (Speetjens *et al*., 1999) especially with regard to its ability to act as a clastogen. It is known that Cr(3+) shows mutagenicity or damages chromosomes in animal cells, therefore, research extend over the second generation is necessary for the risk assessment of Cr(3+). So, there is a crying need to develop the safer form of organic-Cr. It was allowed to uptake the Cr(3+) by the plants, and hypothesized that the inorganic-Cr might be converted to organic-Cr in the plants, where the organic-Cr is the natural convenient, safe and potential way to increase the Cr intake. Therefore the study was designed to investigate Cr uptake by plants, hypoglycemic effect of the organic trivalent Cr(3+) on sreptozotocin (STZ)-induced diabetic mouse and the accumulation efficiency of Cr from dietary inorganic-Cr(3+), yeast-Cr, Cr-picolinate and organic-Cr(3+) in rabbit tissues.

## MATERIALS AND METHODS

### Seeds and fertilizer

Seeds of radish (*Raphanus sativus var*), soybean (*Glycine max*), mung bean (*Vigna radiate*) and alfalfa (*Medicago sativa*) used in this study were purchased from the local market available for hydroponic cultivation. The chemicals for preparation of liquid fertilizer such as, chromic chloride hexahydrate, Cr(3+) sulfate, potassium chromate, gluconic acid, glutamic acid, glycine, glucose, sucrose, hydrogen peroxide, potassium acetate and sodium hydroxide were purchased from Nacalai Tesque (Kyoto, Japan) and Picolinic acid from Tokyo Chemical Industry (Tokyo, Japan).

### Plant cultivation and harvesting

Hydroponic cultivation was carried out in greenhouses equipped with a lighting device and maintained 25±3°C temperature during cultivation. Seeds of the plants were sown on the sponge of the plastic plate in the four day dark state by circulating water to allow germinate. Total five types of liquid fertilizer such as Cr-Gluconic acid 1 (Cr-gluc-1), Cr-Gluconic acid 2 (Cr-gluc-2), Cr-picolinic acid (Cr-pic), Cr-glutamic acid (Cr-glu), Cr-glycine (Cr-gly) were applied for hydroponic cultivation of radish sprout. In addition, soybean, mung bean and alfalfa were also cultivated using Cr-gly and Cr-pic liquid fertilizer to study the efficiency of Cr(3+) uptake. The liquid fertilizers (chemical composition is shown in Table 1) was continuously aerated with pumps in the growth container and repeated with newly made solution by every five days.

These plants were grown with 24 h light, about 25°C temperature and 70% relative humidity in the hydroponic cultivation. These plants were harvested after two-weeks of sowing, then dried, grinded and stored as powder in separate polybags.

### Animal care and treatment

All procedures were performed in accordance with the animal experimentation guidelines of Shinshu University.

***Mouse:*** A total 200 ICR male mice of 5-wk-old (Japan SLC, Inc., Shizuoka, Japan), weighing 20-25 g were used. Animals were housed individually in plastic cages under a controlled atmosphere (temperature 22.0±1°C, humidity 60±5% and light 06:00 to 18:00 h). Deionized water and a commercial diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) of same lot number were available *ad libitum*. The concentration of organic-Cr in the CRF-1 diet was 46.6±4.5 μg/kg as determined by ICP-DRC-MS (inductively coupled plasma dynamic reaction cell mass spectrometry). After 3 days of acclimation period, the mice were injected intraperitoneally with two doses of STZ as 0.1 mg/g body weight (Wako Pure Chemical Industries, Ltd., Osaka, Japan), once after 24 h food deprivation and again at a similar time of the days later (Lenzen, 2008). Control mice were injected with equal volumes of citric acid buffer at the same times as the STZ injections. The day of the last STZ or buffer injection was defined as day 0. At 3$^{rd}$ day (72 h after the last injection), the fasting plasma glucose concentration was measured using tail vein blood samples by a blood glucose monitor (Freedom Lite; Nipro, Oosaka, Japan), and STZ mice were confirmed to hyperglycemic (plasma glucose level >240 mg/dl). Finally the experiment divided the mice as low (control) and high blood glucose levels as 240 ~ <300 mg/dl and >300 mg/dl. The mice were administered organic-Cr as 40, 400, 800 and 2000 μg/head/day. During the feeding trial, glucose level was measured using blood collected from the tail vein. After completion of 10 days feeding trial, the mice were anesthetized with sodium pentobarbital and blood was collected from the abdominal aorta. Then the mice were sacrificed and liver and kidney were collected, washed with saline, blotted and weighed.

