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Hydroxocobalamin (vit b_{12a}) effectively reduced extent of cyanide poisoning arising from oral amygdalin ingestion in rats

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This study investigated the efficacy of hydroxocobalamin in reducing cyanide toxicity arising from amygdalin administration in rats. Amygdalin at its therapeutic dose (20 mg/kg body weight) was co-administered with hydroxocobalamin to rats at two different levels (25 and 50 mg/kg body weight) for 14 days. Symptoms of cyanide toxicity in the blood and liver were monitored in the animals and compared with the control. One of the rats who received amygdalin without the antidote died of cyanide poisoning before the end of the experimental period while no mortality was recorded in rats who received the antidote. There was significant reduction ($P < 0.05$) in blood cyanide and serum lactate concentration in a dose-dependent manner in rats who received the antidote compared with the control. Blood of rats fed amygdalin showed significant elevation in packed cell volume (PCV) and haemoglobin concentration accompanied with significant reduction in blood pH while these abnormalities were reversed by hydroxocobalamin. Histological studies of the liver of amygdalin-fed rats revealed marked alteration in cellular architecture which was not noticeable in rats who received the antidote. We conclude that rats can be protected from the deleterious effects of cyanide poisoning due to amygdalin ingestion by the concomitant administration of hydroxocobalamin.

Key words: Amygdalin, antidote, hydroxocobalamin, cyanide toxicity.

INTRODUCTION

Amygdalin (laetrile) is a cyanogenic glycoside which occur naturally in apricot seed and bitter almond and has been demonstrated to posses both prophylactic and curative anticancer properties with positive results reported in many patients (Moertel et al., 1982; Curt, 1990). The use of the drug was discouraged when it was demonstrated that amygdalin is metabolized in the body to release significant amount of cyanide thus leading to cyanide poisoning (Bromley et al., 2005; Chandler et al., 1984). Side effects of amygdalin ingestion in humans mirror symptoms of cyanide poisoning which includes nausea, vomiting, headache, dizziness, bluish colouration of the skin, liver damage, hypotension, nerve damage, fever, mental confusion, coma and death (Howard- Reuben and Miller, 1984). As a result of its toxicity, the use of amygdalin as a cancer therapy or as a treatment for any other medical condition was discouraged, but the compound continued to be manufactured and administered as an anticancer therapy in many countries, particularly in Mexico.

The incidence of cyanide poisoning is much higher when amygdalin is taken orally compared to intravenous route because intestinal bacteria and some eaten plants contain enzyme (β-glycosidase) that activate the release of cyanide after it has been ingested (Carter et al., 1980). The use of antidotes to reduce cyanide toxicity has long been realized (Way, 1984; Bhattacharya, 2000). One of such compound which has been successfully used as antidote for cyanide poisoning is hydroxocobalamin (Vit. B_{l2a}), a natural form of vitamin B_{12} (Brouard et al., 1987). It acts as a chelating agent by binding cyanide strongly to form cyanocobalamin (Vit. B_{12}) which is non toxic and ea-

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Table 1. Some cyanide poisoning indicators in rats ingested with amygdalin co-administered with hydroxocobalamin for 14 days.

Parameters	Control	Amygdalin only	Amygdalin + 25 mg/kg hydroxocobalamin	Amygdalin + 50 mg/kg hydroxocobalamin
Mortality (%)	0	12.5	0	0
PCV (%)	22.30 ± 2.15	26.52 ± 3.10 *	23.35 ± 1.80	22.85 ± 2.00
Hb conc. (g/dl)	13.20 ± 1.58	16.48 ± 2.23 *	14.00 ± 0.84	13.76 ± 1.25
Whole blood pH	7.31 ± 0.05	6.02 ± 0.03 *	7.11 ± 0.04	7.14 ± 0.07
Blood cyanide (µmol/l)	2.10 ± 0.14	15.23 ± 1.22 **	5.10 ± 0.21 *	4.55 ± 0.42 *
Serum lactate (mmol/l)	0.44 ± 0.04	3.22 ± 0.12 **	1.06 ± 0.06 *	0.95 ± 0.08 *

Values are Mean ± SD, n = 7.
*Values are significantly different from the control group at $p < 0.05$.
**Values are significantly different from the control group at $p < 0.01$.

sily excreted in urine (Forsyth et al., 1993). This study intends to investigate whether co-administration of amygdalin with hydroxocobalamin will reduce the extent of cyanide poisoning arising from oral amygdalin ingestion.

MATERIALS AND METHODS

Drugs/Chemicals

Amygdalin in crystalline form was obtained from Sigma Laboratories, St. Louis. Hydroxocobalamin (5%w/v) is a product of Pharmacie Centrale des Hôpitaux de Paris France. Other chemicals used for the study were of analytical grade (analar) and were obtained from British Drug House, Poole England.

