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In vitro inhibition of glucose transport across the intestinal membrane of mice exposed to trivalent chromium.

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Abstract

Background: Trivalent chromium (Cr^{3+}) supplementation has been used in the management of type-2 diabetes mellitus and the small intestine plays significant role in glucose homeostasis. However, there is dearth of information on the glucose absorption ability of normal gut during Cr^{3+} exposure. In this study, we investigated the effect of Cr^{3+} exposure in the absorption of glucose in the normal gut.

Methodology: Thirty male slc:ddY mice (26.2 ± 1.1 g) were randomly and equally assigned to three groups: Group 1 (control) received drinking water while animals in groups 2 and 3 received 10 and 100 ppm Cr^{3+} respectively for 12 weeks through drinking water. Thereafter, they were sacrificed and their intestines excised, rinsed with ice-cold Ringer solution (RS) and nine everted-sacs were made, with addition of 200 µL RS. The sacs were incubated for 1 hour in 5 mL glucose-free RS and glucose concentrations were determined by spectrophotometry. Transmural potential change (PΔt) was assessed using the short-circuit currents. Data were analysed by one-way ANOVA and $p< 0.05$ was considered significant.

Results: A significant decrease in glucose concentration at the distal jejunum of the serosa in test groups compared with control was observed. The mucosa glucose concentration was elevated at the same region compared with control. The PΔt across the membrane reduced significantly at both the distal jejunum and ileum of Cr^{3+} exposed groups compared with control.

Conclusion: It may be concluded that Cr^{3+} exposure reduced intestinal glucose transport which might probably be a mechanism explored during management of diabetes.

Keywords: *Glucose transport, in vitro, trivalent chromium, transmural membrane, mice*

Résumé

Contexte: La supplémentation en chrome trivalent (Cr^{3+}) a été utilisée dans la prise en charge du diabète de type 2 et l'intestin grêle joue un rôle important dans l'homéostasie du glucose. Cependant, il ya une pénurie d'informations sur la capacité d'absorption du glucose de l'intestin normal pendant l'exposition au Cr^{3+}. Dans cette étude, nous avons étudié l'effet de l'exposition au Cr^{3+} dans l'absorption du glucose dans l'intestin normal.

Méthodologie : Trente rats mâle slc:ddY (26.2 ± 1.1 g) ont été aléatoirement et également assignésà trois groupes:Le groupe 1 (témoin) a reçu de l'eau potable tandis que les animaux des groupes 2 et 3 ont reçu respectivement 10 et 100 ppm de Cr^{3+} pendant 12 semaines par l'administration d'eau potable. Ensuite, ils ont été sacrifiés et leurs intestins excisés, rincés avec une solution de Ringer glacée (SR) et neuf sacs étirés ont été produits, avec addition de 200 µL de SR. Les sacs ont été incubés pendant 1 heure dans 5 ml de SR sans glucose et les concentrations en glucose ont été déterminées par spectrophotométrie. Le changement de potentiel trans-mural (PΔt) a été évalué à l'aide des courants de court-circuit. Les données ont été analysées par ANOVA à sens unique et $p <0,05$ a été considérée comme significative.

Résultats: Une diminution significative de la concentration en glucose au niveau dujéjunumdistal de la sérosa dans les groupes d'essai par rapport au témoin a été observée. La concentration en glucose des muqueuses était élevée dans la même région par rapport au témoin. Le PΔt à travers la membrane a diminué de façon significative à la fois au niveau du jéjunum distal et de l'iléon des groupes exposés au Cr^{3+} par rapport au témoin.

Conclusion: On peut conclure que l'exposition au Cr^{3+} a réduit le transport intestinal de glucose, qui pourrait probablement être un mécanisme exploré lors de la prise en charge du diabète.

Mots clés: *Transport du glucose, in vitro, chrome trivalent, membrane trans-murale, souris*

Introduction

The principal function of the small intestine is to absorb nutrients broken down through digestive processes. Most of these nutrients are absorbed in the jejunum and ileum [1]. Glucose is an important digestive product of carbohydrates and the main source of energy in eukaryotic organism. It plays

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significant role in cellular homeostasis and metabolism [2]. On the other hand, the small intestine play vital role during glucose uptake by the enterocytes of canine [3, 4] and rats [5], while glucose absorption is increased in the insulin-independent pathway especially in the jejunum [6].

It is well established that glucose, a product of digested carbohydrate, is absorbed in the small intestine by two steps format, the sodium dependent glucose transporter (SGLT-1) located on the apical end of the enterocyte which transport glucose into the intracellular space [7]. The second major pathway is through GLUT 2 located on the basement membrane of enterocytes and transport glucose into the interstitial space [8], while acting as a facilitative uniporter with a low affinity but high transport capacity [9]. Corroborating these mechanisms is the report from reverse transcription–polymerase chain reaction (RT-PCR), Northern blot analysis, and a highly specific Glc6Pase assay, suggesting the expression of Glc6Pase gene (mRNA) in human and rat small intestines [10], especially in diabetics or insulinopenia [11]. Thereby confirming that the small intestine has a gluconeogenic capacity apart from the established organs such as the kidneys and liver. Modification involving any of the two major steps stated above will affect intestinal glucose absorption.

