EFFECT OF CINNAMON BARK OIL ON CADMIUM INDUCED TESTICULAR TOXICITY IN MALE ALBINO RATS

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ABSTRACT

Background and Objectives: The present experimental study was designed to investigate the toxic changes in the testes of cadmium ($CdCl_2$) treated rats and evaluate the protection provided by cinnamon bark oil (CBO).

Methods: Thirty male albino rats were randomly divided into three groups A, B and C. Group A served as control and received 1ml/100gm/day of distilled water and 0.5ml/100gm/day of olive oil for 14 days by oral gavage. Group B received 1.5mg/100gm/day of $CdCl_2$ dissolved in distilled water and 0.5ml/ 100gm/day of olive oil for 14 days. Group C received $CdCl_2$, 1.5mg/100gm/day in distilled water and 100mg/kg/day of CBO in 0.5ml/100gm of olive oil for 14 days by oral gavage. Animals were sacrificed on day 15 and testes were removed.

Results: Examination of gross parameters between the control and experimental groups did not show any difference which was statistically significant. Histological sections, stained with H&E were examined under light microscope. The administration of cadmium caused a significant decrease in germinal epithelium thickness and Johnson's score. However, significant improvements were observed in these parameters after the concurrent administration of $CdCl_2$ with CBO.

Conclusion: The results of this study revealed that CBO has protective effect against the toxic effects in rat testes induced by cadmium.

Key words: Cadmium, Cinnamon, Testes, Toxicity, Germinal epithelium, Johnson's score.

INTRODUCTION

Cadmium (Cd) is a heavy metal and environmental toxicant. The general population is exposed to Cd through contaminated food and drinking water.[1,2] The occupational exposure to Cd usually takes place during mining, manufacturing of paints and batteries containing cadmium. Industrial activities such as melting, purifying of metals and incineration of municipal waste also release Cd into the atmosphere as cadmium oxide, chloride or sulfide.[3] Cigarette smoking is another significant source of Cd, heavy smokers have more than double of the Cd body burden[1]. People who live near the factories, which release cadmium as their waste, and those working in the metal refinery Industry, suffer from health problems such as air way diseases, gastrointestinal problems, bone fractures, renal failure, infertility and carcinomas. Cadmium affects multiple organs of the body including kidney, liver, pancreas and reproductive organs.[3]

It has been demonstrated in earlier studies that Cd exposure increased oxidative stress producing imbalance of harmones controlling the reproductive system by disrupting the hypothalamic-pituitary-testicular axis; it also increased production of apoptosis.[4] Cadmium damages the male reproductive organs by causing testicular degeneration and seminiferous tubule damage.[5] Amara observed decrease in weights of testes and seminal vesicle of rats treated with Cadmium which is associated with elevated lipid per oxidation in different organs including kidney, liver and testes.[6,7] Cadmium exposure resulted in decreased antioxidant enzymes, an increase in lipid peroxidation level and histopathological changes in the testes, liver and kidneys of experimental animals.[4] Cadmium is known to enhance reactive oxygen species (ROS) production such as superoxide ion, hydroxyl radicals and hydrogen peroxide. These ROS induce lipid peroxidation, modulation of intracellular oxidized states, DNA damage, alteration in gene expression and apoptosis.[8]

Antioxidants are substances that suppress the production of reactive oxygen species and lipid peroxidation. Spices, herbs and medicinal plants have received increasing interest as sources of beneficial antioxidants against various diseases. The medicinal properties of many spices are well known.[9] Among such spices that possess medicinal wonders is Cinnamon which is

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an evergreen tree that is traditionally harvested in Asian countries. Cinnamon bark is widely used as a spice. It is principally used in cookery as a flavoring material.[10] It is used in traditional medicine, and several studies have tried chemicals extracted from cinnamon for various medicinal effects. Cinnamon is believed to serve as potential dietary source of natural antioxidants for improving nutrition and health.[11]

It has been reported in previous studies that various extracts of cinnamon, such as ether, aqueous, and methanolic extracts show considerable antioxidant activities.[10] Different flavonoids isolated from cinnamon have free-radical-scavenging activities and antioxidant properties.[10] Cinnamon has anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, anti-hyperglycemic effects and its extract is potent antioxidant and free radical scavenger.[9,12,13]

It has been shown earlier that ethanolic extract of cinnamon bark improved reproductive organ weight and sperm quality.[12] Oral administration of cinnamon extract elevated the serum testosterone level, improved sperm motility and alleviated testicular degenerative changes in diabetic rats.[14] Shalaby and Mouneir ascribed the improvement in fertility parameters to antioxidant property of cinnamon. However, there is no reported study to document the effect of cinnamon bark oil on Cd induced testicular toxicity. The present investigations were therefore planned to evaluate the effect of cinnamon bark oil on Cd induced testicular toxicity in albino rats.

