Evaluation the Susceptibility of Metformin Drug and Pollen grain of Typha domengensis (Typha) on Prevention of Damage in octa4 gene Which Induced by Mitomycin c in Some Organs in Male Albino Rats

Ghufran Mershoud Latef Alobaidy (MBChB)[1] **and Zubaida Adnan AL-Jashammy**(PhD)[2]

Abstract

Background: Living organisms are permanently exposed to internal and external factors that damage DNA. This damage, if not repaired, can lead to the mutation which cause verius diseases and cellular death. Therefore,the use of many plants or their products has been adopted as aprotective material against genetic toxicityof certain drugs.

Objective: Examined the susceptibility of both metformin and pollen grain solution to the prevention of damage in octa4 gene and mitomycin C, In order to be adopted as materials available and cheap to prevent damage to genetic material and the result of the use of human drugs.

Patients and Methods: was used 25 white males rats for 4 weeks obtained from the College of Veterinary Medicine / University of Baghdad and ages ranging from (10-12) weeks. The rats were divided into 5 groups. the control group was drip with distilled water as well as injected The second group was treated with mitomycin (0.001ml) was injected subcutaneously weekly for 30 days.while the third group was orally injected with metformin with a concentration of 500 mg / kg with mitomycin , while the fourth group was treated with mitomycin and water solution for pollen at 0.1 g / mL per animal. while the fifth group included rats treated with mitomycin with metformin and water solution for pollen. The animals were anesthetized and the kidney and liver were removed. The DNA extraction process was investigated and the presence of the octa4 gene was investigated using a special technique and PCR technique.The tissues like liver, kidney and testies were collected for study the expression of Oct 4 gene.

Results: The C. papyrus pollon extract along with metformin is effective against mitomicine C induced animals. Oct 4 it has been protected from mitomycin damage in both the kidney and liver tissue compared to the testis of the animals treated with *C. papyrus* pollon extract and metformin. *C. papyrus* pollen grain producing an effective and safe product, and further research is required in addition to the aggregate value to the native plants.

Conclusion: In the present study, *C. papyrus* pollon extract along with metformin is effective against mitomicine C induced animals. Oct 4 expression was significantly higher in the kidney and liver tissue of the animals treated with *C. papyrus* pollon extract and metformin**.** The result of the present study clearly indicates that C. papyrus reduces blood

glucose levels, producing an effective and safe product, and further research is required in addition to the aggregate value to the native plants.

Keywords: DNA damaged, mitomycin c, *Cyperus papyrus*, Oct 4 gene.

Corresponding Author: zubaidabiology@gmail.com

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[1,2] College of Education and Applied Science - University of Tikrit - Salahaddin - Iraq.

Introduction

Every day, genome integrity is challenged by DNA damage from both environmental sources and endogenous agents [1]. As a result of this, the DNA of each cell gathers thousands of lesions every day. These accumulated lessions or extensive damage can cause various diseases or abnormalities such as cancer [2], tumor development, arrest cell cycle progression [3] .This DNA damaged should be removed so that the cell function and proliferate normally [1,4]. This damage essential to be repaired to allow polymerases (RNA and DNA) to precisely read and duplicate the information in the genome. Fortunately, cells contain several DNA repair mechanisms which include mismatch repair (MMR) that recognizes base incorporation errors and base damage, base excision repair (BER) that removes damaged bases, nucleotide excision repair (NER) that removes bulky DNA adducts, and cross-link repair (ICL) that removes interstrand cross-links [4]. Similarly, double strand DNA breaks also repaired by the two pathways viz. nonhomologous end joining (NHEJ) and homologous recombination (HR) [1]. The octa4 gene, also known as Pou5f1, is an important for various cell functions such as differentiation, embryonic growth etc.[5]. In the experimental condition, mitomycin c was used as a DNA damage agent [6]. It inhibits DNA synthesis by producing DNA cross-links which halt cell replication and eventually cause cell death. Various drugs are available to reduce or restore DNA damage to normal via their antioxidant properties. One of such drug is metformin. It is peyquanide compound linked with guanidine and a ring of ammonia. The drug is routinly use to treat various diseases such as diabetes [7], polycystic ovaries syndrome [8], restore human DNA damage and carcinogenicity [9,10]. Na and Yoo [11] dreported metformin used for reduce DNA damage in the insect by protecting against oxidative stress and facilities for aging. Similarly, few plant extracts such as Hertia cheirifolia [12], Rhaponticum carthamoides [13]. Pinus densiflora [14] were also evaluated against DNA damage by various chemical agents [15,16]. Cyperus papyrus (Typhaceae) is growing in the southern Iraq marshes and in saline water [17], However, studies are very limited. So, there is a need for the better treatment for reducing the DNA damage cause by these various agents. With this background current study, aim to evaluate effect of Cyperus papyrus pollen

grains on expression of octa4 gene in mitomycin c induced animal model.