Dietary modifications are important for glucose absorption from the jejunum and in the management of diabetes. This makes medical nutrition therapy essential in the regulation of blood glucose level and in the management of diabetes mellitus [12]. A delay in absorption of carbohydrate may be achieved by dietary fibers, α-amylase inhibitors, or α-glucosidase inhibitors [1]. Literature search shows a strong relationship between diabetes mellitus and trace elements in many research studies. Trace elements such as Cu, Fe, and Se play vital role in insulin action including activation of insulin receptor, serving as cofactor or components for enzyme systems involved in glucose metabolism [13]. Certain study also investigated the correlation of serum level of copper (Cu), zinc (Zn), selenium (Se), iron (Fe) in women with type 2 diabetes mellitus and their possible association with lipid profile [14]. In particular, diabetes mellitus has been shown to be associated with abnormalities in the metabolism of zinc, chromium and magnesium [15].

Chromium is a popular element in the earth's crust with bioavailability in several oxidation state, mostly as trivalent or hexavalent chromium. Trivalent chromium (Cr^{3+}) is present in several foods (e.g. cereals, spices, vegetables) and dietary supplements as essential compound. Hexavalent chromium is generated synthetically from industrial pollutions and from oxidation of trivalent chromium, and has been found to be highly toxic to some tissues of the body [16]. The relationship between chromium and glucose metabolism especially in diabetes has been long reported [17]. Its role in potentiating actions of insulin is traced to about six decades [17, 18], and its anti-hyperglycemic activities have been linked to a glucose tolerance factor (GTF) [17] which was responsible for the plasma glucose lowering ability observed in chromium treated diabetic mice [19]. Aside, there is increase in the daily consumption of Cr^{3+} as it has become a widely popular dietary supplement. In the US, there was evidence of an increase in sales of Cr^{3+} containing dietary supplements to customers and over 85 million dollars realized in 2002. This represented about 5.6% of the total dietary supplement market for the year [20]. The trend in consumption of dietary supplements is on the increase.

Previous reports on blood glucose concentration lowering effect of Cr^{3+} from some *in vivo* studies and its significance in the management of diabetes has been documented [21, 22]. However, the importance of trivalent chromium on intestinal glucose homeostasis in normoglycemic states has not been elucidated. This study sought to investigate the probable effect trivalent chromium exposure in mice might have on intestinal glucose absorption *in vitro*.

Materials and methods

Animals

Thirty, 5 weeks old male slc:ddY mice (26.2 ± 1.1 g) were obtained from Japan SLC Incorporation and housed under standard conditions. They were fed with standard mice pellets and had access to clean drinking water *ad libitum*. The animals were grouped into three, Control (water), test groups 10 ppm (Cr^{3+}) and 100 ppm (Cr^{3+}). Chromium was introduced to the test groups through their drinking water for 12 weeks and the control had only clean drinking water instead for the same period. These experiments were carried out in line with guidelines for animal experimentation in Maebashi Institute of Technology, Japan (No.: 15-009).

Chemicals

Trivalent chromium, potassium chloride, sodium chloride, calcium chloride, magnesium chloride, HEPES-Tris , sodium bicarbonates were purchased from Koshin Chemicals, Japan and Glucose kits (Glucose C2) for everted sac procedure was obtained from Wako, Japan.

Experimental procedures

Everted Sac method

Tissue preparation and mounting – Mice were anesthetized with 2.5% isoflurane and the intestines were isolated by cutting at 5 cm from the caecum distally and at the ligament of Treitz proximally. The mesentery was carefully removed and the excised intestine was quickly rinsed in ice-cold Ringer solution (glucose free) (mM)- 140 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 20 $NaHCO_3$; 10 HEPES-Tris, distilled water, pH 7.4). The entire length was divided into 9 equal segments (a-i segments) of about 3 cm each after the intestine's length had been measured. They were subsequently everted into sacs with a glass rod from where each piece was slipped over the tip of a glass rod 3 mm in diameter and about 20 cm long. The sleeve of tissue was ligated on one end and 200µL of Ringer solution (Glucose-free) was gently released into the serosa end of the sac and again ligated from the opened end. This was then introduced into a test tube containing 5 mL Ringer solution (with 10 mM Glucose), and appropriately gassed with 5% CO_2, 95%, O_2 and incubated at 37°C for 1 hour. During this preparatory phase, mounted and un-mounted tissues were kept in ice-cold Ringer gassed with 5% CO_2, and 95% O_2. On expiration of the incubated period of 1 hour, 20 µL each of fluid in the serosa and that in the test tube (representing mucosa fluid) were added to 3 mL reagent from Glucose-C kit (Wako®, Japan). These were incubated in separate test-tubes for another 5 minutes. The glucose concentration from each test tube was then determined by spectrophotometry after incubation. Weight of each segments post experiment was taken with a digital weighing scale and recorded. The glucose concentrations were used to determine various serosa and mucosa glucose absorptions from the isolated 9 segments which were everted into sacs.

Glucose Concentration (mg/dL) = (Sample Absorbance/Standard Absorbance)* k (200)/ weight of tissue.

