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Synergetic effects of GOS and Cu^{+2} nanoparticles as prebiotics on biochemical and metabolic hormonal profile in alloxan induced diabetic rats model

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Abstract: Keeping in view the increased prevalence of diabetes and challenges in its effective treatment, current study was designed to assess the effect of new formulation {galactose oligosaccharide (prebiotic) and copper nanoparticles (np) (GOS/Cu^{2+})} on glycemic control, insulin levels, thyroid hormone (T_3, T_4) and oxidative stress in alloxan induced diabetic rats model. Normal healthy adult male rats (n=45) divided in three groups; healthy, diabetic control and diabetic treated groups with equal number of rats in each group (n=15). Diabetes was induced by intraperitoneal administration of alloxan at a dose of 120 mg/kg bw. Single oral dose of 200mg/kg bw/day was continued for 30 days to diabetic rats. Serum was separated from blood to measure glucose, insulin, thyroid hormone and oxidative stress level. Pancreatic tissue was collected in formalin (10%) to determine the activity of cellular reactive oxygen species. Treated group showed significantly ($p<0.05$) decreased level of glucose, malondialdehyde and cellular reactive oxygen species activity in pancreatic tissues; whereas significantly ($p<0.05$) increased level of insulin, thyroid hormones and catalase was seen in comparison to control. Conclusively, oral formulation of GOS/Cu^{+2} np have pronounced anti hyperglycemic as well as antioxidant effects that would be helpful to manage diabetic syndrome.

Keywords; Nanoparticle, cellular reactive oxygen species, prebiotic, oxidative stress, thyroid hormone.

INTRODUCTION

Insufficient insulin secretion is a common feature in Diabetes mellitus, a metabolic disorder, which is characterized by insensitivity of the endogenous insulin to its receptors and progressive failure of pancreatic β-cells culminating in hyperglycemia (Rolo and Palmeira, 2006) and become the major health concerns currently in the developed countries (Ogurtsova *et al*., 2015). Although the development of diabetes is strongly predisposed due to genetic factors, life style particularly dietary factors and obesity (Korat *et al*., 2014). Insulin-secreting β-cells of the pancreas are significantly involve in maintaining the homeostasis of glucose and emerging pathophysiology of diabetes. The alteration in insulin secretion acts as a risk factor for developing the diabetes in healthy population. In diabetic patients, a progressive decrease in insulin secretion or inability to produce insulin and an insufficient suppression of glucagon secretion is observed (Petrie *et al*., 2011). Hence, dysfunction of pancreatic β-cells aggravated by insulin resistance is the main determinant for the development of diabetes (Ashcroft and Rorsman, 2012). Conclusively, role of oxidative stress has been established in the development of diabetes (Maritim *et al.,* 2003). Oral administration of yogurt plays a beneficial role to suppress oxidative stress that may delay the onset of induction of diabetes through synthetic chemical compounds (Yadav *et al.,* 2008). It has been suggested that supplementation with prebiotics possess therapeutic and preventive role for immune disease because of its specific immune-modulatory characteristics (Yan and Polk, 2011). Limited studies are available on metal nanoparticles showing antidiabetic effect and need much more attention. α-amylase & α-glucosidase inhibition and free radicals scavenging properties are important determinants in preventing diabetes (Patil *et al.,* 2015). Nanomaterials such as metallic NPs (nanoparticles) have unexpectedly shown enzyme-resembling antioxidant activities such as scavenging of highly reactive free radicals thereby, subsiding level of oxygen derived reactive species. Antioxidant activities of nanoparticles derived from iron, copper, cerium, platinum, silver and gold have been translated similar to naturally occurring antioxidant enzymes (Lushchak *et al*., 2018). Moreover, nanoparticles derived from gold have shown profound

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blood glucose lowing as well as antioxidant properties in chemically induced diabetic subjects (BarathManiKanth *et al*., 2010). In current study, we have investigated the combination of prebiotics (GOS) and nanoparticles (Cu^{+2}) on biochemical as well as metabolic hormones profile in alloxan induced diabetic rats.