MATERIALS AND METHODS

It was an experimental study, carried out at the Experimental and Research Laboratories of University of Health Sciences, Lahore; the rat model for the study was used. The experiment was carried out strictly in accordance with the instruction and guideline of Ethical Committee of UHS.

Male Wistar Albino rats 30 in number, 6-8 weeks old and weighing 200-250gm raised as inbred colony at the University of Health Sciences, Lahore were used; these were examined and weighed to exclude any evidence of disease before beginning the experiment. The rats were housed under controlled temperature of 23 ± 0.3˚C; humidity set at 55 ± 5% and light and dark cycles of 12 hours each. Normal rat chow and tap water ad libitum were allowed to the animals. Rats were kept for a week for acclimatization and were handled from time to time to minimize the stress during experimental period.

Animal Groups

Thirty male albino rats were divided into three groups, randomly of 10 rats each.

Group A was a control group and was given 1ml/100gm/day of distilled water and 0.5ml/100gm/day of olive oil, both by oral gavage, daily for 14 days.

Group B was given 1.5mg/100gm/day of $CdCl_2$ dissolved in 1ml/100gm of distilled water and 0.5ml/100gm of olive oil, both by oral gavage daily for 14 days.

Group C was given 1.5mg/100gm/day of $CdCl_2$ dissolved in 1ml/100gm of distilled water and 100mg/ kg/ day of Cinnamon bark oil (CBO) in 0.5ml/100 of olive oil, both by oral gavage daily for 14 days. The dose of CBO was given daily 2 hrs after Cd.

Earlier studies were used to determine the dose and duration of the study.[12,13,15] Doses were adjusted according to the body weight of the animals. Animals were sacrificed 24hrs after the last dose.

Chemicals

Cadmium chloride was purchased from BDH Laboratory supplies, Poole, BH15 1TD, England.

Cinnamon bark oil was prepared at the PCSIR laboratories, Lahore. Hydrodistillation method was used to prepare cinnamon bark oil. Cinnamon barks were ground into small pieces and mixed with water. The mixture was boiled for 4hrs and condensed to collect essential oil. The extract was then dried over anhydrous sodium sulphate. Cinnamon bark oil was kept at 4˚C until used.[12,13]

The animals were weighed at the end of experimental period and were sacrificed and testes were taken out and weighed. The testes of each animal were divided longitudinally into two parts and were placed in Bouin's fixative for 48 hours; these were washed for 72 hours using required number of changes of 50% and 70% ethanol to remove yellow color. The tissue was then processed in the automatic tissue processor and paraffin blocks were prepared. Sections of 4 µm thickness were obtained using rotary microtome (Shandon Finesse ME+).

Histological Examination: The slides, after staining with haematoxylin and eosin, were examined with light microscope using magnifications of X10 and X40. Sections were examined for Johnson scoring, diameter of seminiferous tubules, thickness of germinal epithelium. Six stained sections of each of the thirty animals were examined and 10 tubules from each of the sections were studied and observed, the diameters and germinal epithelium thickness (from the basal membrane towards the lumen of the tubule) were measured using anocular micrometer, and the meandiameter and germinal epithelial thickness were calculated. Johnson's testicular scoring was done for control and experimental groups.[16] A score between 1 (very poor) and 10 (excellent) was given to each tubule according to Johnson's criteria.[16]

Statistical Analysis: SPSS version 20.0 (Statistical Package for Social Sciences) was used to analyze the data collected from three groups. Mean ± SD was calculated for quantitative variables (weight of rat and

weight of testes), Mean ± SD and median (Inter-quartile range) was calculated for quantitative variables (Johnson's score). One way ANOVA/ Kruskal-Wallis test was applied to compare means of histological, Johnson's scoring and gross quantitative parameters among the groups. Post Hoc Tukey Test was applied to compare which group's mean differs.