Glucose-evoked transmural potential change method

The intestine was isolated and excised as earlier described above, the excised intestine was quickly rinsed in cold Ringer solution (glucose-free) (mM) - 140 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 20 $NaHCO_3$; 10 HEPES-Tris, distilled water, pH 7.4). The intestine was separated into segments of about 3 cm each. The segments were everted and made into sacs with a glass rod and each piece was slipped over the tip of a glass rod 3 mm in diameter and about 20cm long. The sleeve of tissue was ligated on one end and the other opening mounted on a 20 mL graduated syringe. The serosa end was filled with Ringer solution (Glucose-free) and gently lowered into a 20 mL Magnus tube filled with Ringer solution for incubation and aeration with carbogen (95% O_2 and 5% CO_2)

Transmural potential, a potential difference between the electrodes inserted in the serosa fluid and mucosa fluid was determined by attaching the electrodes to the positive and negative ends of the short-circuit current. The potential change following the addition of different concentrations of glucose (1 M, 2 M, 5 M and 10 M respectively), to the mucosa fluid was recorded. The electrodes were linked by means of a salt bridge (a polyethylene tube filled with 3M-KCl / 3% agar) using a modified method of Tasaki et al., [23]. The transmural potential differences were recorded on Sekonic(R) recorder.

Statistical analysis

Results were expressed as Mean ± SEM and one-way ANOVA with Newman-Keuls comparison *hoc* Hoc test was adopted using GraphPad Prism version 5.0 for Windows (GraphPad software Inc., San Diego, CA), $p<0.05$ was considered significant.

Results

Fasting blood glucose concentration before and after exposure to chromium.

There was no significant difference in the blood glucose levels of all the groups prior to and after the period of exposure to chromium (Table 1).

Table 1: Effect of oral chromium exposure for 12 weeks on fasting blood glucose (n=5)

Fasting Blood Glucose (mg/dL)	Control	10ppm	100ppm
Glucose Concentration (Onset)	74.0±4.6	76.0±5.2 ^{ns}	71.8 ± 4.9^{ns}
Glucose Concentration (Final)	81.2±7.1	77.3±4.3 ^{ns}	78.8±7.4 ^{ns}

ns- No significant difference compared with the control

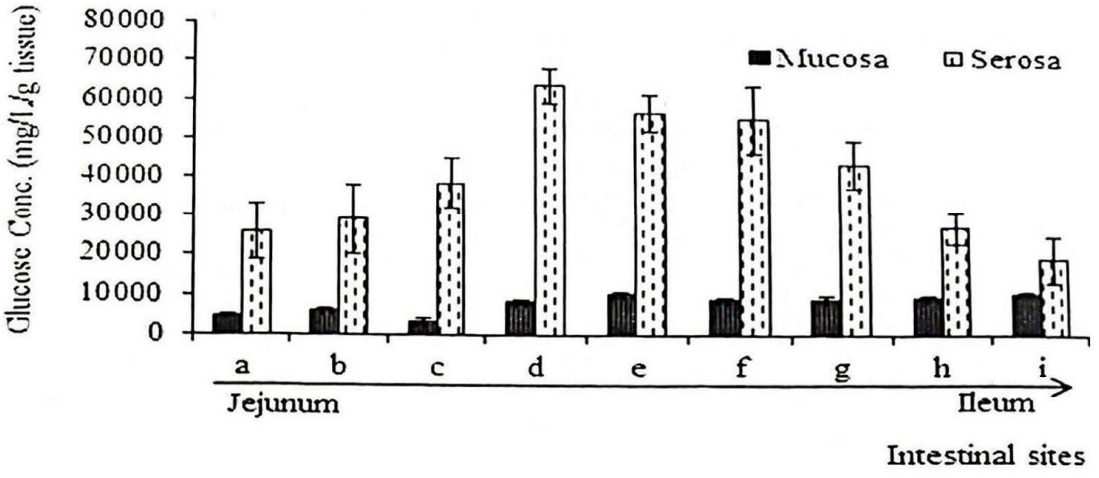

Fig. 1: Glucose concentration at different intestinal sites (a – i) of mucosa and serosa from upper jejunum down to lower ileum in control group using the everted sac method after 12 weeks of exposure to chromium.