***Rabbit:*** A total 75 physically healthy male rabbits of 5 months-old (Japan SLC, Shizuoka, Japan) were randomly divided into five groups as Control, Cr chloride, high-Cr yeast, Cr picolinate and organic-Cr so that 15 rabbits were in each group. The rabbits were housed in individual cage under uniform management conditions with 15 to 20°C temperature and 12L: 12D light schedule. Deionized water and a commercial diet (ORC4; Oriental Yeast Co., Ltd., Tokyo, Japan) were supplied *ad libitum*. The diets administered by the rabbits were supplemented with inorganic-Cr as chromium chloride (+3) hexahydrate chromium chloride ($CrCl_3.6H_2O$, Nacalai Tesque, Inc., Kyoto), yeast-Cr (Health-One, Inc. Bidaka Shoji, Tokyo) and Cr-pic (Nature's Way Prducts, Inc., UT 84043, USA) and organic-Cr incorporated in mung bean cultivated hydroponically with Cr-gly liquid fertilizer (Inshalt®, MI tech Co., Ltd., Nagano, Japan). The rabbits were allowed to take as 200 μg Cr /head/day for 14 days. At the end of the feeding trial, the rabbits were sacrificed and collected blood samples from the femoral artery. The blood samples were centrifuged at 12,000×g for 30 min to obtain serum, and kidney and liver were quickly collected and stored at -180°C.

**Analysis of chromium**

Organic-Cr was determined by inductively coupled plasma mass spectro-metry (ICPMS) using Rh as internal standard. Up to 1 g of the liver, or kidney was heated with 5 ml of metal-free $HNO_3$ in a boiling water bath until the disappearance of insoluble components. The volume of the digest was made up to 25 ml with distilled water. Chromium in the diluted digest was directly nebulized to ICPMS.

**Statistical analyses**

Experimental data were assessed by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference tests (LSD) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). The data were expressed as the mean±SEM. Differences were considered significant at $P<0.05$ and $P<0.01$.

## RESULTS

**Chromium uptake by plants**

Uptake of Cr into the radish sprouts cultivated hydroponically with different types of liquid fertilizer, such as Cr-gluc-1, Cr-gluc-2, Cr-pico, Cr-glu or Cr-gly were shown in Figure 1. Significantly ($P<0.01$) higher uptake of Cr(6+) was occurred in the radish sprouts which grown with Cr-pic (93.0 mg/kg) than the radish sprouts grown with Cr-gluc-1 , Cr-gluc-2, Cr-glu or Cr-gly (7.1, 7.5, 6.4 and 10.0 mg/kg respectively) . The lowest uptake of Cr(6+) was observed in the radish sprouts grown with Cr-gly, however the lowest uptake of total Cr was the radish sprouts grown with Cr-gluc-1 (27.3 mg/kg). The highest uptake of total Cr and Cr(3+) were in the radish sprouts grown with Cr-pic (66.0 mg/kg) and Cr-glu (68.6 mg/kg). Moreover, when radish sprouts and soybean were grown in the presence of Cr-pic liquid fertilizer, the uptake rate of total Cr and Cr(3+) were significantly higher ($P<0.05$) and Cr(6+) was lower ($P<0.01$) in soybean compared to the radish sprouts (Fig. 2).

The results regarding uptake of total Cr, Cr(3+) and Cr(6+) in radish sprouts, soybeans, mung bean and alfalfa grown by using Cr-gly liquid fertilizer are shown in Figure 3. The highest uptake of total Cr and Cr(3+) were observed in soybean (218.0 and 206.8 mg/kg) compared to other plants. Though radish sprouts, soybean and alfalfa uptake little amount of Cr(6+). The uptake of Cr(3+) by mung bean was medium (116.0 mg/kg), but uptake of Cr(6+) was nil.

To examine the converting efficiency of Cr(6+) to Cr(3+) by the influence of mung bean extract. The different levels (0.087, 0.87 and 1.044 mg) of Cr(6+) were added to mung bean extract of 98, 80 and 76 ml and obtain the final concentration (1.085 mg/l) of Cr(6+) in each solution. The mung bean extract of 98 and 80 ml were able to convert the Cr(6+) to 100% Cr(3+) (Table 2).

