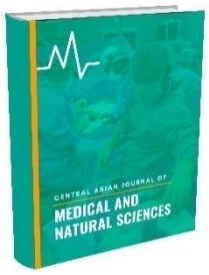
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 05 Issue: 01 | Jan-Feb 2024 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

LIVER TISSUE DAMAGE BY EFFECT OF TEMOZOLOMIDE DRUG

1. Saif Salah Abdul Hassan

Received 20th Nov 2023,
Accepted 28th Dec 2023,
Online 31st Jan 2024

[1] Department of Biology, College of Basic Education, Al-Mustansiriya University, Baghdad, Iraq

Abstract: The new drug, Temozolomide, has been promising in the treatment of malignant gliomas and other tumours. Temozolomide is a new class of imidazotetrazine second-generation medications spontaneously converted into the active alkylating agent under physiological conditions. Use 30 of male albino rats for this purpose, divided into 3 groups, consisting of 10 rats in each group. A physiologic solution (normal saline 0.9 percent) was injected in Group 1 (controls), the drug was given to group 2 for 50 mg/kg/b.w/weekly and drug was received in group 3 for 12 weeks, with 80 mg/kg/b.w/weekly. The animals were killed at the end of the dose; the liver was excised for histologica. Histological exam of Temozolomide group liver tissues showed that histopathological changes increased with the increased dose of the treatments compared with the control group such as shows chronic hepatic edema around blood vessels with fibrosis, As well as there is chronic hepatic edema around blood vessels with fibrosis. Hepatocyte necrosis with ballooning degeneration in hepatocyte. Analysis of DNA damage through the use of the comet assessment kit. This result of comet assay showed that damage in DNA of liver cells at 12 weeks of treatment with Temozolomide different doses in four parameters comet high, comet medium, comet low, and no damage. With 50 mg/kg and 75 mg/kg of Temozolomide groups increase in comet high, comet medium percentage, decrease in comet low, and no damage percentage. The result of the present study indicated that 50 mg/kg/b.w. and 75 mg/kg/b.w. doses of Temozolomide able to induce Male albino rats' DNA liver damage.

Key words: Liver, Histopathology, Temozolomide, Comet assay.

INTRODUCTION

Malignant gliomas (multiform and anaplastic astrocytoma), with a combined incidence of 5–8/100,000 population, occur more frequently than other primary central nervous system tubular types. The median reported survival of less than 1 year even in aggressive treatments with surgery, radiation and chemotherapy (Avgeropoulos, 1999). The new drug

Temozolomide, has been showing promise in the treatment of cancers gliomas and other tumours. Temozolomide is a new class of imidazotetrazin treatments of second generation, spontaneous conversion to the active alkylating agent under physiological conditions. (Stevens, 1987).

Temozolomide as an antitumorous agent is a repercussion of its wide-spectrum activity in tumor models in mouse (Stevens, 1987). The Temozolomide program showed in vitro a variety of malignancies such as glioma, metastatic melanoma and other cancers that are difficult to treat (Plowman, 1994). Temozolomide was shown to be distributed in all tissues, including penetration in preclinical studies the central nervous system; Temozolomide as an antitumorous agent is a consequence of its wide-spectrum activity in tumor models in mouse (Stevens, 1987). The Temozolomide program showed in vitro a variety of malignancies such as glioma, metastatic melanoma and other cancers that are difficult to treat (Plowman, 1994). Temozolomide was shown to be distributed in all tissues, including penetration in preclinical studies.

Temozolomide was synthesised as one of a number of new treatment at Aston University in the early 1980s (Stevens, 1984). They are structurally unique because they contain three adjacent atoms of nitrogen, which convey unique physical and antitumoral properties much higher than previously synthesized bicyclic triazenes, which contain two adjacent nitrogen atoms (Stevens, 1984).

This class of anti-tumor compounds, mitozolomide, had the most effective anti-tumor activity in a large panel of murine tumors (Hickman, 1985). Mitozolomide is a prodrug that spontaneously disintegrates to a highly reactive metabolite with the cross-linking DNA (Bull, 1987).

Temozolomide's cytotoxic mechanism seems to be linked to the failure to find a supplemented basis for methylated guanine by the DNA malapert system. This system involves the formation of a protein complex that identifies, binds and removes methyl guanine (Drummond, 1995). The proposed hypothesis is that when this DNA-strand repair process is targeted against O6-methylguanine, it cannot find the right partner, which results in long-lasting DNA nicks (karan, 1993). These nicks accumulate and persist in the preceeding cell cycle, where they inhibit replication in the cells of the daughter, which eventually blocks the cell cycle (karan, 1992). Tempozolomide sensitivity correlates with increasing fragmentation of DNA and apoptotic cell death in both murine (Taverana 1992) and human (Tentori 1995) leukemia cells. In addition to the cause of cell death, preclinical studies have demonstrated that TemoZolomide adduct DNA and the subsequent alteration and metastatic potential of tumor cell products in particular genes can be reduced by altering tumor cell immunogensity and their cognate protein products (Bianchi, 1992). The definite problem of a high-dose treatment monotherapy (postradiotherapy) with doses ranging from 150 to 200 mg/m2, while a dose of 75 mg/m2 has been defined for concomitant radiotherapy treatment. Temozolomide-related hepatotoxicity in the latter environment is still undefined (Goldbecker, 2011). The aims of this research was to identify the effect on histological structure and nucleic acid function of the liver on different doses of tempozoloamide.