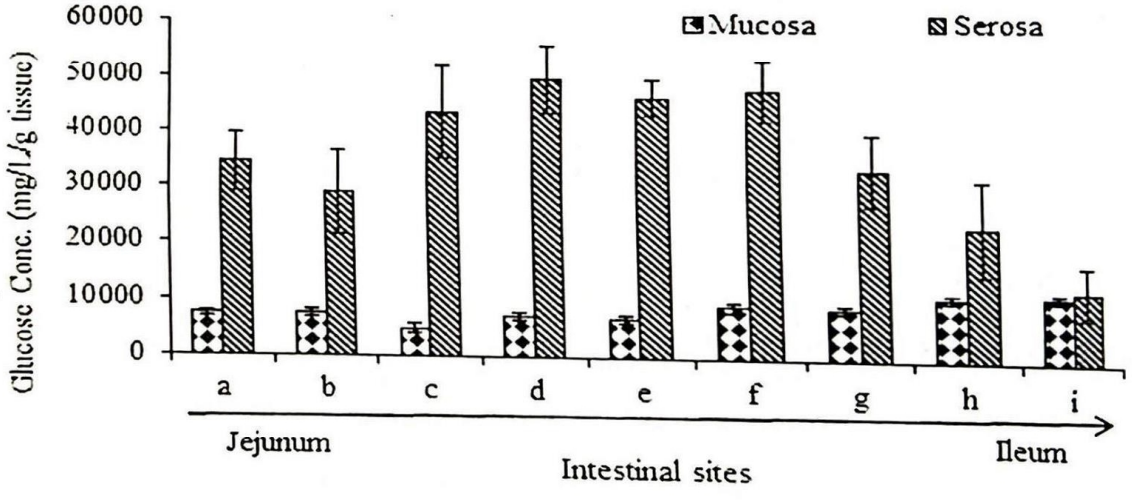

Fig. 2: Glucose concentration at different intestinal sites (a - i) of mucosa and serosa from upper jejunum down to lower ileum in 10 ppm group using the everted sac method after 12 weeks of exposure to chromium

Effect of exposure to chromium on intestinal glucose uptake using the everted sac method

Glucose uptake by the serosa end reduced significantly at the *d* and *e* sites (which constitutes the distal jejunum) for 10 ppm [(49.5 ± 6.2) x 10^3 mg/L/g tissue)], [(46.2 ± 3.1) x 10^3 mg/L/g tissue)] and 100 ppm [(44.5 ± 4.2) x 10^3 mg/L/g tissue), [(45.2 ± 3.8) x10^3 mg/L/g tissue)] compared with control [(63.5 ± 4.4) x 10^3 mg/L/g tissue)], [(56.4 ± 4.7) x 10^3 mg/L/g tissue)], respectively (Figs. 1 to 4). On the other hand, the mucosa glucose concentration increased significantly at three major sites d, e and f (constituting the distal jejunum) of the chromium exposed groups, 10 ppm [(6.1 ± 0.70) x10^3 mg/L/g tissue, (6.9 ± 0.7) x 10^3 mg/L/g tissue and (7.1 ± 8.2) x 10^3 mg/L/g tissue)] and 100 ppm [(6.3 ± 0.6) x 10^3 mg/L/g tissue , (7.5 ± 0.7) x 10^3 mg/L/g tissue and (9.0 ± 0.8) x 10^3 mg/L/g tissue)] compared with control [(3.2 ± 0.90 x 10^3 mg/L/g tissue, (5.2 ± 0.4) x 10^3 mg/L/g tissue and (6.7 ± 0.7) x 10^3 mg/L/g tissue)] respectively, (Figs. 1, 2, 3 and 5).

Effect of 12 weeks exposure to chromium on glucose absorption using the glucose-evoked transmural potential change method

Results for the proximal jejunum were not significant when test groups were compared with control (Plate 1 and Figure 6). However, there was significant

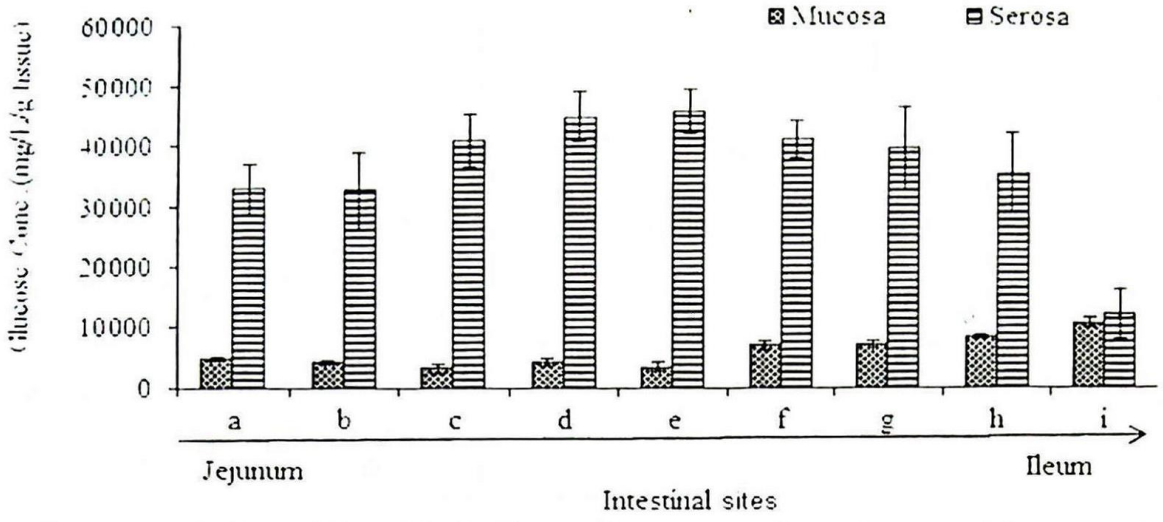

Fig. 3: Glucose concentration at different intestinal sites (a – i) of mucosa and serosa from upper jejunum down to lower ileum in 100ppm group using the everted sac method after 12 weeks of exposure to chromium.