Patients and Methods

Males Albino Rats, obtained from the Faculty of Veterinary Medicine / University of Baghdad at ages 10-12 weeks and weights (325-250) grams. The rats were subjected to appropriate laboratory conditions from a light cycle divided into 12 hours light and 12 hours darkness. Pollen grain powder was obtained from the local markets of the city of Nasiriyah and neighboring markets and was confirmed as locally produced known al-Khrait. Collect the powder and place it in dishes of paper at room temperature. (50 g) of powder added to 500 ml of distilled water to the boiling point and let cool with continuous stirring. Then filter the solution through layers of gauze, then filter paper. The effective dose was determined according to the method of [18]. The laboratory animals were divided into 6 groups and 4 animals per group were administered as follows for 30 days, the control group was injected with distilled water only as injected subcutaneous with distilled water. The second group was injected with mitomycin only with 10 mg / kg injection by subcutaneous injection once a week. The third group gaved metformin at a concentration of 500 mg/ kg by tubular feeding and mitomycin. The fourth group was injected with pollen grain water extract at a concentration of 50 mg / kg body weight with injected by mitomycin, the fifth group was injected with pollen grain water extract and metformin while sixth group injected by mitomycin and gaved metformin with pollen grain water extract. The rats were anesthetized and the kidneys and liver were excised and removed. Sections of the tissue were extracted and the DNA was extracted using a special extraction kit (USA kit extraction DNA Genomic), Which was processed by the United States Geneoid Company and was extracted according to the company's instructions The concentration and purity of the extracted DNA was assessed using the Nanodrop device, and the three-octa4 series was tested (the first package is 120 bp and the second is 220 bp) and the third is the size of bp (410) a For forward sequence(5'CGACCTCCGTTCCTCTCCTC TATT-3')and reverse sequence(3'–AGACGCACAAACCAAAACAAAATTAC A-5') [19]. The reaction conditions using polymerase chain reaction (PCR) were as follows:

Table (1): Conditions of polymerase chain reaction of the oct-4 initiator.

Steps	Temperature	Time	Number of cycles
Initial denaturation	94 C°	7 min	1
Denaturation	94 C°	45 sec	35
Annealing	51 C°	1min	
Extension	72 C°	1min	
Final extension	72 C°	7 min	1

The PCR reaction products were separated by the migration of a sample from each sample to the 2% agrose gel as stated in Maniatis [20]. Lift the gel and was examined with a UV device and photographed for the purpose of study.

Results

In the present study, the expression of the oct4 transcription factor was studied in the rat treated with metformin and *C. papyrus.* The effect was observed on three rat organs viz. liver, kidney and testes.

The kidney, liver and testes of healthy animals showed amplification of three bands. They are about 120, 220 and 410bp Figure (1). While, animals treated with mitomycin showed amplification of only two bands as compared to healthy control Figure (2). The third band which is about 410bp is missing in kidney, liver and testis of mitomycin treated animals.

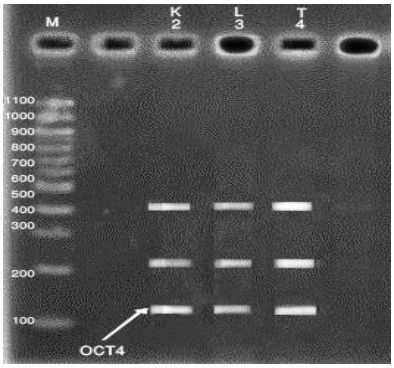

Figure (1): Control animals showed amplification of OCT 4 gene.

*K: Kidney, T: Testis, L: Liver, M: Marker (1Kbp)

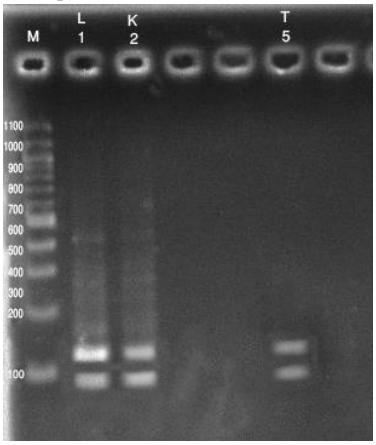

Figure (2): Amplification of OCT 4 gene in the mitomycin treated animals.

*K: Kidney, T: Testis, L: Liver, M: Marker (1Kbp)

Animals treated with mitomycin along with metformin and pollen grains showed no amplification of all band only two dimar bands Figure (3). The individual treatment of metformin and pollen grains does not recover the normal pathophysiology.