The Cr(3+) content in different parts (leaf, stems and roots) of mung bean plant cultivated hydroponically with Cr-gly liquid fertilizer are shown in Figure 4. Among the different parts of the mung bean plants, roots (2.6 g/kg) contained highest ($P<0.01$) level of Cr (3+) compared to the leaves (0.22 g/kg) and stems (0.25 g/kg).

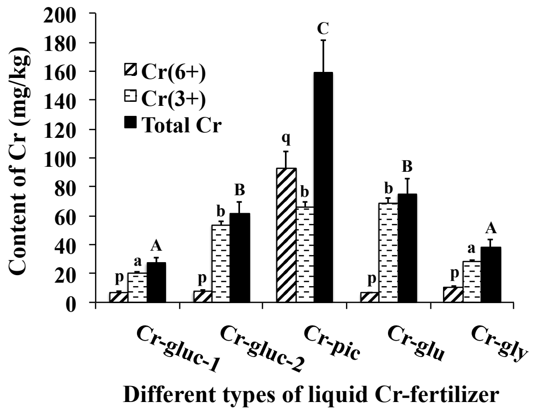


**Fig. 1:** Uptake of Cr into the radish sprouts cultivated hydroponically with different types of liquid fertilizer, such as Cr-gluconate-1, Cr-gluconate-2, Cr-picolinate, Cr-glutamate or Cr-glycine. The content of Cr(3+) was calculated by subtracting the amount of Cr(6+) from the amount of total Cr.Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences ($P<0.05$).

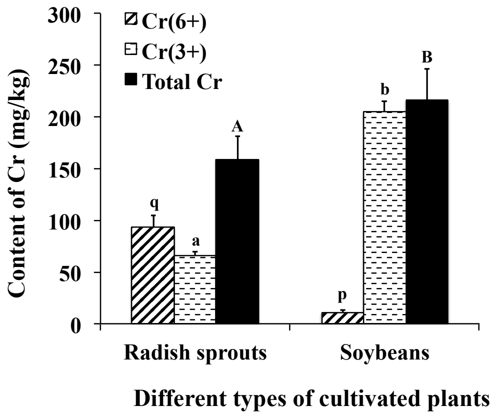


**Fig. 2:** Uptake of total Cr and Cr(3+) into the radish sprouts and soybean were grown in the presence of Cr-picolinate liquid fertilizer. The content of Cr(3+) was calculated by subtracting the amount of Cr(6+) from the amount of total Cr. Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences ($P<0.05$).

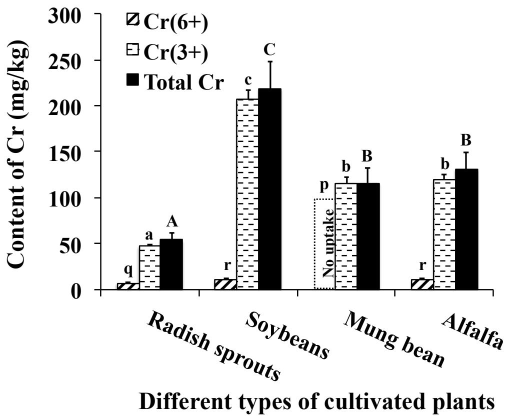


**Fig. 3:** Uptake of total Cr, Cr(3+) and Cr(6+) in radish sprouts, soybeans, mung bean and alfalfa grown with Cr-gly liquid fertilizer. The content of Cr(3+) was calculated by subtracting the amount of Cr(6+) from the amount of total Cr. Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences ($p<0.05$).