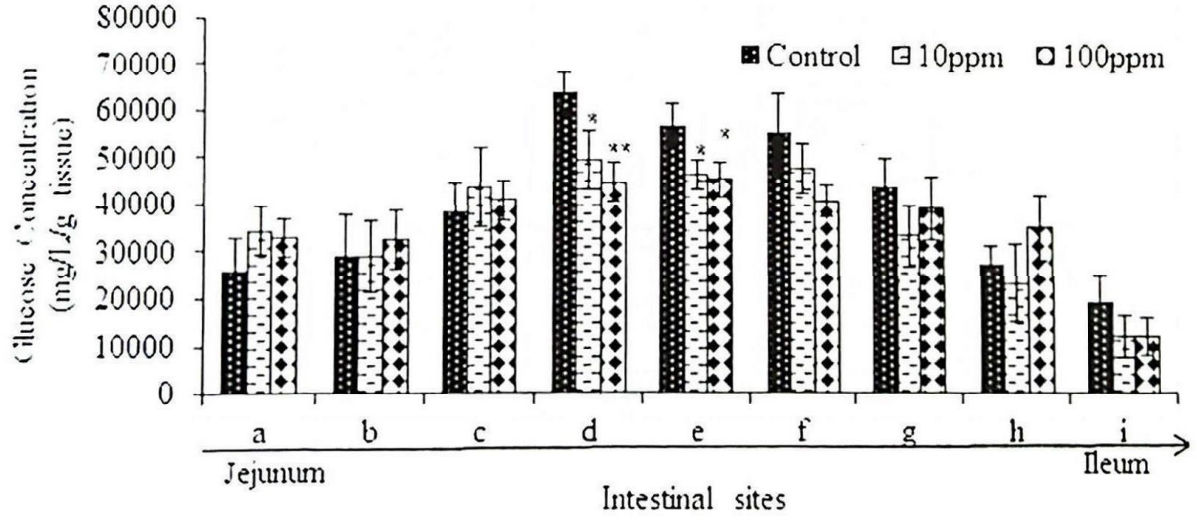

Fig. 4: Serosa Glucose uptake at different intestinal sites from upper jejunum down to lower ileum in the entire group using the everted sac method after 12 weeks of exposure to chromium.
**-significant at $p<0.05$ compared with the control, **-significant at $p<0.01$ compared with the control.*

decrease in potential change at the distal jejunum in all the glucose concentration adopted for the study when test groups were compared with control. A significantly decreased potential change was observed on applications of 1 mM, 2 mM, 5 mM and 10 mM glucose concentration to the mucosa end of the distal jejunum sites in 10 ppm (1.06 ± 0.11mV, 2.0 ± 0.34 mV, 3.38 ± 0.45 mV and 3.88 ± 0.52 mV) and 100 ppm (0.40 ± 0.24 mV, 0.75 ± 0.42 mV, 1.27 ± 0.44 mV and 1.45 ± 0.67 mV) compared with control (2.0 ± 0.22 mV, 3.35 ± 0.54 mV, 5.1 ± 0.82 mV and 5.7 ± 0.88 mV), respectively (Plates 2 and Figure 7).

The ileum part shows significant decrease in the potential change at glucose doses of 1 mM and 2 mM in the 100 ppm (0.38 ± 0.22 mV and 0.95 ± 0.27 mV) compared with control (1.15 ± 0.23 mV and 1.9 ± 0.27 mV), respectively (Plates 3 and Figure 8).

Discussion

The fasting glucose levels pre- and post- exposure to chromium were not significantly different from the control and were within the normal range for rodents [24]. A lot has been said about the role of chromium in glucose metabolism in diabetics. But

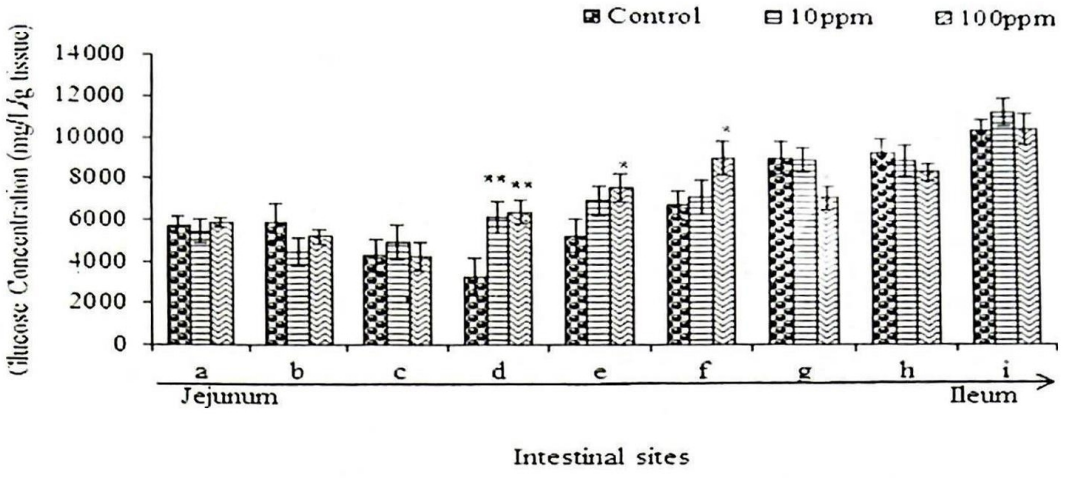

Fig. 5: Mucosa Glucose uptake at different intestinal sites from upper jejunum down to lower ileum in the entire group using the everted sac method after 12 weeks of exposure to chromium.
**-significant at $p<0.05$ compared with the control, **-significant at $p<0.01$ compared with the control*