RESULTS

Gross Parameters: Animals of Groups A, B and C had the mean body weight of 231.16 ± 8.36, 241.45 ± 13.91 and 240.25 ± 12.51gm respectively. No statistically significant difference in the mean body weight was shown when One way ANOVA was applied to the results (p value = 0.124).

The mean weights of the paired testes were 2.27 ± 0.21, 2.37 ± 0.27 and 2.53 ± 0.17gm for groups A, B and C respectively. No significant difference in the mean weights of testes was shown when One way ANOVA was applied to the results (p value = 0.059).

Histological Parameters

Diameter of Seminiferous Tubules

The mean diameter of seminiferous tubules was 256.13 ± 6.24µm; 254.62 ± 4.52 µm and 254.11 ± 3.27 µm in groups A, B and C respectively. One way ANOVA showed no statistically significant difference in mean diameter of seminiferous tubules when compared among groups (p value = 0.629, Fig. 1, 2 and 3).

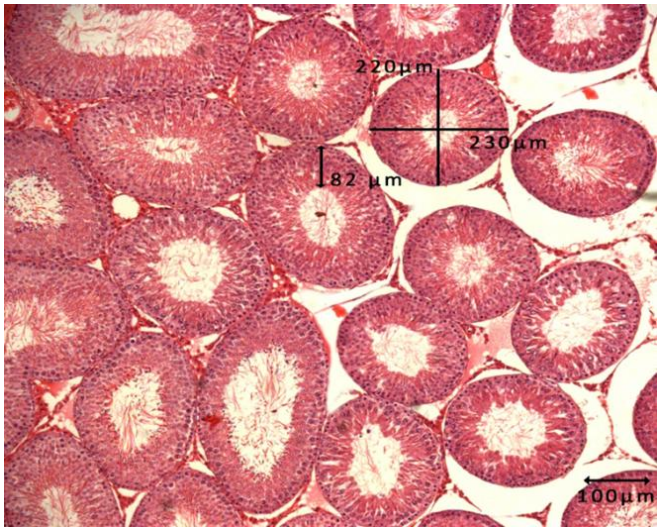

Fig. 1: *Photomicrograph showing transverse section of preparation of testes from group A (control); seminiferous tubules. Diameter of the tubule was measured along both axes (220 and 230µm) and mean diameter was calculated. Germinal epithelium thickness was also measured (82µm). H&E stain. X100.*

Thickness of Germinal Epithelium

The mean thickness of germinal epithelium was 80.73 ± 2.55 µm, 39.89 ± 2.66 µm and 65.20 ± 1.54 µm in groups A, B and C respectively. One way ANOVA showed statistically significant difference in mean thickness of the germinal epithelium when compared among groups (p value = 0.001). Statistically significant difference was seen between groups A and B (p value = 0.001), groups A and C (P value = 0.001) and groups B and C (p value = 0.001), after Post Hoc Tukey test (Tables 1 and 2, Fig. 1, 2 and 3).

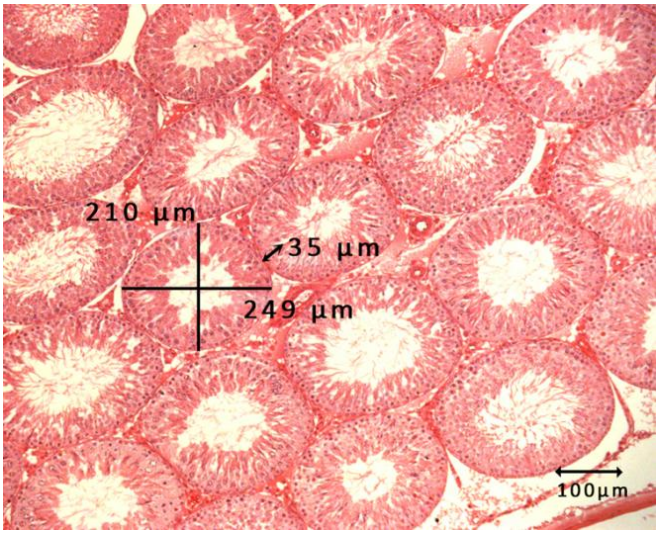

Fig. 2: *Photomicrograph of histological section from group B illustrating transverse sections of seminiferous tubules with degenerating germinal epithelium resulted in decreased thickness of epithelium (35µm). Diameter was calculated at two axes (210 and 240 µm) and mean was calculated. H&E stain. X100.*