**Table 1:** Composition of the liquid fertilizers*[1]

| Ingredients | Cr-gluc-1 | Cr-gluc-2 | Cr-pic | Cr-glu | Cr-gly |
|---|---|---|---|---|---|
| Potassium chromate ($K_2CrO_4$), g | 1.00 | 1.00 | 1.00 | - | - |
| Chromium (3+) sulfate ($Cr_2(SO_4)_3$), g | - | - | - | 1.00 | - |
| Chromium (3+) chloride ($CrCl_3.6\ H_2O$), g | - | - | - | - | 5.50 |
| Gluconic acid ($C_6H_{12}O_7$), ml | 60.0 | 60.0 | 30.0 | - | - |
| Sucrose ($C_{12}H_{22}O_{11}$), g | - | - | - | - | 7.00 |
| Potassium acetate ($C_2H_3KO_2$), g | 20.0 | - | - | - | - |
| Acetic acid ($C_2H_4O_2$), ml | 20.0 | - | 20.0 | - | - |
| L-Glutamic acid ($C_5H_9NO_4$), g | - | - | - | 1.20 | - |
| Glycine ($C_2H_5NO_2$), g | - | - | - | - | 5.00 |
| Nitrobenzene ($C_6H_5NO_2$), ml | - | - | 2.00 | - | - |
| Hydrogen peroxide ($H_2O_2$), ml | - | - | - | 1.00 | 4.00 |
| Water ($H_2O$), ml | 70.0 | 40.0 | 20.0 | 80.0 | 55.0 |

*The pH of the solution was adjusted to 6.5±0.1 with 1 M KOH; [1]The final concentrations of Cr were 0.19, 0.27, 0.18, 0.26 and 0.011% in Cr-gluc-1, Cr-gluc-2, Cr-pico, Cr-glu and Cr-gly, respectively.

**Table 2:** Converting ability of mung bean extract from Cr(6+) to Cr(3+)

| Mixing | | Cr(6+)[b] (mg/l) | Residual Cr(6+)(mg/l) |
|---|---|---|---|
| Mung bean extract[a] (ml) | Cr(6+) (mg) | | |
| 100 | 0.0 | 0.0 | 0 |
| 98 | 0.87 | 1.085 | 0 |
| 80 | 0.87 | 1.085 | 0 |
| 76 | 1.044 | 1.085 | 0.038 |
| 0 | 1.085 | 1.085 | 1.085 |

[a]Extract of raw mung bean (100g/l); [b]Final concentration of Cr(6+) in solutions

**Table 3:** The chemical analysis of organic-Cr(3+) (Inshalt®)

| Nutrients | Content |
|---|---|
| Water | 10.5 ml/100g |
| Protein* | 44.1 g/100g |
| Lipid | 3.4 g/100g |
| Mineral | 5.8 g/100g |
| Cr(3+) | 2300 mg/100g |
| Cr(6+) | 0 mg/100g |
| Carbohydrates[1] | 36.2 g/100g |
| Energy | 352 Kcal/100g |
| Sodium | 327 mg/100g |
| Sodium chloride equivalent | 0.831 g/100g |

*Nitrogen/protein conversion factor: 6.25; [1]100 - (water+protein+lipid+mineral); Energy conversion factor: protein, 4; lipid, 9 and carbohydrates, 4; Sodium chloride equivalent (g) = Na (mg)×2.54÷1000.

The nutrient analysis of organic-Cr contained by legumes cultivated hydroponically using Cr-fertilizers is shown in Table 3. The content of the organic-Cr(3+) **(**Inshalt**®**) was also improved in the legumes cultivated hydroponically in the presence of Cr-fertilizers.

**Effect of dietary organic-Cr(3+) on blood glucose**

Effect of different levels (40, 400, 800 and 2000 μg/head/day) of dietary organic-Cr(3+) on blood glucose levels of STZ induced diabetic mice (having low, medium and high level of blood glucose) are shown in Figure 5. There was no significant (P>0.05) effect of dietary organic-Cr(3+) on blood glucose levels in the mice having lower blood glucose level (control group) during the ten days feeding trial. However, the levels 40, 400, 800 and 2000 μg/head/day of dietary organic-Cr(3+) significantly (P<0.01) begun to decrease blood glucose level in the hyperglycemic mice (both medium and high) from 7$^{th}$ days of feeding trial. The dietary levels 40 and 2000 (μg/head/day) of organic-Cr(3+) potentially decreased the blood glucose level in the medium and high hyperglycemic mice, respectively than that of the other levels. On the other hand, there was no chromium toxicity effect on liver and kidney, even on body weight gain of the diabetic mice due to the dietary intake of organic-Cr(3+) for 10 days. The different levels of organic-Cr(3+) had no significant (P>0.05) effect on body weight gain and the weight of liver or kidney (results haven't been demonstrated).