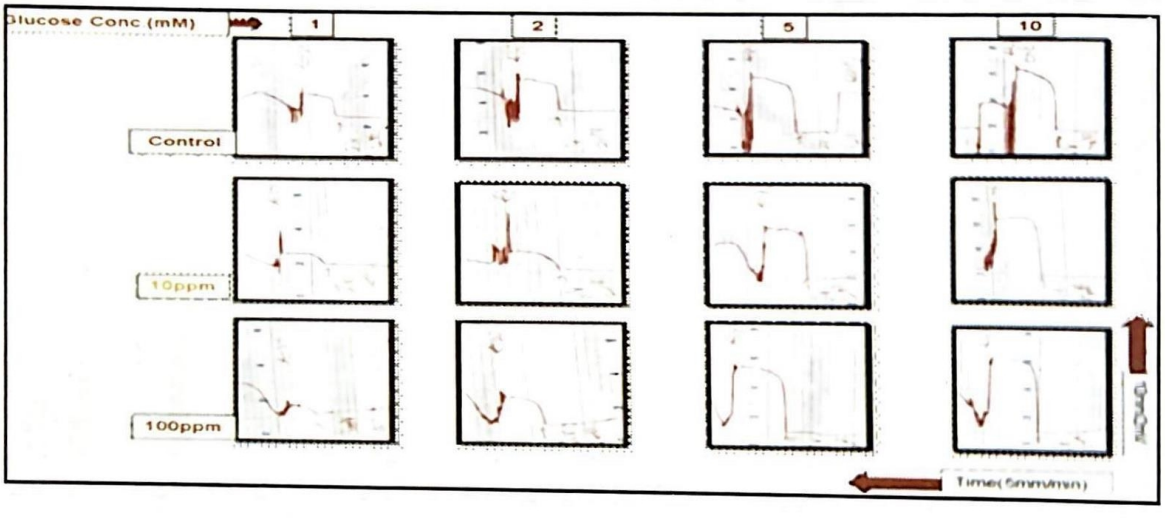

Plate 1: Glucose-evoked potential changes in the proximal jejunum after 12 weeks of exposure to chromium.

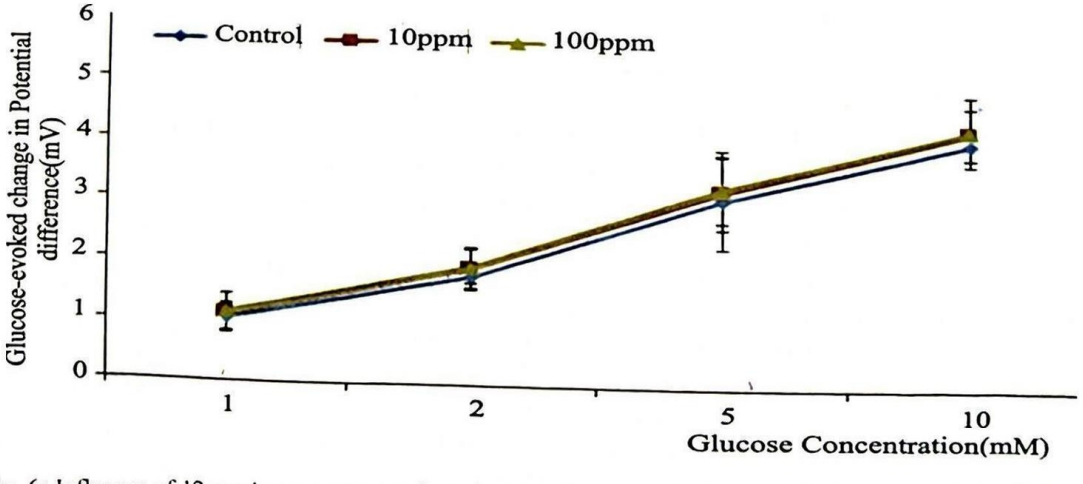

Fig. 6: Influence of 12 weeks exposure to chromium on glucose evoked potential change on proximal jejunum. *No significant difference noted at any point of evaluation.*