MATERIALS AND METHODS

Animals and housing

Thirty male albino rats (Rattus norvegicus) with 230-260g body weight and age 4 – 6 months used in this study. It was purchased from animal house unite, higher institution for the diagnosis of infertility

and assisted reproduction techniques Uruk University, Baghdad, Iraq and housed of the animal house. They kept in plastic cages with a metal network cover under standard laboratory conditions. Rats given water and food ad libitum. Cages cleaned and sterilized weekly.

Preparation of Temozoloamide drug

Injecting 50 mg/kg body weight/week and 75 mg/kg of body was produced of Temozoloamid (international company U.S. Baxter) Injection.

Animals groups were intraperitoneal injected with a single dose of Temozoloamide per week for 12 weeks. group1 (control) received normal saline, group 2 received 50 mg/kg body weight /week and group 3 received 75 mg/kg body weight /week.

After dosages the rats were dissected, the liver was removed and put into a fixator solution for histological examination from each animal.

Histological examination

Histological study the histological sections performed on standard methods according to (Suvarna et al., 2013) as follows:

Protocol to the Paraffin Section

1. Fix tissues dissected at room temperature with 10% formalin for 24 hours.
2. To remove formaldehyde, rinse your tissue with tap water for 30 to 40 min.
3. The following order is used to dehydrate the tissues with ethanol: 70%, 80%, 90%, 95% and the 100% ethanol.
4. Three times clear the tissue with xylene.
5. Samples of infiltration place in xylene and liquid paraffin wax at a ratio of 1:1 at a temperature of 60°C for 30 min, then left for paraffin wax by two changes 30 min per changes.
6. Melt the paraffin wax in oven 60 °C to adding the tissue.
7. Pour melted paraffin into paraffin metal brass mold.
8. Wait for its cooling (15 min) and then transfer to refrigerator.
9. Sect the blocks by the microtome in a 4-5 micronutrient thickness and float in a distilled water bath of 50°C.
10. After one drop of Mayer's albumin (albumin and glycerine 1:1) is put on slide, float the sections on to clean slides and spread them over 10 min., and then the hot plate will bind the tissue to the slide.
11. Slides may be stored at room temperature overnight or used immediately in histopathological stain.

Protocol for Hematoxylin and Eosin Staining:

1. Removing paraffin on a hot plate for 20 minutes at 60 °C, and then moving through three xylene changes to be dewaxed in the electrical oven for 10 minutes.
2. Rehydration, passing through various ethanol alcohol concentration levels 100% for 5 minutes, 90% 80% and 70% for 30 sec, then washing in tap water for 2 minutes.
3. Hematoxylin and Eosin (H&E) (hematoxyl tin for 3–5 minutes, rinsing for 2-3 minutes with running tap water, staining for 30 seconds with eosin stain and dipping for 2 min. in tap water).
4. Dehydrating by passing slides through gradual ethanol alcohol levels of 70%, 80%, 90% for 30 sec and 100% for 5 min.

5. Clear by two xylene changes for 20 minutes per change.
6. Place a drop of Canada balsam on the slide by cover slides, so as not to leave any bubble. Add the cover, drop on the slide and hold it dry at room temperature.
7. Under light microscopic (40 X Lens) microscopic examination sections and pictures of the microscope taken immediately via camera.

Comet test

DNA damage was assessed by the comet. The kit for the comet test (Trevigen) was used to perform the test according to (De Boeck et al., 2000). The way of quantifying was done by using image analysis software comet score, the analysis software calculated different parameters for each comet. Fifty randomly selected cells of hepatocytes were counted per sample to quantify the comet cell.

The result is calculated from the comet ratio to calculate the Comet Index (CI). The range was 1.2 to 2 with low damage to DNA (LD), 2.1 to 3 with medium damage to DNA (MD) and up to 3 with high damage to DNA (HD) accordingly (Al-Jewari, 2010).

Tissue Preparation

Blood rich organs (liver), chopped tissue into large pieces (1-2 mm), let settle for 5 minutes then aspirated and discarded medium. Ice cold 1-2 ml and 20 mM EDTA was add added with 1X PBS and the fabric was cut into very small pieces (Ca++ and Mg++ free) and 5 minutes left to stand. The cell suspension has been removed, thus preventing debris transfer.2.

Alkaline Comet test

1. Lysis Solution prepared and cooled at 4°C for at least 20 minutes before use.
2. LM Agarose melted for 5 minutes, loosening the cap, in a boiling beaker of water. For a minimum of 20 minutes, the bottle is placed in the 37°C water bath to cool.
3. 1 x 105/ml of the cells combined with the molten LM Agarose (at 37°C) and at 1:10 (v/v), piping 50 µl on the Comet Slide at once. Agarose/cell spread across the sample area was used side of the pipette tip to ensure full sample coverage when the sample does not spread evenly. Before application, the slide heated to 37°C.
4. Flat slides set for 10 minutes in the dark at 4°C (e.g. in the freezer). On the edge of the slide area of the Comet was a 0.5 mm clear ring. (Enhance sample adherence in high humidity environments by increasing gelling time to 30 minutes).
5. Slides immersed for 30-60 minutes in 4°C lysis solution. It should be incubated overnight at 4°C for more sensitivity or convenience.
6. Excess tampon is drained from slides and immersed in the Alkaline Unwinding solution, freshly prepared, pH>13. Gloves were worn when this solution was prepared or handled.
7. Comet slide immersed in Alkaline Solution at room temperature for 20 minutes, or at 4°C for 1 hour in the darkness.
8. Sliding placed inside the brick slide (slide labeled adjacent to Black Cathode) and covered with slide Tray Overlay for Comet Assay Electrophoresis (ES-unit) ~850 mL 4°C Alkaline Electrophoresis Solution. The power was set to 21 volts and 30 minutes of voltage were applied.
9. Drained the excess electrophoresis solution, dipped twice in 5 minutes in DH2O, and then 5 minutes in 70% ethanol.