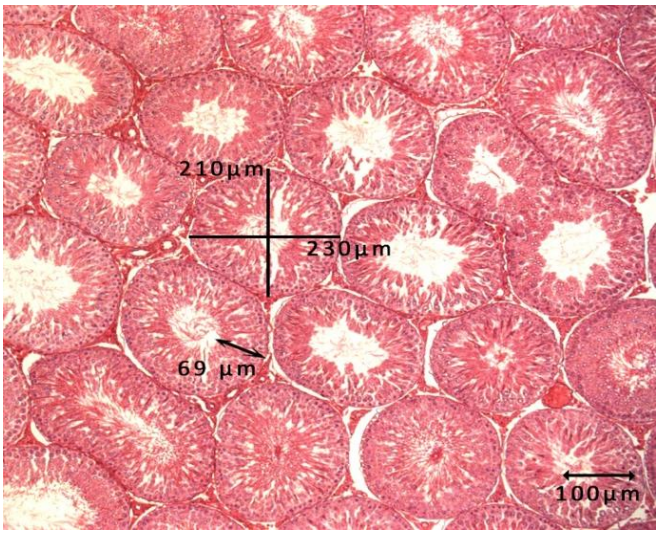

Fig. 3: *Photomicrograph of histological section from group C illustrating transverse sections of seminiferous tubules with improvement in germinal epithelium thickness (69µm). And a cross section of tubule with diameter (210 and 230µm) at two axes is illustrated. H&E stain. X100.*

Mean Johnson's Score

In the control group of animals, mean Johnson's score was 10 ± 0.00; in the experimental groups B and C mean Johnson's scores were 5 ± 0.00 and 7.3 ± 0.483 respectively. Kruskal Wallis test showed statistically significant difference in mean Johnson's score (p value = 0.001). Statistically significant results were observed between groups A and B (p value = 0.001), groups A and C (P value = 0.008) and groups B and C (p value = 0.008, Tables 3 and 4, Fig. 4, 5 and 6).

Table 1: *Shows comparison among mean thickness of germinal epithelium among animals of groups A, B and C.*

Parameter	*Group A Mean ± SD*	*Group B Mean ± SD*	*Group C Mean ± SD*	*p-value*
Mean thickness of germinal epithelium (μm)	80.73 ± 2.55	39.89 ± 2.66	65.20 ± 1.54	0.001*

*p value ≤ 0.05 is statistically significant.

Table 2: *Post hoc Tukey's test showing multiple comparisons of mean thickness of germinal epithelium among animals of groups A, B and C.*

(I) Groups	*(J) Groups*	*Mean Difference (I-J)*	*SE*	*p-value*
Group A	Group B	40.83800	1.03175	0.001*
	Group C	15.53200	1.03175	0.001*
Group B	Group A	-40.83800	1.03175	0.001*
	Group C	-25.30600	1.03175	0.001*
Group C	Group A	-15.53200	1.03175	0.001*
	Group B	25.30600	1.03175	0.001*

*p value ≤ 0.05 is statistically significant

The germinal epithelium in the control group A appeared normal with a Johnson's score of 10. Histological examination of cadmium treated group B, showed degeneration and sloughing of the germinal epithelium of the tubules. There was decrease in the Johnson's score and thickness of germinal epithelium. An improvement in these parameters was observed in the cinnamon bark oil treated group C.

DISCUSSION

In the current investigation the animals were weighed before and towards the end of the experimental period to evaluate the difference in weights which were statistically insignificant, both in the beginning and at the end, when compared among groups. The animals however, maintained good state of health throughout the period of experiment.