MATERIALS AND METHODS

Animal selection and housing

Healthy male *Wistar albino* rats (n=45) aged 6 week having weight of (140 ± 10) gms were randomly picked from Animal rearing nursery, Department of Physiology, Government College University, Faisalabad. All chosen rats were kept in separate cages in isolated experimental room for an acclimatization period (1 week) in controlled environmental conditions temperature (25°C ± 5°C) and relative humidity (45 ± 5%) while maintaining equal light and dark period (12-hours). Experiment was conducted in accordance with the permission letter having reference. No. GCUF/ERC/130, following the regulations of ERC (Ethical Review Committee) established in Government College University, Faisalabad.

Grouping

Rats were divided into three groups; healthy control (HC), diabetic control (DC) and diabetic treated (DT) group. Each group (n=15) consists of equal number of rats.

Treatments and dosing protocols

Diabetes was induced by administering alloxan through intraperitoneal route at dose rate of 120mg/kg body weight followed by 100mg/kg body weight in second and third dose one dose/day. Sucrose solution (5 %) was administered orally to avoid hypoglycemia if developed. Blood sugar level was measured/monitored through glucometer (Accu-Chek Performa, Roche) by piercing blood through pricking the rat tail. Rats having more than 250mg/dl sugar level were considered as diabetic. Both HC and DC groups received no treatment except rodent diet while DT group received rodent diet and oral dose of GOS/Cu^{2+} formulation (98.5:1.5) at the dose rate of 200mg/kg body weight through gastric tube for 30 days. At the end of experiment, rats were sacrificed by adopting decapitation procedure to gather blood sample to harvest the serum. Tissue sample of pancreas from each rat was preserved in formalin solution (10 %) for detection of cellular ROS.

Serum glucose

Serum glucose was quantified through Bioclin® Glucose Monoreagent diagnostic kit having limit of detection (LOD) within the range of 2-500mg/dl with CV% <3.11.

Serum insulin

Commercially available Elisa kit with catalog # E-EL-R2466, purchased from E-Lab Sciences, sensitivity; 0.47µlU/mL showing detection range of 0.78-50µlU/mL & CV%<10 was used to estimate serum insulin. The ELISA kit followed Sandwich-ELISA principle. Rat insulin specific antibody has been coated to the micro ELISA plates provided in this kit. Samples and standards were added to the micro ELISA plate wells the insulin in them will combine with the precoated antibodies. Then a biotinylated rat insulin and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well. After incubation, the optical density (OD) was measured on spectrophotometer at a wavelength of 450 nm ± 2 nm.

Thyroid (T_3 & T_4) hormone

Thyroid hormones ELISA Kits {T_3 PISHTAZTEB T_3 diagnostic ELISA kit (PT-T_3-96) showing sensitivity 0.94pg/mL, LOD 1.56-100pg/mL & CV%<10} and for detection of T_4 {T_4 PISHTAZTEB T_4 diagnostic ELISA kit (PT-T_4-96) having sensitivity 0.94pg/mL, LOD 1.56-100pg/mL and CV%<10} were obtained from PISHTAZTEB, Tehran. The competitive ELISA is the test principle of this technique. Microplate wells were coated by certain amount of anti-T_3 and T_4 monoclonal antibody (mAb Anti-T_3 & T_4). Absorbance was measured spectrophotometrically at 450 nm after adding the measured amount of rat serum and standards to the microtiter wells.

Oxidative stress index (MDA and Catalase)

Spectrophotometer (Biolab 310, Biolab Scientific Ltd. Canada) was used to estimate levels of serum catalase and MDA according to previously described procedure by Hadwan and Abed (2018) and Al-Assaff and Takruri (2019).