**Growth performance of rabbit**

The dietary effects of inorganic-Cr(3+), yeast-Cr, Cr-pic or organic-Cr(3+) for 14 days on body weight, and liver and kidney weight of rabbits are shown in Figure 6. There were no significant (P>0.05) differences in case of body weight, as well as liver and kidney weight of the rabbits fed diets supplemented with inorganic-Cr(3+), yeast-Cr, Cr-pic or organic-Cr(3+) for 14 days feeding period.

**Chromium accumulation in serum and tissues**

The accumulation of Cr in serum, liver and kidney of the rabbits fed the diets supplemented with different types of Cr such as, $CrCl_3.6H_2O$, yeast-Cr, Cr-pic or organic-Cr(3+) are demonstrated in Figure 7. The concentration of Cr in serum was higher in the rabbit fed organic-Cr(3+) (1.3 μg/l) compared to the other rabbits (0.3, 0.6, 0.8, and 0.8 μg/l) fed without Cr (Control), $CrCl_3$, yeast-Cr or Cr-pic supplemented diets, respectively. Among the rabbit groups fed diets supplemented with $CrCl_3$, yeast-Cr, Cr-pic and organic-Cr(3+), the accumulation of Cr in kidney was lowest in the rabbit group fed diets with yeast-Cr. The accumulation of Cr in liver was lower in the rabbit groups fed yeast-Cr or Cr-pic diets than that of the other groups of rabbits. The accumulation of Cr in the serum, liver and kidney were higher in the rabbits fed organic-Cr(3+) diet than other groups of rabbits. The Cr content in the liver of the rabbits fed organic-Cr(3+) supplemented diet was 1.6, 2.2 and 2.2 times higher than the rabbit groups fed inorganic-Cr, yeast-Cr, and Cr-pic diets, respectively.

## DISCUSSION

In the natural environment, heavy metals, including Cr, affect all elements of the food chain, from soil microorganisms to plants, animals, and humans (Bååth, 1989; Jordao, *et al*., 1999; Liu *et al*., 2009). Chromium is a non-essential element for plant growth and development (Huffman and Allaway, 1973; Zayed and Terry, 2003). It may be absorbed by plant roots as Cr(3+) or Cr(6+), it is poorly translocated and largely retained in roots, independently of Cr form that has been taken up (Cary, 1977; Zayed *et al*., 1998; Zayed and Terry, 2003). The two ions do not share a common uptake mechanism: the uptake of Cr(3+) is largely a passive process, whereas the uptake of Cr(6+) is mediated by low affinity sulphate carriers, specific for the uptake of essential metals (Skeffington *et al*., 1976; Cervantes *et al*., 2001; Shanker *et al*., 2005) and quickly converted to Cr(3+) in roots by Fe3+ reductase enzymes (Zayed *et al*., 1998). In fact conversion of Cr(6+) to Cr(3+) occurs in the roots where Cr(3+) is the most predominant form: once Cr is transformed, translocation is very little, being Cr(3+) a form with low solubility (Zayed and Terry, 2003; Zayed et, al., 1998). Nevertheless plants absorb it and the impact on the physiology of plants depends on Cr oxidation state, responsible of its fate and the resultant toxicity in plants (Shanker, *et al*., 2005).

In hydroponic cultivation of radish sprouts under this study with the presence of gluconic acid, picolinic acid, glycine and others, it was observed that radish sprouts accumulated Cr(6+) in case of all chelating agents. Radish sprouts accumulated the highest amount of total Cr and Cr(6+) when cultivated with specially, picolinic acid (Fig. 1). The Cr(+6) uptake was higher in the radish sprouts than that of soybean in the presence of picolinate fertilizer. Hexavalent Cr content of plants in the same liquid fertilizer was considered different depending on the type of plants. An early chelator that was used for mineral nutrition in plants was ethylene-diamine-tetra-acetic acid (EDTA, Huang *et al*., 1997). The EDTA molecule forms bonds with metals that are very strong, occasionally, EDTA chelated minerals are used as foliar nutrients, but their high stability decreases the release of their minerals at the plasma membrane. If some of the minerals are released at the plasma membrane, the strongly chelating EDTA scavenges calcium out of the cell walls of the leaves and contributes to leaking cytoplasm, cell damage, and disease. The EDTA problem is inability to biodegrade in the environment.

