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THE EFFECT OF HYOSCINE-N-BUTYLBROMIDE (BUSCOPAN) ON OXIDATIVE STRESS PARAMETERS IN ALBINO RATS

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Abstract

Effects of Hyoscine-N-butylbromide (Buscopan) on oxidative stress parameters of albino rats were studied. Catalase, reduced glutathione, superoxide dismutase and malonylaldehyde levels were determined using standard methods. The rats were placed into two groups A and B where group A was sub-divided into three sub-groups. Sub-group A_1 received normal recommended dose of 10mg/60kg body weight while A_2 and A_3 received two and four times the recommended dose respectively. Group B was used as control. The results revealed no significant difference ($p>0.05$) in the activity of reduced glutathione, superoxide dismutase and malonylaldehyde in the test groups. The effect on the activity of catalase showed a significant increase ($p<0.05$). The result showed that there may be some side effects of overdose as it might lead to increased oxidative stress.

Keywords*: Buscopan, catalase, glutathione, malonylaldehyde and superoxide dismutase.*

Introduction

Oxidative stress is the destruction caused by free radical molecules in the living system. This results in an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Halliwell and Gutteridge, 2000). Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage components of cells including proteins, lipids and DNA (Block, 2003). Sources of oxidative stress include exogenous factors such as cigarette smoke, prescription drugs and toxic chemicals such as pesticides and endogenous factors such as the oxidative burst from activated macrophage (Obata *et al.*, 2000). Hence oxidative stress results when oxidative force exceeds the antioxidant system due to loss of the balance between them. Among the antioxidants include vitamin C (ascorbate), vitamin E (tocopherol), selenium, lycopene, enzymes (superoxide dismutase and catalase), reduced glutathione and carotenoids (Halliwell and Gutteridge, 2000).

Oxidative stress contributes to tissue injury following irradiation and hyperoxia (Block, 2003). Many clinical conditions such as atherosclerosis, Parkinson's disease, heart failure and Alzheimer's disease are associated with increased indices of oxidative stress. This suggests that overwhelming the antioxidant system initiates and propagates processes involved in the pathogenesis of many diseases (Halliwell and Gutteridge, 2000). Short term oxidative stress may be important in the prevention of aging by induction of a process called mitohormesis. Reactive oxygen species can be beneficial as they are used by the immune system as a way to attack and kill pathogens and are also used in cell signaling (Gems and Partridge, 2008).

Certain biological markers have so far been identified as basis for assessing oxidative stress. They include lipid hydroperoxides, isoprostan, 4-hydroxynonemol, malondialdehyde, 8-hydroxyguanine and ubiquinol-10. These biomarkers have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions as well as to evaluate the efficiency of drugs (Trevisan *et al.*, 2001).

Buscopan is an antispasmodic drug which contains Hyosine N-butylbromide. It is used to relieve painful spasm in the stomach, intestines and bile duct (gastrointestinal tract), the reproductive organs and urinary tract (genitourinary tract) (Tygat, 2007). Hyosine works by relaxing the muscle that is found in the wall of the gastrointestinal tract and genitourinary tract. This type of muscle is called the Smooth muscle; it normally contracts and relaxes in response to natural body chemicals called neurotransmitters. The contractions are caused by a neurotransmitter called acetylcholine (Mokoto *et al.*, 2000). Hyosine stops the spasm in the smooth muscle by preventing acetylcholine from acting on the muscle. It does this by blocking the receptors on the muscle cells that the acetylcholine would normally act on (muscarinic or cholinergic receptors). Buscopan is prescribed for conditions such as irritable bowel syndrome and severe menstrual cramps (Tygat, 2007).

Oxidation reactions are crucial to life, they can also be damaging, hence plants and animals maintain complex systems of multiple types of antioxidants such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Valko *et al.*, 2007). Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill the cells (Skrzydlewska *et al*., 2001). It becomes important to assay for the activities of some of these enzymes (such as catalase, superoxide dismutase), reduced glutathione and lipid peroxidation (malonylaldehyde level).

Glutathione is one of the most important cellular antioxidants (Sahin *et al*., 2001). The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione *s*–transferase (Schafer and Buettner, 2001).

This system is found in animals, plants and microorganisms. Glutathione peroxidase catalyses the breakdown of hydroperoxides and organic hydroperoxides to water and alcohol respectively (Sahin *et al.,* 2001).

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals steal electrons from the lipids in the cell membranes, resulting in cell damage (Skrzydlewska *et al.,* 2001). This process proceeds by a free radical chain reaction mechanism. It most often affects poly unsaturated fatty acids, because they contain multiple double bonds in between which lie methylene groups that possess especially reactive hydrogens (Mukai and Goldstein, 2004).