Cellular ROS activity

Reactive oxygen species (Superoxide dismutase) in pancreatic tissues were determined by using Cellular ROS/Superoxide dismutase detection assay kit (cell based) (abcam ab139476) to determine intracellular ROS production (Peng et al., 2017; Seto et al., 2017). Where NAC (N-acetyl-L-cysteine) was used as general ROS inhibitor and pyocyanin was used as ROS inducer. Two fluorescent dyes including total ROS concentration detecting (Green, Ex/ Em 490/ 525nm) and Superoxide Detection Reagent (Orange, Ex/ Em 550/ 620nm) were used in the kit. To conduct cellular ROS activity, tissue samples from the pancreas were fixed on glass slides and incubated at 37ºC with superoxide/ROS detection mix for 30 min. By using confocal microscope, superoxide dismutase production levels were monitored in green channels.

STATISTICAL ANALYSIS

The SPSS software version 23 was used for statistical analysis. All datasets were statistically demonstrated as

mean ± SEM. One way Analysis of Variance (one way ANOVA) was observed to seek the significance of difference among groups followed by post hoc tukey's test. The level of significance was actively considered at $p<0.05$.

RESULTS

Fasting blood glucose (mg/dl), serum glucose (mg/dl) and insulin level (µIU/dl)

Diabetic control (DC) group had significantly ($p<0.05$) higher mean fasting blood glucose (mg/dl), serum glucose (mg/dl) and decreased insulin level (µIU/dl) as compared to HC group. While DT group showed a significant ($p<0.05$) decrease in mean fasting blood glucose, serum glucose and higher level of insulin as compared to diabetic group (figs. 1a, 1b and 1c).

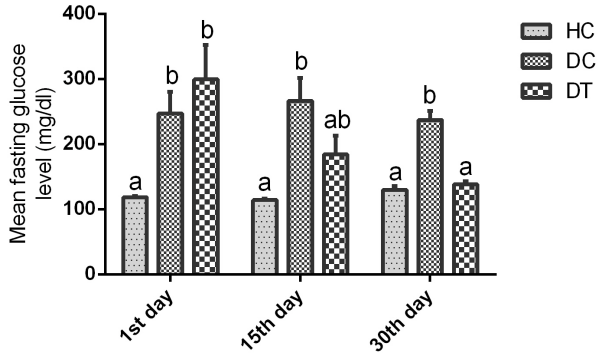

Fig. 1a: Mean fasting glucose level (mg/dl±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 1st, 15th and 30th day post treatment. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

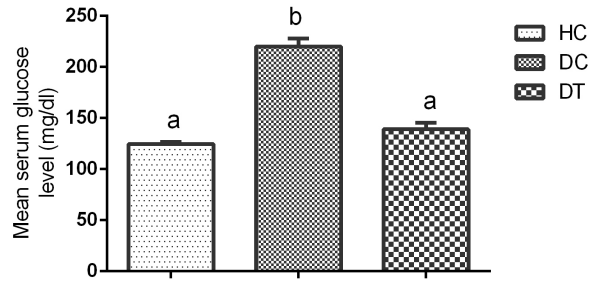

Fig. 1b: Mean serum glucose level (mg/dl±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

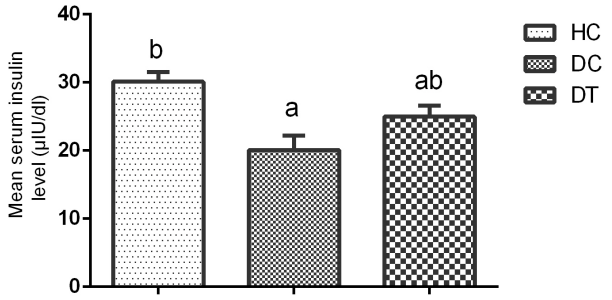

Fig. 1c: Mean serum insulin level (µIU/dl±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

Thyroid hormone T_3 (ng/ml) and T_4 (µg/ml) levels

The mean serum T_3 (ng/ml) and T_4 (µg/ml) concentration was measured significantly ($p<0.05$) lower in DC group as compared to HC group. Whereas DT group depicted a significantly ($p<0.05$) higher T_3 and T_4 levels in comparison to DC group (figs. 2a and 2b).

