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Effect of Kombucha tea on chromate(VI)-induced oxidative stress in albino rats

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Abstract

The effect of Kombucha tea (KT) on oxidative stress induced changes in rats subjected to chromate treatment are reported. KT feeding alone did not show any significant change in malondialdehyde (MDA) and reduced glutathione (GSH) levels, but did enhance humoral response and delayed type of hypersensitivity (DTH) response appreciably over control animals. Chromate treatment significantly enhanced plasma and tissue MDA levels, decreased DTH response considerably, enhanced glutathione peroxidase and catalase activities; however, no change in GSH, superoxide dismutase and antibody titres was noticed. KT feeding completely reversed the chromate-induced changes. These results show that Kombucha tea has potent anti-oxidant and immunopotentiating activities.

Keywords: Chromate; Oxidative stress; Immunity; Kombucha tea

1. Introduction

People all over the world claim drinking Kombucha tea (KT), a traditionally fermented beverage, provides relief from many physical ailments. Presently, its consumption is popular in the US, mainly due to its refreshing power and speculative curative effects (Steiger and Steinegger, 1957; Stadelman, 1961). Some of the reported benefits are: it helps in digestion, it gives relief from arthritis, it acts as a laxative, it prevents microbial infections, it helps in combating stress, it vitalises the physical body, cures cancer, etc. It is believed that it enhances immunity. Mayser et al. (1995) have reported the microbiology of Kombucha culture. The tea fungus 'Kombucha' is a symbiosis of *Acetobacter xylinum* and various yeasts. Yeasts of the genera *Brettanomyces*, *Zygosaccharomyces* and *Saccharomyces* were identified in 56, 29 and 26%, respectively.

Although there have been few clinical studies performed in America, the Russians, the Germans, the Swedes and others have compiled the benefits of KT for nearly 100 years. In the book

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on Kombucha, Frank (1991) lists much evidence from the Russian and German doctors who have claimed many benefits to their patients consuming KT regularly. Despite so many testimonials and endorsements by herbalists regarding the clinical benefits of KT, there is no scientific evidence supporting any clinically relevant pharmacological activity of Kombucha tea.

In view of these facts, the present study was undertaken to find out the anti-oxidant activity of Kombucha tea in rats subjected to chromate(VI) treatment.

2. Materials and methods

2.1. Preparation of Kombucha tea

2.1.1. Preparation of the medium

One hundred grams of sugar was added to 1 litre distilled water, and the solution allowed to boil for 15 min in a sterile conical flask. Later, 12 g/l tea powder (Brookbond, India) was added, and the flask allowed to cool to room temperature for 1 h. Later, the media containing the extracted tea decoction was filtered through a sterile nylon mesh.

2.1.2. Fermentation

The Kombucha culture was obtained from a known commercial source 2 years ago. Since then, it has been maintained under aseptic conditions in our laboratory. The fermentation was initiated by adding 10% of Kombucha culture and the incubations were carried out at 28 ± 1°C for 8–10 days. Later, the medium was centrifuged at 7000 rpm for 30 min aseptically and stored in polypropylene vials at -20°C until further use.

2.2. Animals

The experiments were conducted on male Sprague–Dawley albino rats weighing 180–200 g maintained at 25 ± 2°C with food and water ad libitum. The tea was given to rats orally, 0.6 ml/200 g body weight, with the help of gastric cannula. Two control groups were maintained throughout the study: (1) saline fed group; and (2) unfermented medium (tea decoction) fed group.

2.3. Oxidative stress

It was induced by force feeding of 1 ml sodium dichromate(VI) daily, 1 5 mg/kg body weight, for 30 days. Later, the animals were anaesthetised and the blood was collected by retro-orbital puncture. The animals were then sacrificed by cervical dislocation and various tissues were processed for biochemical analyses. Reduced glutathione (GSH) was estimated in blood by the method of Kum-Talt and Tan (1974). Malondialdehyde (MDA) was determined by the method of Dousset et al. (1983). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in erythrocytes were determined as per manufacturer's instruction using kits (RANDOX). Catalase (CAT) in red blood cells was determined by the method of Aebi (1984). The chromium content in liver homogenate was determined by atomic absorption spectrophotometer.