Chromium picolinate is currently a very popular nutritional supplement; however, its safety has recently been questioned, especially with regard to its ability to act as a clastogen. The rate of Cr(3+) uptake was high, and Cr(6+) was also unusually high when radish sprouts were cultivated with picolinate fertilizer. Uptake of heavy metals in the edible parts of plants represents a direct pathway for their incorporation into the human food chain (Florijn and van Beusichem, 1993). Hexavalent Cr is highly dangerous if ingested into the body for its toxic effects. Its compounds are easily dissolved and highly absorbed by intestinal wall compared to the Cr(3+) compounds. However, extracellular conversion of Cr(6+) to Cr(3+) in gastric fluid is a well-recognized detoxification process (De Flora and Boido, 1980; De Flora, 2000) thought to protect against systemic toxicity of ingested Cr(6+) at doses that do not overwhelm reductive capacity. As demonstrated by numerous studies, once Cr enters human cells, it causes DNA damage and mutagenesis (McCarroll, 2010; Zhitkovich, 2011).

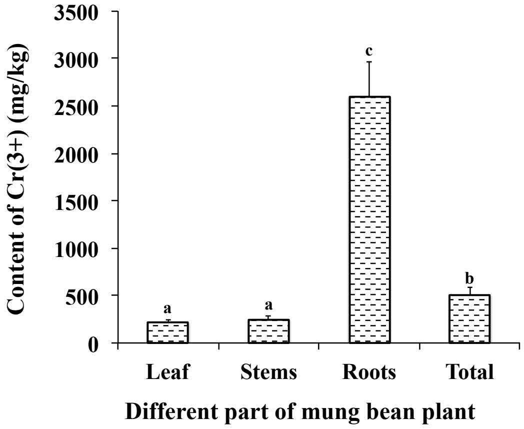


**Fig. 4:** Cr(3+) content in different parts (leaf, stems and roots) of mung bean plant grown with Cr-gly liquid fertilizer. Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences (P<0.05).

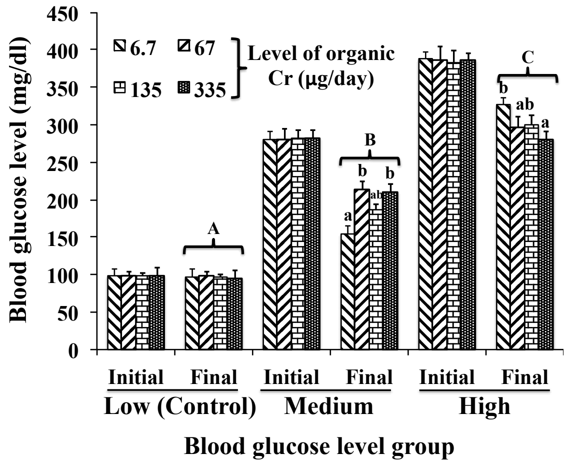


**Fig. 5:** Effect of different levels (40, 400, 800 and 2000 µg/head/day) of dietary organic Cr(3+) on blood glucose levels of STZ induced diabetic mice (having low, medium and high level of blood glucose). Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences (P<0.05).

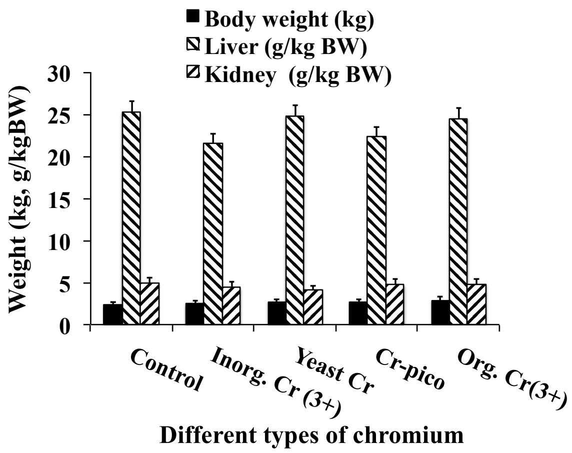


**Fig. 6:** The dietary effects of inorganic Cr(3+), yeast Cr, Cr-pic or organic Cr(3+) (200 µg/head/day) for 14 days on body weight, and liver and kidney weight of rabbits. Each bar with error bar represents the mean±SEM values.