Catalase is an antioxidant enzyme that catalyses the conversion of hydrogen peroxide to water and oxygen using either iron or manganese as a cofactor (Valko *et al.,* 2007). Catalase also uses hydrogen peroxide to break down potentially harmful substances in the body and can be referred to as hydrogen peroxide oxidoreductase. This protein is localized to peroxisomes in most eukaryotic cells (Block, 2003).

Materials and Methods

Male albino rats of over eight weeks old were used in the investigation. The rats were weighed and the amount of drug solution administered was calculated based on the body weight. A quantity of 0.17mg/kg body weight (corresponding to 10mg recommended dose for adult) of Buscopan was administered by intubation three times daily to rats in group A consecutively for seven days. Group A was divided into subgroups A_1, A_2 and A_3 containing 4 rats each. The rats in sub group A_1 were given the recommended dosage (based on two tablets of 10mg per 60kg body weight) while animals in groups A_2 and A_3 were given times two (2x) and four (4x) the recommended dosage. Group B was used as the control.

After the period of administration, the animals were sacrificed and blood samples were collected into different sterile bottles. The blood samples were centrifuged at 1500rpm for 15 minutes and the serum was separated from the whole blood.

The catalase activity was determined according to UV assay method of Hugo (1974). In the UV range hydrogen peroxide shows a continual increase in absorption with decreasing wavelength. The decomposition of hydrogen peroxide can be followed directly by the decrease in extinction at 240nm. The difference in extinction (ΔE_{240}) per unit time is a measure of the catalase activity.

Lipid peroxidation was determined spectrophotometrically according to the method of Wallin *et al.* (1993) by assessing the level of thiobarbituric acid reactive substances (TBARS). Some quantities of malondialdehyde (MDA) are produced during lipid peroxidation. These react with thiobarbituric acid (TBA) to generate a pink coloured complex which in acid solution absorbs maximally at 532nm.

Superoxide dismutase activity was determined by Epinephrine method of Pajovic *et al.,* (2003) which is based on the ability of superoxide dismutase to inhibit the auto oxidation of adrenaline and the absorbance taken at 480nm at 15 seconds for 1 minute 30seconds.

Reduced glutathione level was determined according to Phospho-18-tungstic acid method of Snell and Snell (1962) which is based on the presence of free sulfhydryl group since reduced glutathione forms the bulk of non-protein sulfhydryl groups and the absorbance taken at 610nm within 10minutes.

Results

TABLE 1: The mean levels of Catalase, Glutathione, Superoxide Dismutase and Malonylaldehyde in Rats Treated with Buscopan.

GROUP	Average activity of catalase in K (mol/sec)	Average glutathione level (mg/100ml)	Average level of superoxide dismutase (mg/100ml)	Concentration of malonylaldehyde (mg/100ml)
Group A_1	0.0006±0.0001	0.24±0.03	2.05±0.01	0.22±0.03
Group A_2	0.0009±0.0002	0.28±0.07	2.09±0.08	0.26±0.02
Group A_3	0.0007±0.0002	0.23±0.02	2.10±0.08	0.29±0.06
Group B (control)	0.0005±0.0001	0.20±0.02	2.05±0.04	0.30±0.01

Values are mean ± standard deviation.

The effect of buscopan on the level of glutathione, superoxide dismutase, malonylaldehyde did not show any significant differences ($p > 0.05$) when compared with the control. There was a significant increase ($p<0.05$) in the activity of catalase.

Discussion

The effect of buscopan on oxidative stress indices showed that there was a decrease in physical activity and food intake in the treated animals. This observation could be due to the fact that the drug decreased their metabolic rate by decreasing appetite for food (Tygat, 2005).

The glutathione activity of the treated animals in table 1 showed an increase that was not significant ($p>0.05$). Superoxide dismutase activity of the treated animals showed no significant difference ($p> 0.05$) when compared with the

control. This is in line with the findings of Tygat, (2005) that the constituents of buscopan may play no role in superoxide dismutase activity.

The mean malonylaldehyde (MDA) levels in the treated animals showed no significant difference ($p > 0.05$) when compared with the control. Halliwell and Gutteridge (2000) stated that cellular damage through oxidation of proteins, DNA and lipids may result if free radicals accumulate in the cell. Catalase activity in the treated animals increased significantly ($p < 0.05$) and this result did not differ from the report by Gaetani *et al.*, on the role of catalase in disposal of hydrogen peroxide within the human erythrocyte (1996).

Conclusion

The observations made in this research suggest that Buscopan may produce no significant side effects and the increased activity of catalase and reduced glutathione may be helpful in mopping up free radicals generated from oxidative stress. However this study is subject to further investigation and more precise findings.

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