2.4. Antibody titre

Separate groups of eight rats each were immunised by injecting 20 μl of 5×10^9 ml^{-1} sheep red blood cells (SRBC) subcutaneously (s.c.) into the right hind foot pad. Seven days later, the rats were challenged with the same number of SRBC intradermally (i.d.) into the left hind foot pad. Blood samples were collected from individual rats by retro-orbital puncture on the 14th day for determination of antibody titre. Antibody levels were determined by haemagglutination as described earlier (Sai Ram et al., 1997). Briefly, 25 μ1 of 0.1% SRBC were added to 2-fold dilutions of serum samples made in saline containing 0.1% bovine serum albumin in V-bottomed Takasty microtitration plates. After mixing, the erythrocytes were allowed to settle down at 37°C until the control wells showed a negative pattern (small button). The value of the highest serum dilution causing visible haemagglutination was taken as the antibody titre.

2.5. Delayed type of hypersensitivity response

It was induced by the method of Atal et al. (1986). Groups of eight rats each were immunised

by injecting 25 μl of 1×10^{10} SRBC ml^{-1} s.c. into the right hind foot pad. Seven days later, the thickness of the left hind foot pad was measured with the help of a Vernier Caliper and then the rats were challenged by injecting 25 μl of 1×10^{10} SRBC ml^{-1} i.d. into the left hind foot pad. Foot thickness was measured again 24 and 48 h after the challenge. The difference between pre- and post-challenge foot thickness was taken as a measure of delayed type of hypersensitivity (DTH) response.

All the experiments were conducted on two different occasions and data were analysed using Student's *t*-test. Since there is no significant difference in any of the parameters in the saline fed and tea decoction fed (two controls) groups, the comparison of the results was made between saline fed and Kombucha fed rats only.

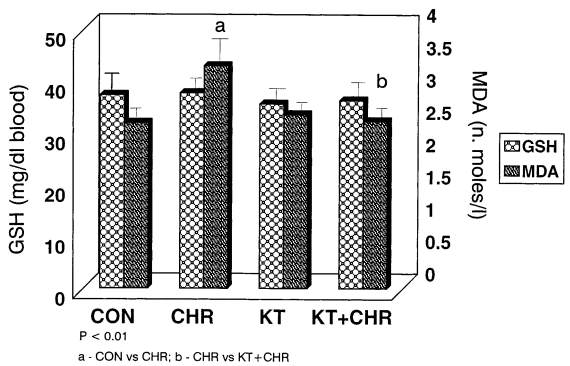

Fig. 1. Effect of Kombucha tea on plasma MDA and erythrocyte GSH levels in rats subjected to chromate(VI) treatment. Con, Control; CHR, chromium; KT, Kombucha tea; KT + CHR, Kombucha tea + chromium.

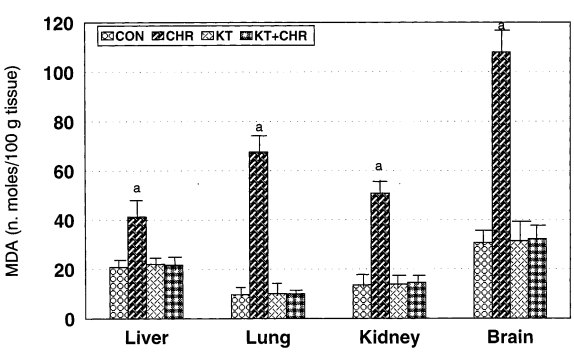

Fig. 2. Effect of Kombucha tea on tissue MDA levels in rats subjected to chromate treatment. Con, Control; CHR, chromium; KT, Kombucha tea; KT + CHR, Kombucha tea + chromium.