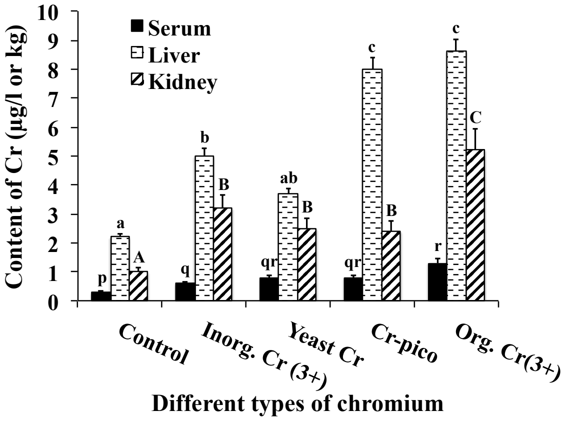


**Fig. 7:** The accumulation of Cr in serum, liver and kidney of the rabbits fed (200 µg/head/day) the diets supplemented with different types of Cr such as, $CrCl_3.6H_2O$, Cr yeast, Cr-pic or organic Cr(3+). Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same type of bar indicate significant differences (P<0.05).

The Cr(6+) uptake in soybean and mung bean were predominantly lower than radish sprouts cultivated hydroponically with Cr-gly liquid fertilizer. It was observed in this study that the content of Cr(6+) in mung bean was nil. The mung bean have the highest potentiality to convert Cr(6+) to Cr(3+). Both enzymatic and nonenzymatic pathways may be involved in Cr(6+) reduction, although at normal physiological conditions nonenzymatic reduction is believed to dominate. The primary reductants of Cr(6+) are ascorbic acid, glutathione, and cysteine, where ascorbic acid being the main reductant (NTP, 2008). However, the antioxidant property of the soy isoflavones, namely, genistein and daidzein are well established in different experimental models and also in clinical studies. Lytle *et al.* (1998) reported that reduction of heavy metals in situ by plants might be a useful detoxification mechanism for phytoremediation. The conversion of Cr(6+) to Cr(3+) appeared to occur in the fine lateral roots. The Cr(3+) was subsequently translocated to leaf tissues. In the experiment, considering zero level uptake of Cr(6+) and medium level uptake of Cr(3+), mung bean performed as the best plant for the uptake of Cr(3+) when cultivated in the presence of Cr-gly liquid fertilizer.

In this study, dietary organic-Cr begun to reduce blood glucose level in the STZ induced hyperglycemic (medium and high diabetic) from 7 days, and after 10 days of feeding trial the blood glucose level was significantly reduced. Mertz (1993) reported that Cr supplementation improved some measure of glucose utilization or had beneficial effects on blood lipid profiles. Impaired glucose tolerance refers to a prediabetic state and is currently defined by the presence of impaired fasting glucose (fasting plasma glucose concentration of 110-125 mg/dl) and impaired glucose tolerance status (plasma glucose concentration of 140-199 mg/dl during a two-hour challenge test with a 75 g oral glucose load; WHO, 2006). Impaired glucose tolerance is associated with modest increases in risk of cardiovascular disease, as well as other traditional microvascular complications of diabetes (Singleton *et al.*, 2003). Anderson *et al* (1997) reported a dose-response relation between dietary Cr supplementation and a decrease in Hb A1c concentrations in the control group compared with the treatment groups (200 and 1000 µg Cr-pico/day). Such tendency was observed in this experiment too, where mice was administered to the organic-Cr from 40 to 2000 µg/day. But there was no significant difference in body weight and organs weight of the mice fed even high dose of organic-Cr for 10 days. Moreover, some other mice were fed 800 µg of organic-Cr over 50 days, which did not show any abnormal physiology, that seemed organic-Cr is safe for animal being. Althuis *et al.* (2002) reported that one hundred and eighty participants were randomized to receive either a placebo or Cr supplements in the form of Cr-pic either 200 or 1,000 µg/day. After four months of treatment, fasting blood glucose concentrations were found to be 15 to 19% lower in those who took 1,000 µg/day of Cr compared to those who took the placebo. Recently, Vincent (2013) suggested that greater doses of Cr might be required to observe beneficial effects of Cr supplementation. There is a little evidence that Cr(3+) is toxic to humans, but the toxicity from oral intakes is considered to be low because ingested Cr is poorly absorbed, and most absorbed Cr is rapidly excreted in the urine (Nielsen, 2012). The studies included in this review contribute only an estimated 220 person-years of data (both treated and control groups) and <35 person-years in subjects who received high doses of Cr (≥800 µg). Several case series in the literature have reported Cr toxicity (Lukaski, 1999). The Food and Nutrition Board (FNB) of the Institute of Medicine did not set a tolerable upper intake level for Cr. Yet, despite limited evidence for adverse effects, the FNB acknowledged the possibility of a negative impact of high oral intakes of supplemental Cr(3+) on health and advised caution (FNB, 2001). Thus, future studies of Cr supplementation should establish the long-term safety of Cr therapy, particularly at high doses.