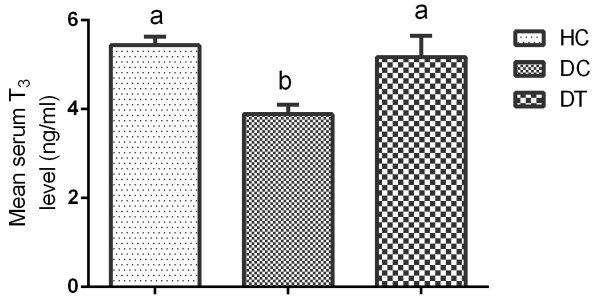

Fig. 2a: Mean serum T_3 level (ng/ml±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

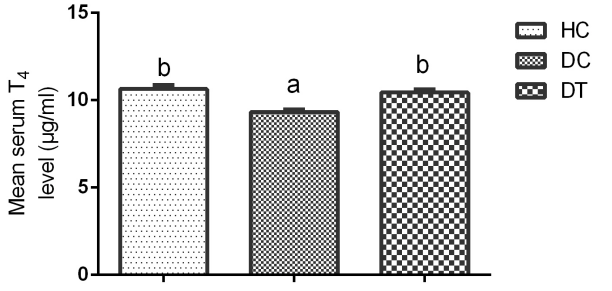

Fig. 2b: Mean serum T_4 level (µg/ml±SEM) in different groups; HC=Healthy control group, DC=diabetic control

and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

Oxidative Stress; catalase (IU/ml) and MDA (pmol/dl)

The DC group showed a significantly (p<0.05) increased level of MDA and decreased level of catalase in comparison to HC group. After treatment period, the DT group depicted significantly (p<0.05) reduced level of MDA and increased level of catalase when compared to DC group (figs. 3a and 3b).

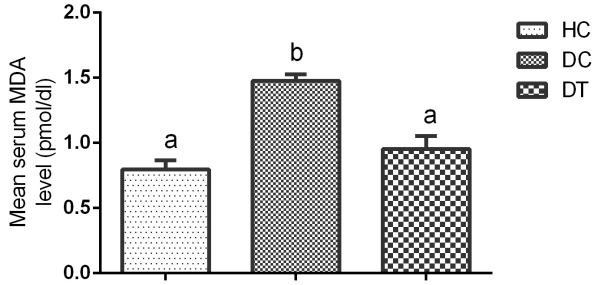

Fig. 3a: Mean serum MDA level (pmol/dl±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

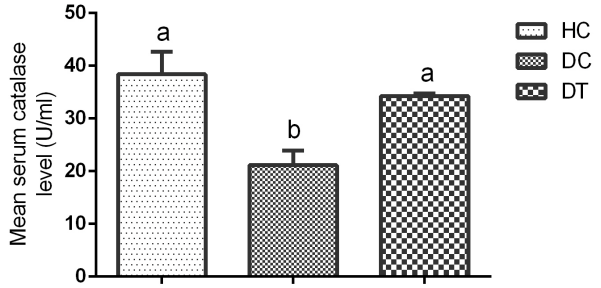

Fig. 3b: Mean serum catalase level (IU/ml±SEM) in different groups; HC=Healthy control group, DC=diabetic control and DT= diabetic treated group (oral administeration of single dose of 200mg GOS/Cu^{2+} per kg body wt per day) after 30 days. Different alphabets on the mean bars shows statistical difference between groups (P≤0.05).

Cellular ROS activity

Results showed (fig. 4) that there is marked damage mediated by the reactive oxygen species (Superoxide dismutase) generation in pancreatic tissues of DC group rats. GOS/Cu2+ nanoparticle treatment suppressed this alloxan-induced ROS production (in DT group rats) as shown in fig. 4 B.