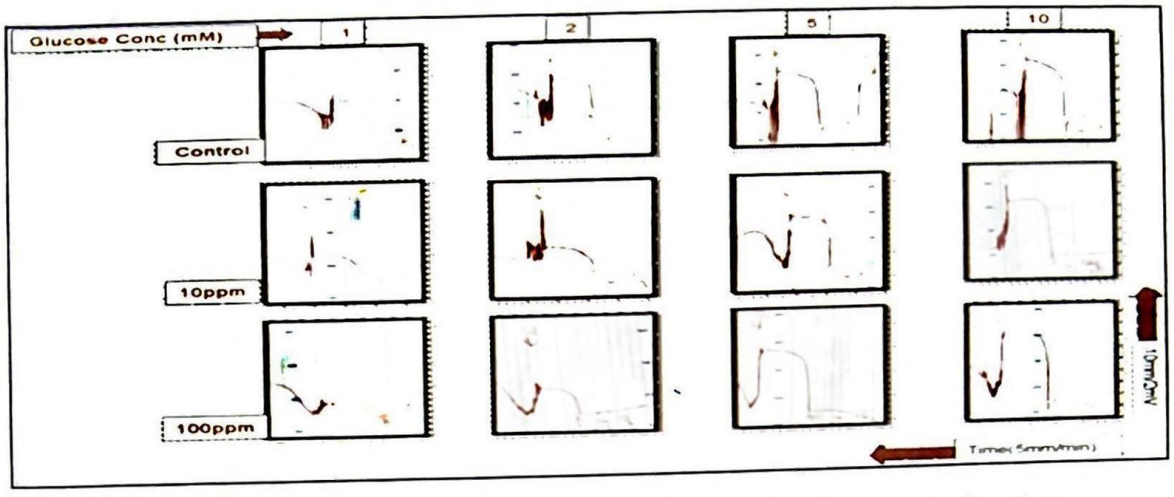

Plate 2: Glucose evoked potential changes in the distal jejunum after 12 weeks of exposure to chromium.

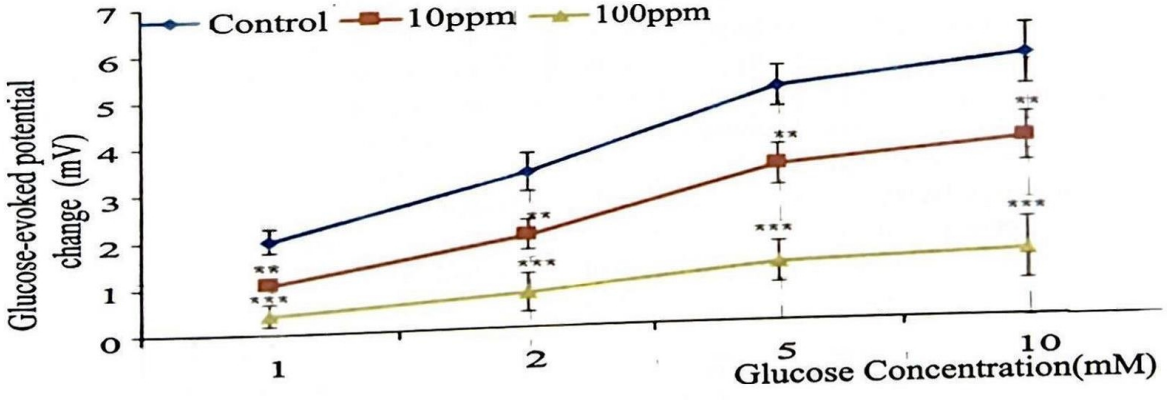

Fig. 7: Influence of 12 weeks exposure to chromium on glucose evoked potential change on distal jejunum. ***significant at P<0.01, ***significant at P<0.001 compared with the control.*

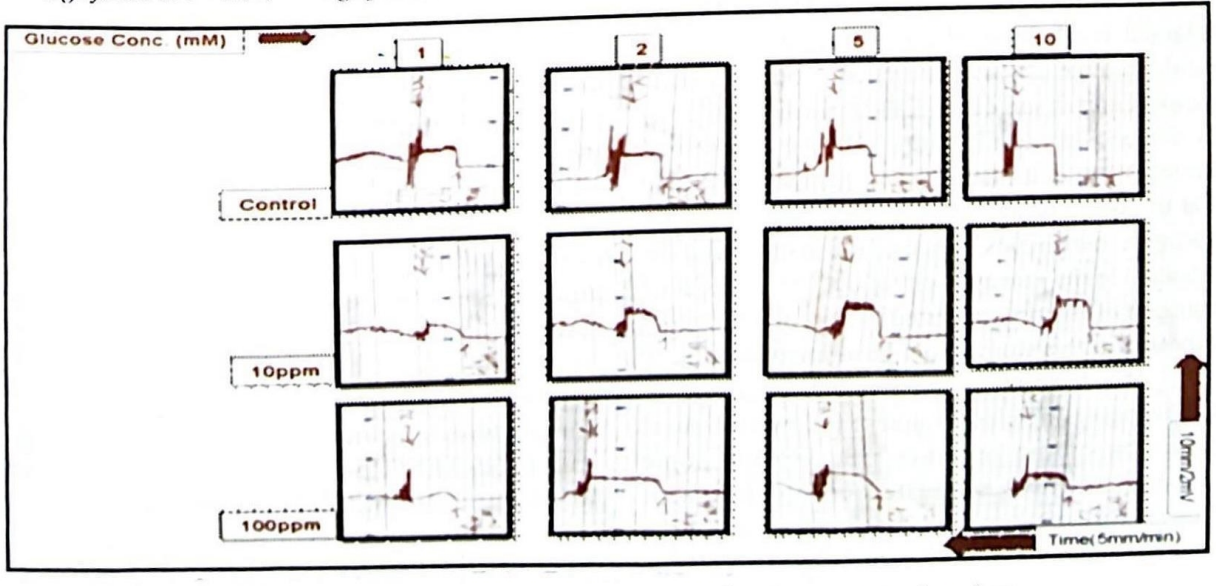

Plate 3: Glucose-evoked potential changes in the ileum after 12 weeks of exposure to chromium.