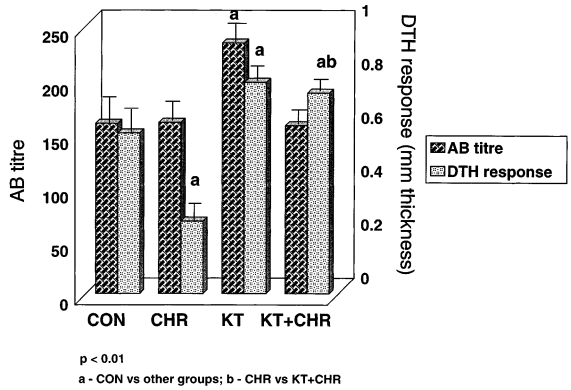

Fig. 3. Effect of Kombucha tea on immune response in rats subjected to chromate treatment. Con, Control; CHR, chromium; KT, Kombucha tea; KT + CHR, Kombucha tea + chromium.

3. Results

The effect of Kombucha tea on the oxidative stress induced by chromate(VI) is shown in Fig. 1. There is no change in plasma MDA and blood glutathione (GSH) levels in the animals fed with KT alone as compared with the control group. Chromate significantly elevated the plasma MDA level over control rats; however, in KT fed rats, there was no significant change in MDA by chromate treatment. Interestingly, there is no significant change in blood GSH levels across all the four groups studied (Fig. 1).

Fig. 2 depicts the MDA level in various tissues in rats exposed to chromate. As expected, the MDA level was significantly increased in all the tissues especially in the brain over the control. However, KT feeding arrested the MDA increase appreciably. Like in erythrocytes, there is no appreciable change in the GSH levels in all the tissues in the presence of chromate over the control rats (data not shown).

The effect of KT feeding on chromate-induced immunosuppression is shown in Fig. 3. KT feeding significantly enhanced antibody titres and

DTH response over control rats. Chromate treatment did not alter the humoral response (antibody titre) but inhibited the DTH response severely. KT feeding completely reversed the immunosuppressive effect of chromate on DTH response (Fig. 3).

The anti-oxidant enzyme levels in the four groups of animals studied are shown in Table 1. There was no significant change in SOD across all the groups studied, whereas there was a significant increase in GPx and CAT activities in animals subjected to chromate treatment. However, KT feeding arrested the increase in the GPx and CAT levels by chromate and the values were very much similar to that of the control.

The chromium content in the liver of various groups of animals studied is shown in Table 2. The chromate content was much higher in animals fed with chromate over control rats, while in KT + chromate fed animals there was a marked reduction in the chromium content, their levels still being significantly higher than the control values.

Table 1
Effect of Kombucha tea on anti-oxidant enzyme levels in red blood cells of rats exposed to sodium chromate(VI) ($P<0.01$)

Group	Haemoglobin U/g		
	SOD	Glutathione peroxidase	Catalase
Control	2566 ± 283	651 ± 42	636 ± 61
Chromium	2646 ± 394.8	850 ± 50[a]	842 ± 72[a]
KT	2410 ± 146.8	644 ± 86	619 ± 51
KT + chromium	2541 ± 195	681 ± 51[b]	558 ± 57[b]

[a] Control versus other groups.
[b] Chromium versus KT + chromium.

Table 2
Effect of Kombucha tea on the chromate content in the liver ($P<0.01$)

	Chromate content (ng/g tissue)
Control	1.75 ± 0.8
Chromium	56 ± 17[a]
KT	1.80 ± 0.5
KT + chromium	28 ± 11[a,b]

[a] Control versus other groups.
[b] Chromium versus KT + chromium.

