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عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	دراسات in vitro خارج الجسم على التأثير الوقائي للكيوريسيتين على التغيرات الناتجة من بروميت البوتاسيوم في المؤشرات الأوكسده ومضادات الأوكسده في خلايا الدم في الجرذان
Title of the proposed project in English	In vitro studies on the protective effect of quercetin on potassium bromate induced alterations in oxidative stress and antioxidant defenses indices in rat erythrocytes
المشرف الرئيس - PI	Dr Nikhat J Siddiqi
التخصص الدقيق للمشرف الرئيس - Specialty of PI	Collagen biochemistry and oxidative stress
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	12
Abstract of the proposal (No more than 200 words)	Potassium bromate (KBrO ₃) is used in the disinfection of drinking water and conditioning of dough. Bromate has been detected in tap and bottled water. Exposure to KBrO ₃ results in generation of oxidative stress in vivo. Increased production of reactive oxygen species (ROS) and free radicals has been

implicated in mediating KBrO₃-induced toxicity. These radicals can cause extensive tissue damage by reacting with macromolecules like proteins, nucleic acids and membrane lipids .

Quercetin, is a flavonoid naturally occurring in plant extracts and phytochemicals. It also exhibits a wide range of biological functions including anti-carcinogenic, anti-inflammatory, antiviral, and psychostimulant activities, in addition to the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis (Aguirre, et al , 2011). In the proposed study the protective effect of quercetin on KBrO₃ induced oxidative stress in rat erythrocytes in vitro will be investigated.

Hypothesis of the proposal

Potassium bromate (KBrO₃) is known to cause erythrocyte damage in vivo and in vitro. Addition of quercetin to erythrocytes along with potassium bromate (KBrO₃) in vitro would mitigate potassium bromate (KBrO₃) induced erythrocyte damage. This project will test the above hypothesis.

Specific objectives

- 1- Study the in vitro oxidative stress induced damage caused by potassium bromate (KBrO₃) to rat erythrocytes.
- 2- Study the protective effect in vitro of quercetin on oxidative stress induced damage caused by potassium bromate (KBrO₃) to rat erythrocytes.

Methodology & Major Techniques to be used

Erythrocytes will be isolated from rat blood. They will be incubated with different concentrations of KBrO₃ and quercetin and incubated at 37°C for sixty minutes. Four groups of hemolysates will be prepared viz.,

1. Control group,
2. Lysates treated with four different concentrations of KBrO₃,
3. Lysates treated with two different concentrations of quercetin,
4. Lysates treated with quercetin followed by KBrO₃.

Lysates will be prepared from control and experimental groups and used for determination of various biochemical parameters

- 1- Lipid peroxidation will be determined by the method of Utlley, Berheim et al. (1967).
- 2- Reduced glutathione will be estimated by the method of Beutler, Duron et al. (1963).
- 3- Protein carbonyl levels in the samples was quantified as per the method Levine, Garland et al. (1990).

4- Glutathione reductase activity will be assayed by the method developed by

Goldberg and Spooner (1987).
5-Superoxide dismutase will be estimated by the method of Kakkar, Das et al. (1984).
6-Catalase was assayed by the method Aebi (1984).

7-Total antioxidant capacity will be measured using commercial kit.
8-The protein content in the sample was measured by the modified method of Lowry Markwell, Haas et al. (1978)

Availability of Samples

No

Kindly justify

The samples will be obtained from animals after the project is approved and ethical approval is obtained.

Availability of Chemicals

No

Kindly justify

The chemicals will be purchased after the project is approved and funds are allotted.

Availability of Instruments

Yes

Ethical Approval

In the process

Recent References

Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. J Lab Clin. Med ; 61: 882-888.
Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J.

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Goldberg, D.M., Spooner, R.J (1987). Glutathione reductase. NAD(P)H: oxidized Glutathione oxidoreductase (EC 1.6.4.2), third ed. In: Bergmeyer, H.U., Bergmeyer, J., Graßi, M. (Eds.), *Methods of Enzymatic Analysis*, vol. III Verlag Chemie, Weinheim. 258–265.

Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 1984 Apr;21(2):130-2.

Levine RL, Garland D, Oliver C, Amici A, Climent I, Lenz A (1990) . Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 186:464–478.

Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) . A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem.* 87: 206-210.

Utley HG, Berheim F, Hochstein P (1967). Effect of sulfhydryl reagent on peroxidation in microsomes. *Arch Biochem Biophys.* 118: 29-32.