10. Dry samples for 10-15 minutes at a temperature of 37° C. Drying led to the observation of all cells in one plane. Samples stored at room temperature before scored at this stage with desiccant.
11. 100 µl diluted SYBR Green placed in the dark for 30 minutes (room temperature) on each circle of dried agarose. The slide has been carefully taped to remove excess SYBR solution and washed in water briefly. At 37°C the slides were fully dried.
12. Microscopy viewed diaphragms. 12. (The maximum excitement/emission of SYBR Green is 496nm. Appropriate fluorescein filter).

Statistical analysis

The Statistical Analysis System (2012) program used to determine the effect of different factors in study parameters. All the values statistically analyzed by analysis of variance (ANOVA) Least significant difference (LSD) test. Data expressed as the mean ± SD. $P \leq 0.05$ considered as significant difference (Rosner, 2010).

RESULTS

1. Histological Examination

Control group animals

The control animals show the normal liver with hematoxylin and eosin stain (Figure 1).

Animals treated with Temozolomide (50 mg/kg)

Animal infection Section in Temozolomide (50mg/kg b.w.) treated animal livershows chronic hepatic edema around blood vessels with fibrosis (Figure 2). As well as there is chronic hepatic edema around blood vessels with fibrosis (Figure 3). Cell fatty degeneration with macrophage and lymphocyte aggregation (Figure 4). Small multiple aggregation of mononuclear cell scatter in liver parenchyma with congested central vein (Figure 5).

Animals treated with Temozolomide (75 mg/kg)

Histological section in the liver of animal treated with Temozolomide (75mg/kg b.w.) shows hepatocyte fibrosis (Figure 6). As well as there, is massive congested of blood with acute hepatic edema (Figure 7). Hepatocyte necrosis with ballooning degeneration in hepatocyte (Figure 8). Hepatocyte necrosis (Figure 9).

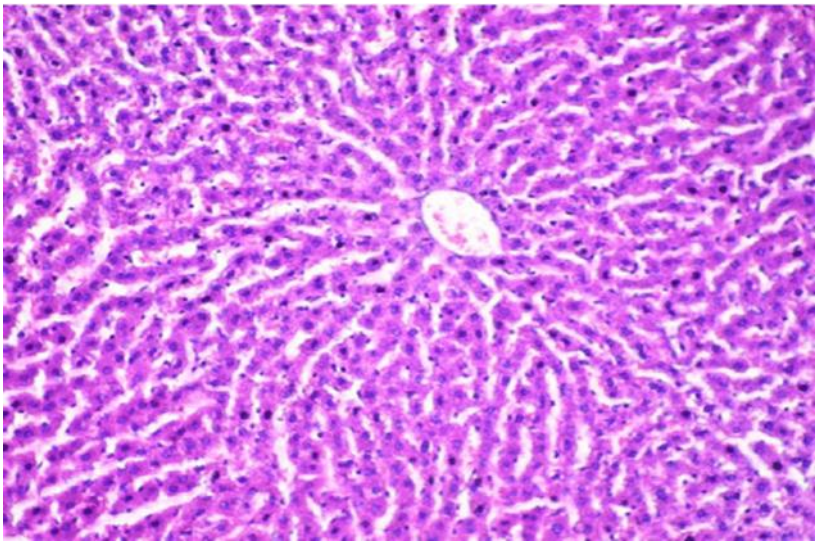


Figure 1: The control animals show the normal liver with hematoxylin and eosin stain

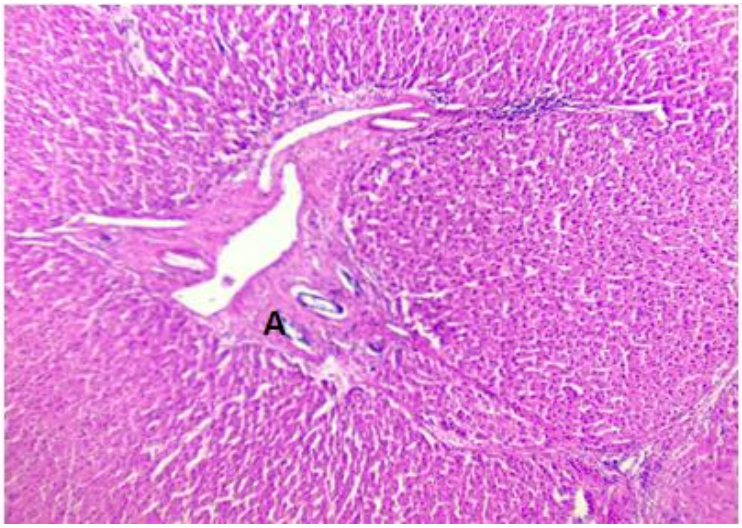


Figure 2: Cross-section in the liver treated with Temozolomide (50mg/kg b.w) show chronic hepatic edema around blood vessels with fibrosis (A) (H&E 40X).