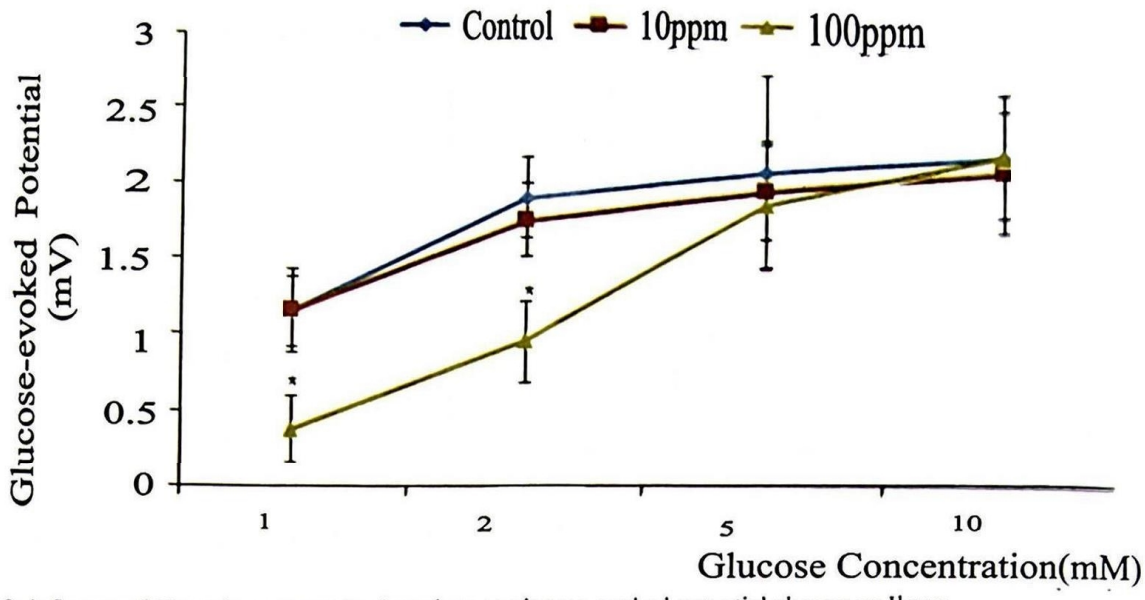

Fig. 8: Influence of 12 weeks exposure to chromium on glucose evoked potential change on Ileum.
**significant at P<0.05 compared with the control.*

there is no report on its normoglycemic state and its effect on intestinal regulation of glucose uptake. A number of studies on human [25, 26], pigs [27] and rats [28] have reported the possibility of chromium influencing glucose tolerance and insulin resistance after supplementation. Everted sac and transmural potential change findings from this current study, mirror each other with similar inhibition of glucose uptake at the distal jejunum compared with control. Thus, buttressing the importance of jejunum in glucose uptake and its' possible use in providing treatment interventions in deranged glucose states.

Previous works on effect of chromium supplementation on fasting blood glucose showed no significant change in diabetic patients [29, 30]. In our study using normal mice, similar results were obtained. It is believed that reducing glucose uptake would reduce its availability in blood which is classical means some anti-diabetic medications such as α-glucosidase inhibitors [31] were designed among many other mechanisms [32]. It is possible that trivalent chromium might be reducing glucose transport in diabetics, hence its use in managing the disease. In the management of diabetes, a reduced transport of glucose suggested by trivalent chromium exposure in this study might be of importance.

The ileum plays an important compensatory role in increasing glucose uptake following clinical resection of the jejunum [33, 34] and some researchers have suggested up-regulation of intestinal hexoses transporters as a major mechanism of ileum improved sugar uptake following resection [35, 36]. What is not certain at this point is the clinical relevance these findings of chromium inhibiting glucose transport especially in the jejunum could suggest in cases requiring re-sectioning of the small intestine. In which case, the compensatory effort of ileum in increasing glucose uptake for instance, during short bowel syndrome [37] might be compromised in persistent chromium supplementation. This may impair or derange the adaptive processes to glucose uptake by ileum, especially when different modulatory mechanisms are in place suggesting the compensatory effect of ileum in glucose absorption following major intestinal resection [33, 34, 38, 39], might be affected.

In conclusion, it is unclear at this point how chromium suppresses intestinal glucose uptake and is unlikely to be due to physical inhibition or presence of dietary fibers. Chromium may inhibit glucose absorption by either reducing or suppressing cellular proliferation (hypoplasia) or acting on SGLT-1 gene by down regulating its expression which was not determined in this present study. This might be a mechanism to be verified.

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