Experimental animals

Thirty two (32) male Wistar strain albino rats weighing between 150 and 165 g were used for this study. The animals were housed in groups in metabolic cages and given food and water *ad libitum*. They were randomly divided into four experimental groups (eight in a group). Group A served as the control and was given distilled water. Group B rats were administered with 20 mg/kg body weight amygdalin daily for 14 days while groups C and D received the same dose of amygdalin co-administered with 25 and 50 mg/kg hydroxocobalamin respectively.

Amygdalin was dissolved in distilled water and administered orally to rats by the use of canular while hydroxocobalamin was given by intravenous injection.

Collection of blood and liver from rats

Rats from each group were anaesthetized at the close of experiment by putting them in jar containing cotton wool soaked in diethyl-ether vapour. They were allowed to go into unconscious state after which they were sacrificed by cutting their jugular veins to collect blood. Part of the blood was collected into EDTA coated bottles to prevent clotting and preserved for heamatological analysis. The other part of the blood was collected in plain bottles and allowed to clot for the preparation of serum. The clotted blood was centrifuged at 3000 rpm for 5 min. The clear supernatant (serum) was then separated from the pellet and kept frozen till required. After bleeding, animals were quickly dissected and the liver removed and rinsed in ice-cold 0.25 M sucrose solution for histological study.

Measurement of blood parameters

Blood cyanide level was determined by the Konig reaction (Lundquist, et al., 1985) while Drabkins method of Alexander and Griffith (1993) was used for the measurement of haemoglobin concentration. Serum lactate concentration was measured by the lactate oxidase method (Hadzivassiliou and Pieder, 1968). Whole blood pH was determined using Jenway pH meter N-512 connected with ERH 111 electrode.

Histological procedure

The liver was sectioned transversally in its biggest diameter with a steel blade and fixed in 10% formaldehyde solution for 24 h. After fixation, the specimens were rinsed with water and immersed in a 70% alcohol solution after which the specimen were sent to the histology lab to undergo routine histological procedure. 7 - 10 mm semi-serial cuts slice were made and embedded with paraffin. It was stained with haematoxylin and eosin and mounted in a conventional optic microscope using X40 magnification after which the slides were assessed by two trained observers using criteria earlier described (Bancroft and Stevens, 1982). Microphotographs of each slice were also taken using Nikon- Labophot 2.

Statistical analysis

Data was analyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) at two levels of significance ($P < 0.01$ and $P < 0.05$) as described by Montgomery (1976).

RESULTS

Table 1 shows some cyanide poisoning indicators in rats ingested with amygdalin after 14 days compared with control. One (12.5%) of the eight rats who received amygdalin without the antidote died before the end of the experiment while no mortality was recorded in rats who received hydroxocobalamin. There was significant reduction ($P < 0.05$) in a dose dependent manner in blood cyanide and serum lactate levels in groups C and D rats who received the antidote compared with animals in group B. PCV and haemoglobin concentration in the blood of amygdalin-fed rats were significantly elevated while these