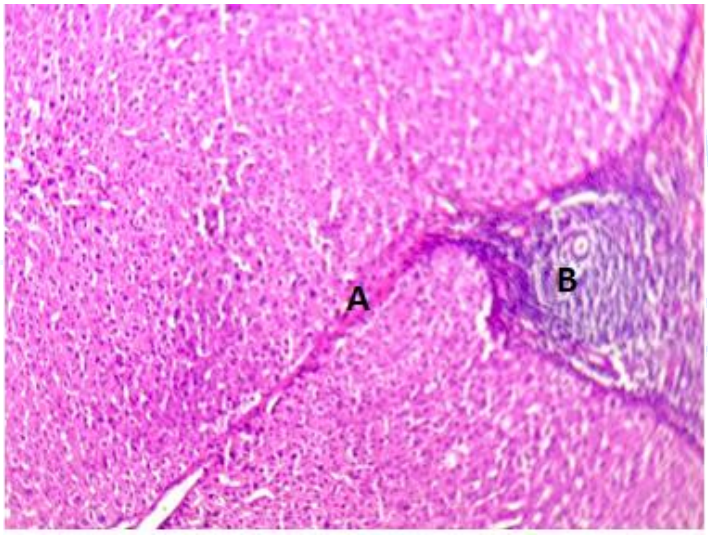


Figure 3: Cross-section in the liver treated with Temozolomide (50mg/kg b.w) show chronic hepatic edema around blood vessels with fibrosis (A, B) (H&E 40X).

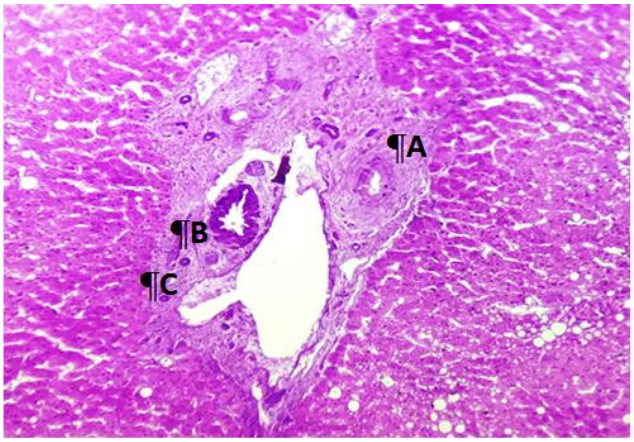


Figure 4: Cross-section in the liver treated with Temozolomide (50 mg/kg b.w) show cell fatty degeneration (A) with macrophage and lymphocyte aggregation (B, C) (H&E 40X).

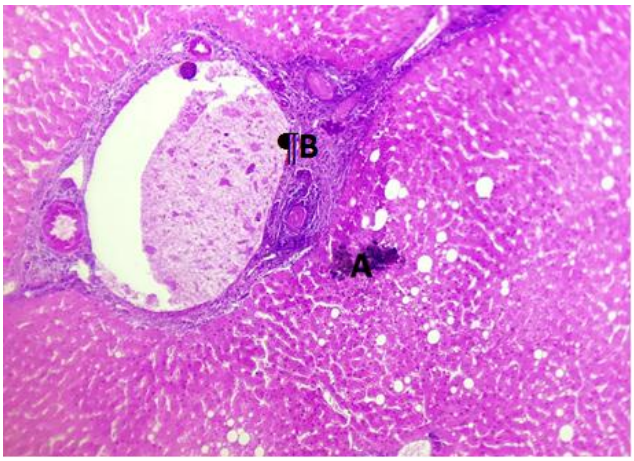


Figure 5: Cross-section of the liver treated with Temozolomide (50 mg/kg b.w.) shows the congested central vein (B) in the liver parenchyma (A) with small multiple aggregations of mononuclear cell scatter (H&E 40X).

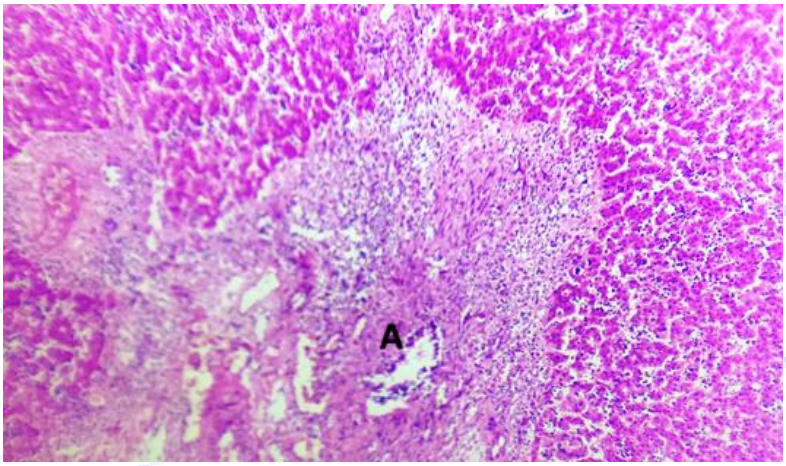


Figure 6: Cross-section in the liver treated with Temozolomide (75 mg/kg b.w) show hepatocyte fibrosis (A) (H&E 40X).