Table 3: *Shows comparison among Johnson's score of groups A, B and C.*

Johnson's score	*Group A*	*Group B*	*Group C*	*p-value*
Mean ± SD	10±0.00	5 ± 0.00	7.3 ± 0.483	0.001*
Median (Q1-Q3)	10	5	7 (7 – 8)	0.001*

*p ≤ 0.05 is considered statistically significant
Note: Median was calculated as data was not normally distributed

The difference in the weights of testes when compared among the groups was statistically insignificant. These findings agree with those previously reported that the low doses of Cd for shorter duration of time had no effect on the body weight and that of the testes.[5]

Table 4: *Post Hoc test showing multiple comparisons of mean values of Johnson's score among groups A, B and C.*

(I) group	*(J) group*	*Mean Rank Difference*	*Std. Error*	*p- value**
A	B	20.00	3.762	0.001*
	C	5.00	3.762	0.008*
B	A	-20.00	3.762	0.001*
	C	-10.00	3.762	0.008*
C	A	-5.00	3.762	0.008*
	B	10.00	3.762	0.008*

*p ≤ 0.05 is considered statistically significant

During the current investigation, the histological examination of testis showed no statistically significant difference in the mean diameters of the seminiferous tubules among various groups. Statistically significant difference in the result was observed when the means of germinal epithelium thickness and Johnson's score were compared among various groups. There was a significant decrease in the germinal epithelium thickness in group B when compared to group A (p value = 0.001). This finding is in accord with previous studies in which degeneration of spermetogenic cells and decrease in germinal epithelium thickness in rats treated with cadmium was observed.[5,17,18] The thickness of germinal epithelium improved in group C, which was treated with CBO, when compared to group B treated with $CdCl_2$ (p value = 0.001). However germinal epi-

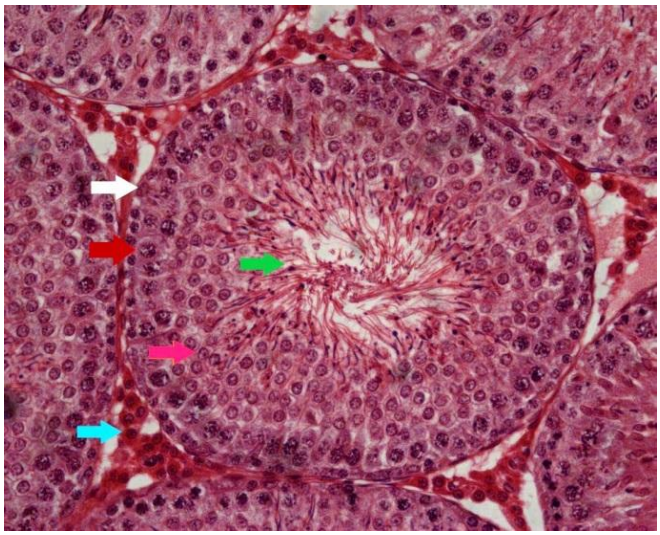

Fig. 4: *Photomicrograph of histological section of testis from group A (control) illustrating a seminiferous tubule with complete spermetogenesis and Johnson's score of 10. All major types of germ cells are present. Spermetogonia (white arrow), primary spermatocyte (red arrow), round spermatids (pink arrow), spermatozoa (green arrow). Leydig cells (blue arrow). H&E stain. X400.*

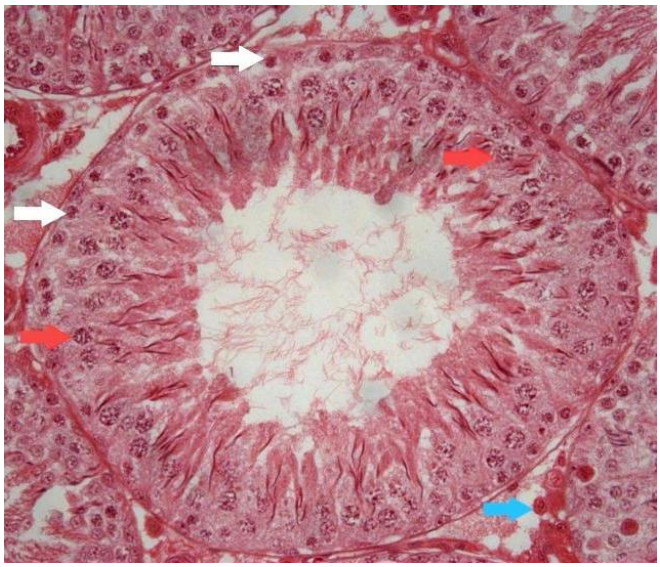

Fig. 5: *Photomicrograph of a histological section from group B illustrating a transverse section of seminiferous tubule. The surface layers of the germinal epithelium are degenerated with slough in the lumen. Basal layers with spermetogonia (white arrow) and many primary spermetocytes (red arrows) are seem with a Johnson's score of 5. Leydig cell (blue arrow). H&E stain. X400.*