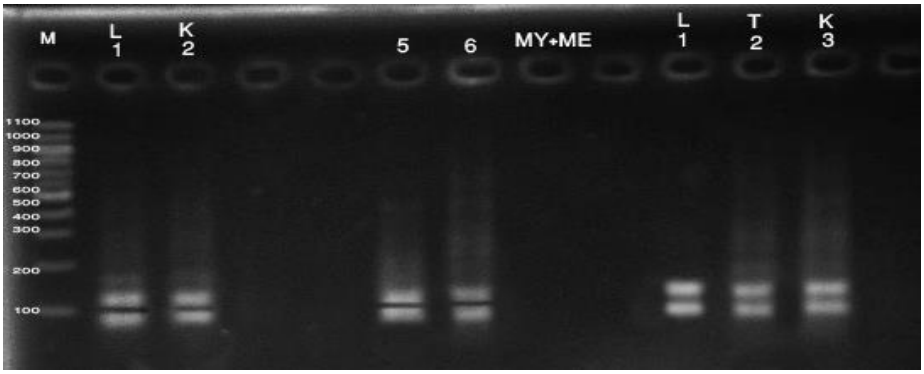

Figure (3): Amplification of OCT4 gene in animals treated with mitomycin along with metformin and pollen grains, respectively.

* P: Pollen grains, K: Kidney, T: Testis, L: Liver, M: Marker, MY: mitomycin, ME: metformin

Kidney and liver of animals treated with a combination of mitomycin, metformin, pollen grains showed amplification of all three bands (120, 220 and 410bp). The complex structure of the testis tissue may not have helped to introduce both metformin and pollen into the organ to protect it against the mitomycin C Figure (4).

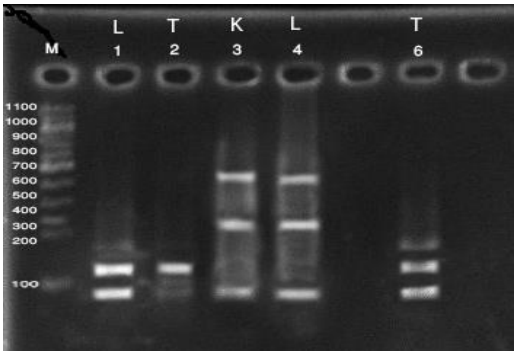

Figure (4): Amplification of OCT 4 gene in animals treated with a combination of mitomycin, metformin, pollen grains.

Discussion

Oct4 (encoded by Pou5f1) was first identified in mice as an ESC-specific and germline-specific transcription factor [21,22,23]. Oct4, a member of POU homeobox gene family, is a transcription factor capable of binding to an octameric consensus sequence to activate its target genes [11]. In humans, OCT4 is the product of the OTF3 gene, and three isoforms, OCT4A, OCT4B and OCT4B1, have been reported [24].

Oct4 dosage is playing a key role in the murine embryonic carcinoma (mES) cell fate determination. Oct 4 concentration decides the mES for maintaining their pluripotency or towards differentiate lineages viz. trophoblast, primitive endodermal, mesodermal [5,25,26]. Several reports are

available about the Oct 4 gene regarding its role in the cell proliferation [5,22,23,24,26]. The results showed the presence of three packages of the gene under study (the first package is 120 bp and 220 bp) and the third was the size of bp (410) in both the kidney and liver tissue samples of the standard sample as in Figure (1).However, the absence of octa4 gene band does not appear in the rats genome ,the three bands that appeared in the standard samples are genetically modified, and the presence or absence there will be confirmed as evidence of the availability of both pollen extract and metformin in the preventio of mitomycin damage . The Figure (2) showed the treatment with mitomycin, the disappearance of the three band in each of the DNA samples of the kidneys and liver. This indicates a a damage in the genome, which led to the difficulty of knowing the initiator under study to the complementary areas of the gene. This is required. Mitomycin is used to cause this damage. High for this property and used [27],. The third group, which was injected with mitomycin and gaved pollen grain water extract, We note the inability of the pollen to prevent of damage to the gene. The results were similar in the group treated with mitomycin and metformin because metformin had no ability to prevent damage in each of the two organs under study Figure (4). While some studies indicated that metformin was able to prevent and repair damage His DNA [28]. This may be due to the inability of both kidney and liver tissue to metabolize metformin [29]. The fifth group, which was injected with mitomycin and stimulated with metformin, in addition to the water extract of pollen. This treatment showed a good prevent of the gene manifested by the re-emergence of the three bands as in the standard sample result. This confirms the enhancement of the pollen interaction between the pollen and metformin. This may be due to the fact that pollen contains effective anti-oxidant agents for pollen that can be diagnosed by containing substances that act as Scavenger free radicals and as inhibitors of lipid peroxidation. These substances interact with the free root of the compound (DPPH) 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reduce its effectiveness as a result of -blocking the free radicals of this compound .

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