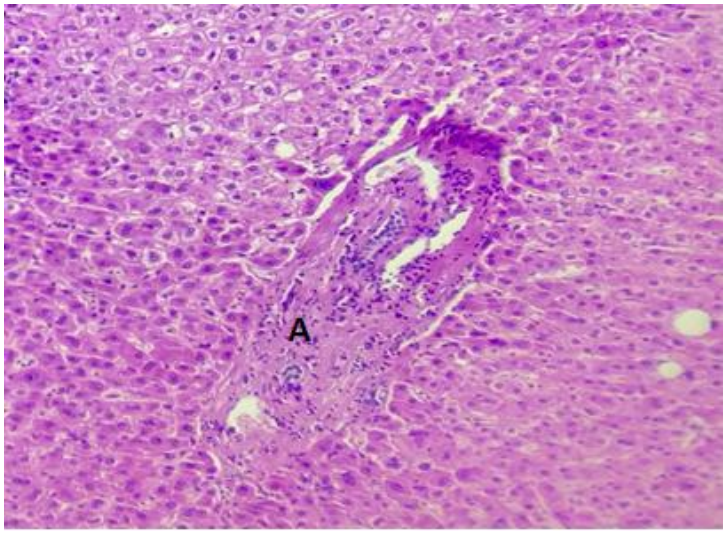


Figure 7: Cross section in the liver treated with Temozolomide (75 mg/kg b.w) show massive congested of blood with acute hepatic edema (A) (H&E 40X).

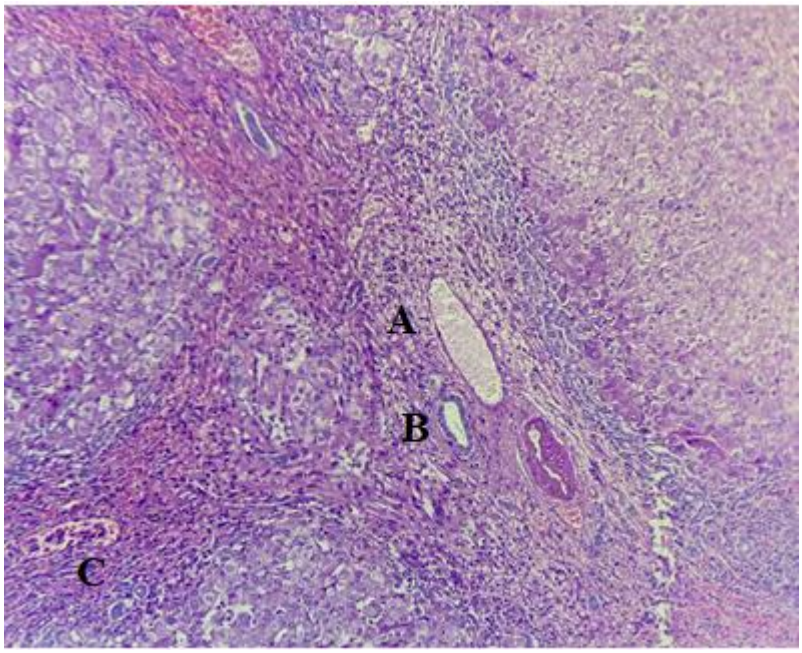


Figure 8: Cross section in the liver treated with Temozolomide (75 mg/kg b.w) show hepatocyte necrosis (A, B) with ballooning degeneration in hepatocyte (C) (H&E 40X).

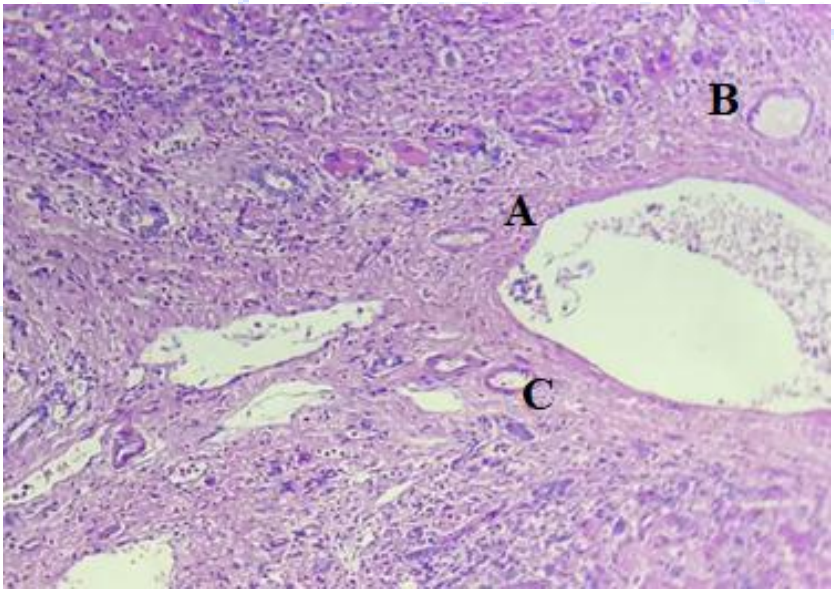


Figure 9: Cross section in the liver treated with Temozolomide (75 mg/kg b.w)

the present study in table (1) showed that damage in DNA of liver cells at 12 weeks of treatment with Temozoloamide high and low dose in four parameters comet high, comet medium, comet low, and no damage.

Comet high percentage

The results show significant increase in comet high of Temozolomide (50 mg/kg) group was (21.26) % and Temozoloamide (75 mg/kg) group was (34.10) % compared with control group was (9.67) %. (table1) (Figure 12)

Comet medium percentage

The results show significant increase in comet medium of Temozolomide (50 mg/kg) group was (27.74) % and Temozoloamide (75 mg/kg) group was (29.60) % compared with control group was (11.65) %. (table1) (Figure 11).

Comet low percentage

The results show significant decrease in comet low of Temozolomide (50 mg/kg) group was (26.86) % and Temozoloamide (75 mg/kg) group was (18.38) % compared with control group was (32.01) %. (table1) (Figure 11).