thelium did not completely recover in group C, the results were comparable to those reported earlier, which stated that the germinal epithelium thickness did not completely recover by CBO when it was given

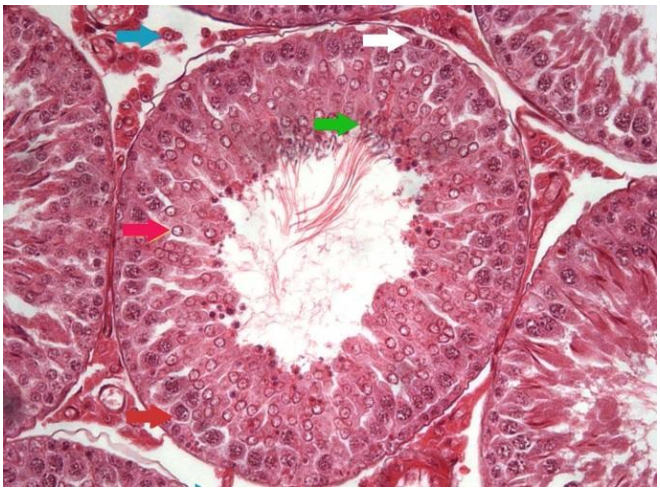

Fig. 6: *Photomicrograph of histological section from group C illustrating seminiferous tubule lined by germinal epithelium with spermetogonia (white arrow), primary spermetocyte (red arrow), round spermatids (pink arrow) and a Johnson's score of 8 as some spermatozoa (green arrow) are seen. Leygig cell (blue arrow). H&E stain. X400.*

along with carbon tetra chloride.[13]

In experimental animals, the toxic effects of cadmium is reported on various organs, such asthe kidney, liver, pancreas, testes and lungs[2]. Cadmium had been demonstrated to produce reactive oxygen species (ROS), resulting in oxidative toxic effect on lipids, proteins and DNA; leading to various pathological consequences.[17] Oxidative stress is known to play a crucial role in the testicular toxicityon account of the production of reactive oxygen species and decrease in levels of antioxidants. Farombi reported loss of testicular function to oxidative stress as a result of cadmium.[4] His findings were comparable to previous study by Mudathir, who reported that the production of ROS as a result of cadmium exposure affects the testicular structure and function resulting in infertility.[15] The current study showed marked histological changes in testes of rats exposed to cadmium like loss of spermetogenic cells, decrease in germinal epithelium thickness resulting in low Johnson's scoring. These results were similar to previous studies of Mudathir, Farombiand El-Sahat, who reported necrosis and sloughing of spermetogenic cells after treatment with cadmium.[4,15,19]

Cinnamon is being used as a traditional medicine for years. The volatile oils and oleoresins of cinnamon leaf and bark have anti-microbial and antioxidant properties.[16] The antioxidant and free radical scavenging activity of cinnamon bark oilhave been reported in different experimental studies.[12,13,20] Present study revealed that CBO administration ameliorated histopathological lesions of testis induced by cadmium; it increased the germinal epithelium thickness, improved the spermetogenic cells resulting in improved Johnson's score,

these are the parameters indicative of protection provided by CBO against testicular toxicity induced by cadmium.

It ***concludes*** the use of cinnamon bark oil improved the condition of testicular lesions as suggested in the present study.The observations of this study highlight medicinal properties of cinnamon. Cinnamon, a cheap and common household herb, thus can be used to combat against the toxic effects of cadmium.

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Authors' Contribution

AM: Conception and design of the work. Data collection. Data analysis and interpretation. Drafting the article. MT: Conception and design of the work. Critical revision of the article. Final approval of the version to be published. NN: Data interpretation. Critical revision of the article.

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