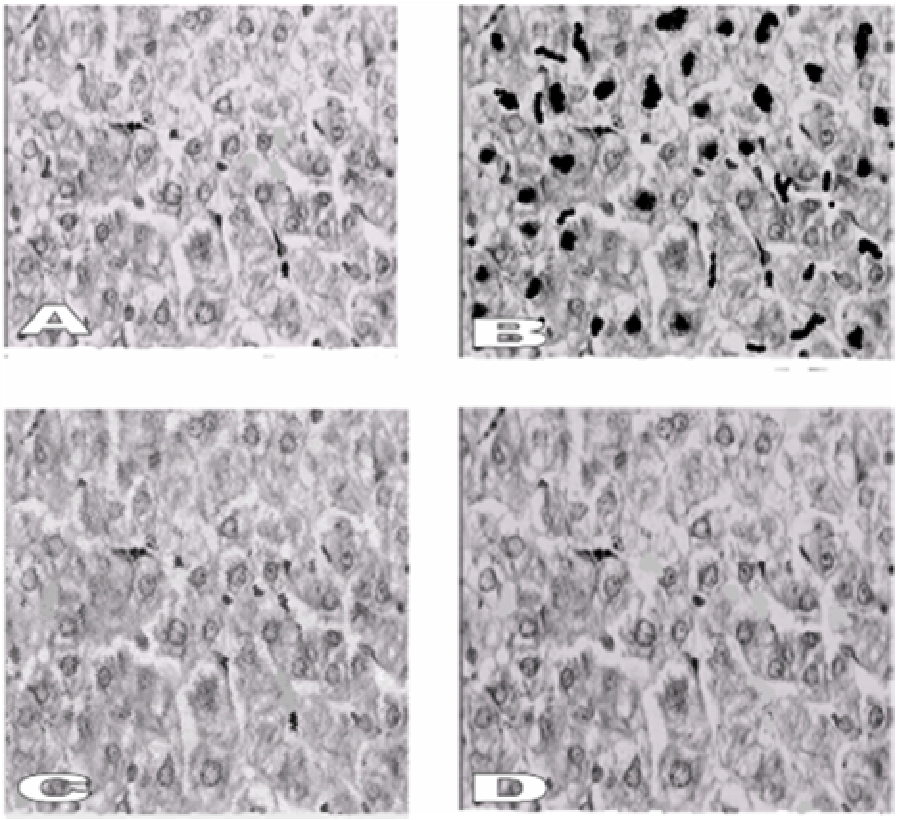

Figure 1. Haematoxylin and eosin stained (X40) histological structure of the liver of rats after 14 days.
A= Normal control rats administered with distilled water. Picture shows normal architectural structure of the liver.
B= Rats ingested with 20 mg/kg body weight amygdalin only. Picture reveals parenchyma necrosis, marked portal inflammation and colour alteration on the hepatic surface of the liver.
C= Rats ingested with 20 mg/kg amygdalin co-administered with 25 mg/kg body weight hydroxocobalamin respectively. Picture show no significant histological alteration in the colour and size of the liver compared with that of normal control.
D= Rats ingested with 20 mg/kg amygdalin co-administered with 50 mg/kg body weight hydroxocobalamin respectively. Picture show no significant histological alteration in the colour and size of the liver compared with that of normal control.

blood parameters were significantly reduced close to the normal control rats in those who received the antidote. The acidic blood pH (6.02) recorded in amygdalin-fed rats was also normalized close to normal in rats co-administered with hydroxocobalamin.

Figure 1 shows the histological stained slides of 4 groups of the animals (X40 magnified, haematoxylin and eosin stained). There was parenchyma necrosis, marked portal inflammation and color alteration on the hepatic surface of the liver in rats given oral amygdalin (group B). The picture also indicates canicular proliferation and presence of hepatic fibrosis in this group of animals. There was no significant histological alteration in the colour and size of the hepatic lobes in groups C and D animals who received the antidote.

DISCUSSION

The mortality recorded in rats fed with amygdalin occurred due to complications from cyanide poisoning. Amygdalin is metabolized by the body to produce cyanide, a very rapid poison which impairs cellular respiration leading to a cascade of events culminating in death (Ballantyne, 1987). Victims having a blood cyanide level above 60 µmol/l frequently succumb to respiratory cessation within 20 - 30 min of exposure and may die within 3 h (Salkowski and Penney, 1994). The morbidity or mortality depends upon the magnitude of poisoning, which varies with the dose, time of exposure, form of cyanide and the route of poisoning (Marrs et al., 1996; Hall and Rumack, 1986). Amygdalin fed to dogs produced 60% mortality

due to cyanide-poisoning with 100% of the animals showing signs of respiratory, cardiac and neurologic impairment (Schmidt et al., 1978).

Amygdalin ingestion to rats caused elevation of PCV and haemoglobin probably because the drug induced hypoxaemia in the rats. Hypoxaemia is a condition of low oxygen in the blood which can be caused by cyanide intoxication (Perutz, 1979) and the condition is characterized by abnormally high blood PCV and haemoglobin concentration (Grimes, 1980). The significant elevation in serum lactate levels and reduction in whole blood pH in rats ingested with amygdalin indicates that the drug might have impaired cellular respiration and caused tissue hypoxia and metabolic acidosis in the rats (Hussain et al., 2003). Lactate is elevated in the blood during anaerobic metabolism caused by inhibition of cytochrome oxidase and interruption of electron transport chain and oxidative phosphorylation (Isom and Way, 1982).

The significant reduction in blood cyanide, serum lactate, PCV and Hb concentration in a dose dependent manner and zero mortality recorded in rats administered with hydroxocobalamin indicated its effectiveness as antidote to cyanide poisoning. The antidote also corrected the acidic pH recorded in rats fed with amygdalin. Hydroxocobalamin contains cobalt which binds to cyanide with greater affinity than cytochrome oxidase to form cyanocobalamin (Sauer and Keim, 2001). Hydroxocobalamin effectively decreased the low whole blood cyanide levels found in heavy smokers (Brouard et al., 1987) and was also found to be capable of preventing toxic symptoms and death in mice due to cyanide (Van Heijst and Meredith, 1990). Mice injected with potassium cyanide were found to recover rapidly when administered with hydroxocobalamin (Brouard et al., 1987).

The histological alterations observed in the liver of amygdalin-fed rats indicate cholestasis characterized by biliary pigment retention within the hepatic lobes; while the observed increased in the size of the hepatic parenchyma in the histological cuts of the liver lobe indicate disruption of cellular architecture. All these abnormalities might have resulted due to tissue hypoxia caused by cyanide which disrupted normal cellular functioning of the liver. Microscopic analyses of the liver of rats in group C and D show no architectural alteration in the examined area demonstrating that the antidote prevented amygdalin from causing any sufficient damage to the liver cells.

Conclusion

Oral administration of amygdalin at its therapeutic dose to rats for 14 days resulted in death and toxicity due to cyanide poisoning. Co-administration of amygdalin with hydroxocobalamin prevented mortality and significantly reduced amygdalin toxicity in the blood and liver of rats. We therefore conclude that co-administration of amygdalin with hydroxocobalamin effectively reduced the extent of cyanide poisoning arising from oral amygdalin ingestion in rats.

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