Comet low percentage

The results show significant decrease in comet low of Temozolomide (50 mg/kg b.w.) group was (24.13) % and Temozoloamide (75 mg/kg b.w.) group was (17.82) % compared with control group was (46.06) %. (table1) (Figure 10).

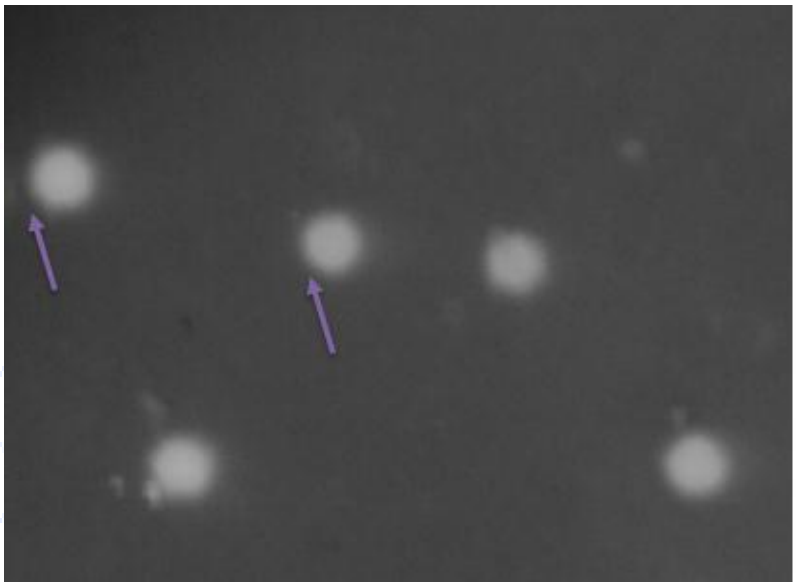


Figure 10: scoring of the comet assay no damage in hepatocytes cell of control animals.

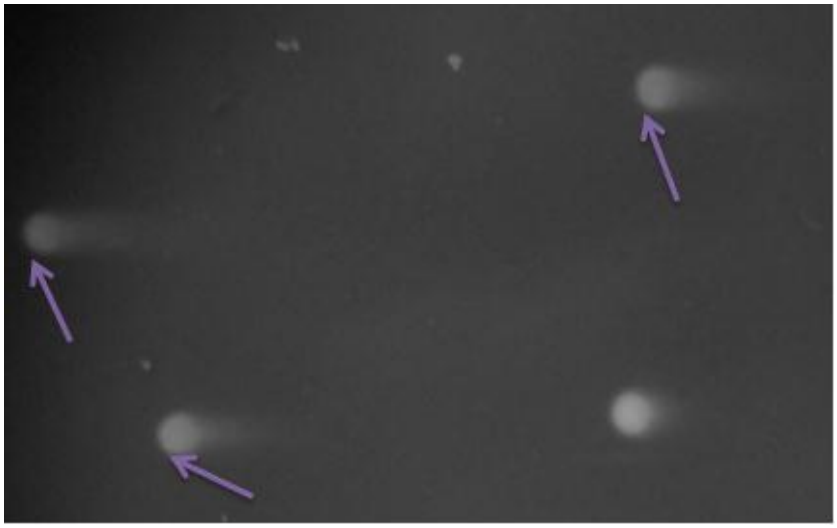


Figure 11: scoring of the comet assay low damage and medium damage in hepatocytes cell of animals treated with Temozolomide (50 mg/kg b.w)

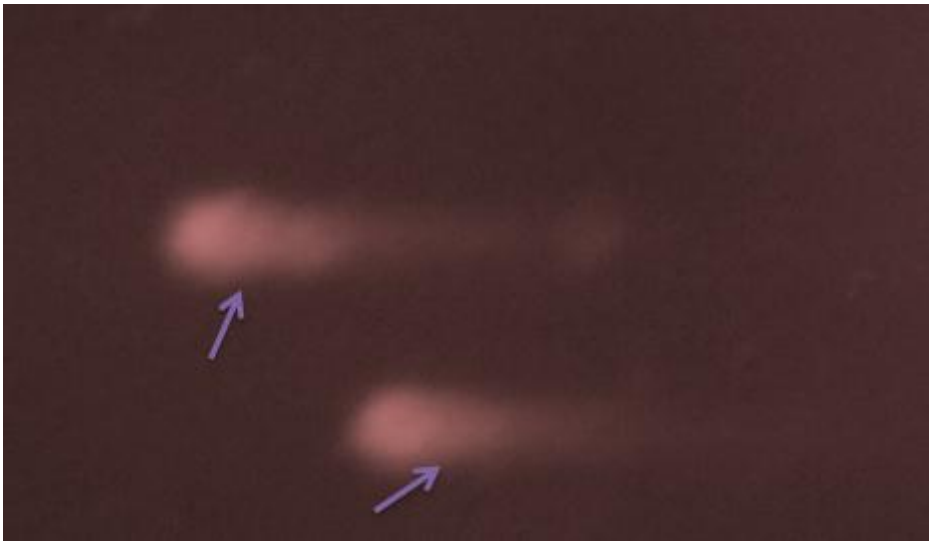


Figure 12: scoring of the comet assay high damage in hepatocytes cell of animals treated with Temozolomide (75 mg/kg b.w).

Table 1: Effect of Temozoloamide on DNA of hepatocytes.