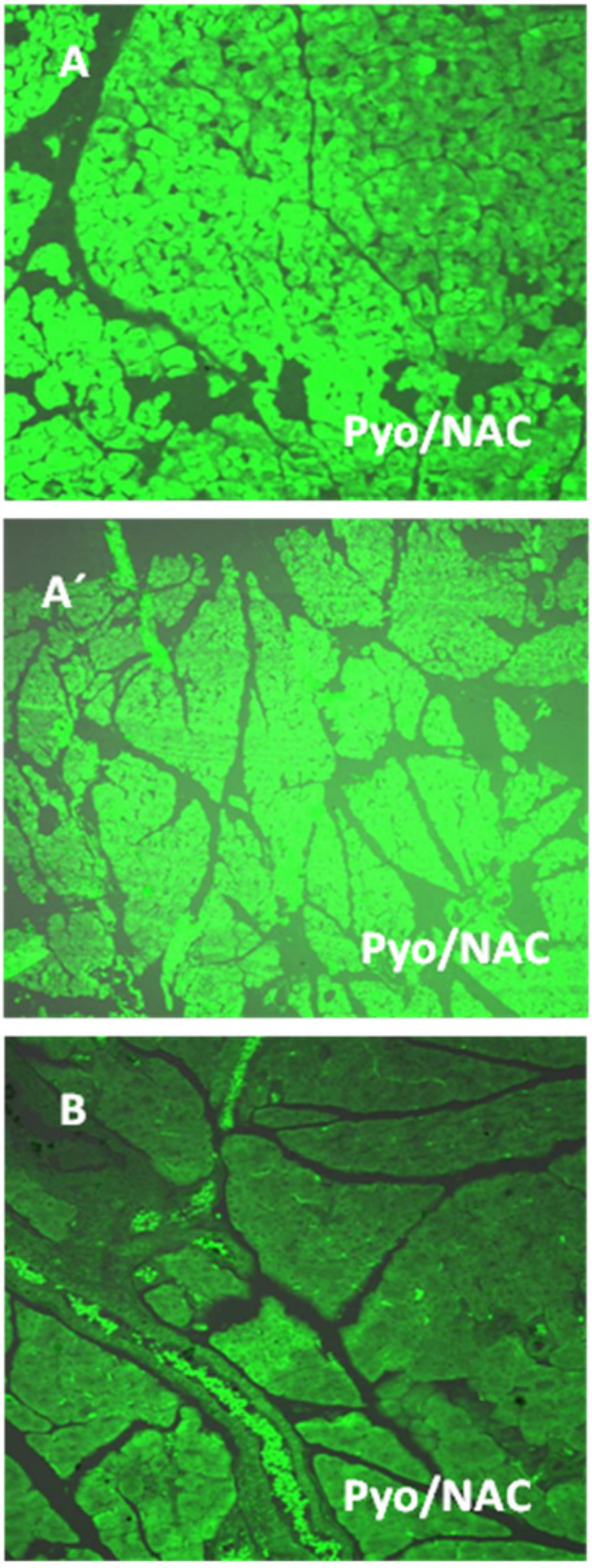

Fig. 4: Pancreatic tissue in different groups; HC; healthy control group = **A**, DC; diabetic control = **A´**, and DT; diabetic treated group (oral administeration of single dose

of 200mg GOS/Cu^{2+}/kg body wt/ day) = **B** after 30 days post oral treatment.

DISCUSSION

To achieve and maintain the normal blood glucose level is the primary goal while treating diabetic patient with oral hypoglycemic agents and insulin. Such treatment strategies have proven inadequate rather more threatful by eliciting their toxicities. Major research of hypoglycemic remedies is currently focused to discover hypoglycemic agents that are safe with minimum or no side effects. Plenty of plants, herbs, fermented foods, its components and microorganisms have been presently utilized and tested as a treatment option for the diabetics (Patel *et al.,* 2012).