4. Discussion

There has been a lot of attention regarding the possible benefits and toxicity of KT. Recently, Phan et al. (1998) reported lead poisoning in two people who consumed Kombucha tea fermented in a ceramic pot. Since ceramic pots contain lead and KT is strongly acidic, it was evident that the lead has leached into the tea resulting in contamination. Sadjadi (1998) has reported the presence of *Bacillus anthrax* in Kombucha tea that was fermented in unhygienic conditions. Srinivasan et al. (1997) has reported gastrointestinal toxicity of Kombucha tea in four patients. However, all these reports are isolated cases and a very low number of persons (two to four) are involved. Furthermore, in all these cases, there is no scientific evidence that Kombucha tea is directly responsible for the observed toxicity.

We have evaluated scientifically the acute and chronic toxicity of the tea at various doses. The studies carried out at our laboratory and by another group at our sister laboratory have revealed that the tea prepared under proper hygienic conditions has no significant toxicity as revealed by various haematological, histopathalogical and biochemical studies (results are under publication). Few laboratory tests were carried out by Kappa laboratories in Miami, FL in 1995 and they reported that the tea was fit for human consumption. In 1995, the Food and Drug Administration also conducted few microbiological tests and found no pathogenic micro-organisms in the tea but warned Kombucha brewers of possible contamination when proper conditions are not maintained. In our studies, we found that KT feeding alone had no significant effect on lipid peroxidation, antioxidant enzyme level, but enhanced antibody titres to SRBC and DTH response significantly over control indicating that KT has immunopotentiation activity.

In the present study, oral feeding of chromate(VI) resulted in significant elevation in plasma

and tissue lipid peroxidation (malondialdehyde) levels and decreased DTH response to SRBC over control animals. The increased levels of GPx and CAT in chromate-treated animals indicate that the cells have induced these enzymes to cope with oxidative stress as an adaptive phenomenon. Chromate(VI) compounds are well known to be potent toxic and carcinogenic agents. Chromium(VI) is taken up by cells easily and is subsequently reduced to the other forms such as chromium(III, IV and V), which in turn are believed to cause adverse biological effects (Shi and Dalal, 1994). Kotenkamp et al. (1990) reported that chromium(V)-induced DNA breaks were predominantly due to the production of OH radicals. The arrest in the increase in plasma and tissue MDA, and anti-oxidant enzyme levels in red blood cell KT supplementation indicate that it has strong anti-oxidant activity. Interestingly, there was no change in blood GSH content in chromate-treated rats. The reason for this anomaly is not known at present.

In the present study, we found that DTH response (cell-mediated immunity), which is mediated by T lymphocytes, is severely inhibited by chromate while humoral immune response (B-lymphocyte mediated) remained unaffected. Chromium has been reported earlier to inhibit the immune response (Tanigawa et al., 1991; Cohen et al., 1998). It is found to enhance the production of reactive species and inhibit phagocytosis in macrophages (Johansson et al., 1986), inhibit lymphocyte proliferation, T lymphocytes being more prone than 'B' lymphocytes (Snyder and Valle, 1991). The immunosuppressive effect of chromate is attributed to the production of oxygen free radicals, since anti-oxidants like vitamin E are known to attenuate the chromate-induced cytotoxicity (Bagchi et al., 1995). Studies by Meydani et al. (1995) revealed that oxidative stress significantly inhibits the T-cell response. It is therefore speculated that KT relieved immunosuppressive activity of chromium due to its anti-oxidant activity.

Many testimonials on Kombucha tea reveal that it contains glucuronic acid that binds with poisons and helps to eliminate them through urine. To test this hypothesis, we have determined chromium content in liver in all the groups. Interestingly, the chromium content in liver of KT + chromium fed rats is significantly lower than the respective control group (only chromium fed). This indicated that KT helps in excretion of chromium from body tissues. Chromate removal from the tissues may also be responsible for decreased oxidative stress by chromate. However, the level of chromium in the liver in KT fed rats is still significantly higher than that in the control (without chromium) animals indicating that the decreased lipid peroxidation and improved DTH response in KT fed animals would be both due to its anti-oxidant activity and chromate elimination from the body. Experiments are in progress to characterise the bioactive components in Kombucha tea.

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