Groups	Mean±SD			
	No damage %	**Low damage %**	**Medium damage %**	**High damage %**
Control	A 46.06±3.52	A 32.61±1.18	C 11.65±3.62	C 9.67±1.14
(50mg/kg)	B 24.13±2.03	B 26.86±1.76	B 27.74±8.60	B 21.26±2.20
(75mg/kg)	C 17.82±1.40	C 18.38±1.64	A 29.60±6.67	A 34.10±2.56
LSD	**3.07**	**4.09**	**2.33**	**3.25**
Different letters: Significant difference between groups.				

DISCUSSION

Histological examination

This result in the present study shows noticeable distortion in the architecture in the liver of animals treated with different doses of temozolomide. The histological section in the liver of animal treated with Temozoloamide (50mg/kg b.w.) appear low damage and animal treated with Temozoloamide (75 mg/kg b.w.) appear high damage in tissue of liver compare with control animals.

We report a case of serious cholesterol hepatitis in a glioblastoma-affected patient treated with the standard dose of temozolomide. Pursuant to the present guidelines on drug-induced liver injury management and the diagnosis (Tajiri, 2008) The absence and the absence of other medications in the temporal relationship with the beginning of the treatment are a likely cause of the observed hepatic injury to temozolomide.

Liver toxicity is currently under debate. While (Newands, 1997) could not find a liver metabolism of temozolomide, liver affections reported in literature (Grewal, 2007). In one case, temozolomide applied together with valproic acid; in the two other cases, a viral hepatitis reactivated. We have seen two patients with liver toxicity, which led to interruption of chemotherapy, reactivation of hepatitis.

Adverse event reporting system and seven temozolomide-induced liver injuries were identified by the Food and Drug Administrator (FDA) but two cases involved other treatments that could potentially cause liver toxicity. Among other reported cases, Goldbecker seems to be the only case due to the absence of potential other treatments and the about two-month latency (Goldbecker, 2011). The role of medicines in interaction and the potential interaction with alternative and complementary medicines as recently reported must be discussed further (Melchardt. 2014).

Comet Assay

The results presented here show DNA damage analysis in liver of male albino rats treated with different doses of cyclophosphamide intravenously for 12 weeks by using the comet assay. In (50 mg/kg b.w.) dose group show significantly increase in comet high percentage (high damage) and the comet medium percentage (medium damage) and show significantly decrease in comet low percentage (low damage) and no damage percentage in the DNA of the liver compared with the control group. In (75 mg/kg b.w.) dose group show, significantly increase in comet high and medium percentage, and show significantly decrease in comet low and no damage percentage in the DNA of the liver compared with control group.

Alkylating agents were some of the oldest chemotherapy treatments developed in the early 20th century as chemical weapons. Their anti-neoplastic aspects were examined only after the Second World War (Colvin, 2010). The production of DNA crosslinkages, inhibiting both DNA and cellular replication, acts as traditional alkylating agents. Similar drugs such as temozolomide are used to work with methylating DNA, which also lead to cell replication and inhibited DNA (Colvin, 2010). All of these agents are unspecific and affect cancer cells as well as normal cells. But cancer cells divide faster than normal tissues and should therefore be more sensitive to these effects (Colvin, 2010).

A DNA damage, such as single or double beach brisk are known to cause chemical agents such as alkylating agents, heavy metals, antimetabolites, cytotoxic and topoisomerase DNA inhibitors (Losa, 2016) and DNA Protein Reparation Inhibitors can be combined to overcome such resistance (Puigvert, 2016). Temozolomide (TMZ) is an imidazotetrazine converted to an alkylating DNA compound. In physiological pH and degrade to methyldiazonium cation TMZ (5-3-(methyltriazen-1-yl imidaszole-4-carboximide) is chemically converted to MTIC that deliver methyl to the purine bases of the DNA and thus stop DNA replication and result in cytotoxicity of cells that propagate (Zhang, 2012).

The action of methylation due to the TMZ was suggested at N7, adenine, O3, and guanine (Bobola, 2015) but its defined role is still open for discussion in cancer cytotoxicity. There is a correlation between tumor cell line sensitivity to TMZ and activation of the DNA reparation protein when certain carcinomas are involved (Bobola, 2015).

CONCLUSION

The results of this study indicated that the dose of Temozolomide in the liver of male albino rats is 50 mg/kg/ and 75 mg/kg Owing to the more harmful effects on liver tissue and DNA. 75 mg/kg dose of TMZ should be not used because of its more negative effects on the liver tissue and DNA.

RECOMMENDATION

Study the effect of Temozoloamide on various body organ at different doses and investigate histological tissue changes.

REFERENCES

1. Al-Jewari, H.S.J. (2010). In vitro Cytotoxic Activity of the L-aspara ginase Extracted and Purified from Pathogenic Escherichia coliagainst four eukemic cell lines. PhD Thesis, Genetic Engineering Biotechnology for Post Graduate Studies, University of Baghdad, Iraq

2. Avgeropoulos, N. G., and Batchelor, T. T. (1999). New treatment strategies for malignant gliomas. Oncologist, 4: 209–224,

3. Bianchi, R., Citti, L., Beghetti, R., Romani, L., D'Incalci, M., Puccetti, P., and Fioretti, M. C. (1992). O6 -Methylguanine-DNA methyltransferase activity and induction of novel immunogenicity in murine tumor cells treated with methylating agents. Cancer Chemother. Pharmacol., 29: 277–282,

4. Bobola, M. S., M. Alnoor, J. Y. Chen, D. D. Kolstoe, D. L. Silbergeld, R. C. Rostomily, et al. (2015). O 6-methylguanine-DNA methyltransferase activity is associated with response to alkylating agent therapy and with MGMT promoter methylation in glioblastoma and anaplastic glioma. BBA Clin. 30:1.