In present investigation combination of prebiotic and nano-particles formulation (GOS/Cu^{2+}) was used to investigate their anti diabetic, antihyperglycemic and antioxidant capacity in alloxan induced diabetic rat models. In current study, rats in DC group were identified by higher blood glucose levels along with lower serum insulin level when compared to HC group. Deliberately, blood glucose level was found decreased in nanoparticles formulation (GOS/Cu^{2+}) treated group (DT). Extended antidiabetic activity of current nanoparticles formulation (GOS/Cu^{2+}) was further strengthened by achieving higher levels of insulin in DT group thereby establishing an insulinogenic potential of post oral administration of current formulation. Thyroid hormone exerts a salient part in regulating pancreatic hormone as well as carbohydrate metabolism. It has been reported that Diabetes severely effects the functional capability of thyroid glands. Hyperthyroidism has been considered as a key promoter of hyperglycemia (Wang, 2013). In hyperthyroidism; half-life of insulin is reduced due to higher degradation rate & release of insulin precursor which is biological inactive (Hage *et al.,* 2011). In current study, level of T_3 was decreased in untreated diabetic group (DC), such T_3 state may be explained by impaired T_4 conversion into T_3 which is involved in the improvement of glycemic control (Ogbonna and Ezeani, 2019). However, serum T_3 level was found higher in DT group in comparison to HC group. In present study, serum T_4 level was found lower in DC as compared to HC group. Different studies correlate with our findings in which diabetic patients have decreased T_4 level as compared to healthy individuals (Swamy *et al.* (2012). Oxidative stress indicates an imbalance between pro & anti oxidants in the body which favors pro-oxidant species. Oxidative stress develops due to increased free radical production and decreased antioxidant level. A number of different mechanisms which increase intra & extra cellular glucose concentration resulting in oxidative stress having a defined role in the development of secondary diabetic complications, including vascular complications as well (Fancesco *et al.,* 2004). The current study demonstrated increased cellular ROS production in the pancreas of DC group rats. A major cause of insulin resistance could be insulin disruption via cellular ROS production by mitochondria (Barazzoni *et al.,* 2012). Catalase is an enzyme which have a double role, firstly, catalytic function i.e. catalyses the breakdown of hydrogen peroxide (H_2O_2) to produce water and oxygen. In current study, plasma catalase level in HC group was significantly higher than DC group which is incongruent with previous outcomes highlighted by Engwa *et al.* (2018). Catalase concentration was higher in DT as compared to DC group also support our study by the findings of Sangwan *et al.* (2014) who discuss the increased serum catalase level in diabetic rats were observed when treated with GOS. Similar results were also founded by Sanja *et al.* (2015), who describe increased catalase level in broiler after administration of prebiotics. Malondialdehyde (MDA) is the major by-products of lipid peroxidation and can be used as indicator of cell membrane injury in obesity leading to development of insulin resistance (Jackson, 2015). In current study plasma MDA level in HC group was found lower as compared to the DC group. Previously it is reported that the diabetic patients of both type 1 & 2 have increased malondialdehyde levels as compared to normal healthy volunteers (Maritim *et al.,* 2003). Previously increased serum MDA was observed in diabetics as compared to healthy individuals (Telci *et al*., 2000). In our study the DT group showed a significant decrease in MDA concentration in comparison to DC group. These results were supported by the findings of Lin *et al.* (2019) confirming that MDA level was significantly decreased in type 2 diabetic mice treated with Neoagaro-Oligosaccharides. This decreased level in treated group may be due to antioxidant capacity of copper nanoparticles and insulin stimulatory effect of galactose oligosaccharides acted in a synergism.

CONCLUSION

Conclusively, it is founded that the formulation (GOS/Cu^{2+}) have antidiabetic as well as antioxidant effect and would be helpful to manage diabetic syndrome and prevent progression to diabetic associated complications.

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