Anderson *et al.* (1996) showed that the concentration of Cr in the kidneys was more than ten times higher than in other tissues of the rats fed diets containing 63% cornstarch supplemented with different Cr(3+) compounds (5 mg elemental Cr/kg diet). The Cr content was highest in the kidneys, followed by the lungs, the gastrocnemius muscle, the liver, the spleen, and the heart of the rats that received a diet with $CrCl_3$. In this study, Cr content in the serum was higher in the rabbits fed CrCl3, yeast-Cr, Cr-Pic or organic-Cr3+ supplemented diets than the rabbits fed diet without Cr for 14 days. Anderson *et al.* (1985) have found the basal serum concentration of Cr in adults to be 0.13 ± 0.02 µg/l, followed by a significant increase to 0.38 ± 0.02 µg/l after 3 months of Cr supplementation. Ultmann *et al.* (1965) examined the bio-distribution by intravenous injection of sodium chromate ($^{51}Cr$) in mice and rats and observed that in mice of Cr levels is in the spleen (14.4%), liver (11.6%) and the other organs (2%) and in rat of Cr levels is in the spleen (22%), liver (4.5%) and the bone marrow (4.5%). In this experiment, Cr content in kidney and liver of all rabbits fed is higher than that of the rabbits fed diet without supplementation of Cr. Lindemann *et al.* (2004) measured the content of Cr in sows after supplementing different amounts of Cr-pic (0, 200, 600 and 1000 µg/kg Cr as-fed basis). The concentrations of Cr were measured in the adrenal gland (18.4, 20.0, 34.0 and 48.4 μg/kg), the kidneys (35.8, 56.4, 132.6 and 176.0 μg/kg) and in the liver (22.8, 37.4, 87.6 and 92.2 μg/kg). The higher dose of Cr supplementation increased the accumulation rate of Cr. Many of the mouse and rat experiments, the dose of Cr is less than 100 μg, the period of administration was more than 30 days. In this study, blood glucose levels were dramatically reduced within 10 days due to the administration of higher dose of organic-Cr (2000 μg). As there was no health hazard effect on animal, higher doses of Cr might be beneficial effect on lowering blood glucose levels. Moreover, this study revealed that the sequential order of the Cr accumulation in serum, kidney and liver of these rabbit was inorganic-Cr <yeast-Cr <Cr pic < organic-Cr(3+). Olin *et al*. (1994) investigated that the absorption/retention of Cr(3+) compounds $CrCl_3$, Cr nicotinate (Cr-nic), Cr-pic over a 12 h period in a rat model. They found that 3-8 times more Cr-nic was absorbed and retained than that of Cr Pic or $CrCl_3$. Anderson *et al*. (1996) investigated the incorporation of nine different Cr compounds on accumulation of Cr in rat's tissues and found the relative absorption/retention as follows: Cr-nic>Cr-pic>Cr $Cl_3$. Anderson *et al*. (1997) reported that Concentrations of Cr-pic in the liver and kidney were found to be 2-6 times higher than for $CrCl_3$ or Cr-nic-fed rats. These results were corresponded with the results of this study.

**Conclusion**

The study conclude that hydroponically cultivated mung bean plants using Cr-Gly liquid fertilizer may accumulate potentially the organic-Cr, which has 100% ability to convert the toxin Cr(6+) compounds to non-toxin Cr(3+) compounds. Moreover, the dietary organic-Cr(3+) have the hypoglycemic effect and may be considered as a safe and excellent element for animal physiology compared to any other Cr supplements.

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