5. Bull, V. L., and Tisdale, M. J. (1987). Antitumour imidazotetrazines-XVI macromolecular alkylation by 3-substituted imidazotetrazinones. Biochem. Pharmacol., 36: 3215–3220,

6. Colvin M. (2010). Alkylating agents and platinum antitumor compounds. In: Kufe DW, Frei E, Holland JF, et al, eds. Holland-Frei Cancer Medicine. 8th ed. Shelton, Connecticut: People's Medical Publishing House.

7. De Boeck, M., Touil, N., De Visscher, G., Vande, P.A. and Kirsch– Volders, M. (2000). Validation and implementation of an internal standard in comet assay. Mutat.Res.469, 181-197.

8. Drummond, J. T., Li, G. M., Longley, M. J., and Modrich, P. (1995). Isolation of an hMSH2–p160 heterodimer that restores DNA mismatch repair to tumor cells. Science (Washington, DC), 268: 1909–1912,

9. Goldbecker A, Tryc AB, Raab P, et al. (2011); Hepatic encephalopathy after treatment with temozolomide. J Neurooncol. 103:163–166.

10. Goldbecker A, Tryc AB, Raab P, et al. (2011); Hepatic encephalopathy after treatment with temozolomide. J Neurooncol. 103:163–166.

11. Grewal J, Dellinger CA, Yung WK. (2007), Fatal reactivation of hepatitis B with temozolomide. N Engl J Med 356:1591-1592.

12. Hickman, J. A., Stevens, M. F. G., Gibson, N. W., Langdon, S. P., Fizames, C., Lavelle, F., Atassi, G., Lunt, E., and Tilson, R. M. (1985). Experimental antitumor activity against murine tumor model systems of 8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)- one (mitozolomide), a novel broad-spectrum agent. Cancer Res., 45: 3008–3013,

13. Karran, P., and Bignami, M. (1992). Self-destruction and tolerance in resistance of mammalian cells to alkylation damage. Nucleic Acids Res., 20: 2933–2940,

14. Karran, P., Macpherson, P., Ceccotti, S., Dogliotti, E., Griffin, S., and Bignami, M. (1993). O6 -methylguanine residues elicit DNA repair synthesis by human cell extracts. J. Biol. Chem., 268: 15878–15886,

15. Losa, M., F. Bogazzi, S. Cannavo, F. Ceccato, L. Curtò, L. De Marinis, et al. (2016). Temozolomide therapy in patients with aggressive pituitary adenomas or carcinomas. J. Neurooncol. 26:519–525.

16. Melchardt T, Magnes T, Weiss L, et al. (2014); Liver toxicity during temozolomide chemotherapy caused by Chinese herbs. BMC Complement Altern Med. 14:115.

17. Newlands ES, Stevens MF, Wedge SR, Wheelhouse RT, Brock C: (1997). Temozolomide: a review of its discovery, chemical properties, preclinical development and clinical trials. Cancer Treat Rev 23:35-61.

18. Patel, M., McCully, C., Godwin, K., and Balis, F. (1995). Plasma and cerebrospinal fluid pharmacokinetics of temozolomide. Proc. Am. Soc. Clin. Oncol., 14: 461,

19. Plowman, J., Waud, W. R., Koutsoukos, A. D., Rubinstein, L. V., Moore, T. D., and Grever, M. R. (1994). Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res., 54: 3793–3799,

20. Puigvert, J. C., K. Sanjiv, and T. Helleday. (2016). Targeting DNA repair, DNA metabolism and replication stress as anti-cancer strategies. FEBS J. 283:232–245.

21. Rosner, B. (2010). Fundamentals of Biostatistics; Brooks/cole / cengage learning. Inc. Boston, USA.

22. Stevens, M. F. G., Hickman, J. A., Langdon, S. P., Chubb, D., Vickers, L., Stone, R., Baig, G., Goddard, C., Gibson, N. W., Slack, J. A., Newton, C., Lunt, E., Fizames, C., and Lavelle, F. (1987). Antitumor activity and pharmacokinetics in mice of 8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (CCRG 81045: M & B39831), a novel drug with potential as an alternative to dacarbazine. Cancer Res., 47: 5846–5852,

23. Stevens, M. F. G., Hickman, J. A., Stone, R., Gibson, N. W., Baig, G. U., Lunt, E., and Newton, C. G. (1984). Antitumor imidazotetrazines. 1. Synthesis and chemistry of 8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one, a novel broad-spectrum antitumor agent. J. Med. Chem., 27: 196–201,

24. Suvarna, S.K., Layton, C. and Bancroft, J.D. (2013). Bancroft theory and practice of histological techniques.7th ed. Elsevier Lim, China.

25. Tajiri K, Shimizu Y. (2008). Practical guidelines for diagnosis and early management of drug-induced liver injury. World J Gastroenterol. 14:6774–6785.

26. Taverna, P., Catapano, C. V., Citti, L., Bonfanti, M., and D'Incalci, M. (1992). Influence of O6 -methylguanine on DNA damage and cytotoxicity of temozolomide in L1210 mouse leukemia sensitive and resistant to chloroethylnitrosoureas. Anticancer Drugs, 3: 401–405,

27. Tentori, L., Graziani, G., Gilberti, S., Lacal, P. M., Bonmassar, E., and D'Atri, S. (1995). Triazene compounds induce apoptosis in O6 -alkylguanine-DNA alkyltransferase deficient leukemia cell lines. Leukemia, 9: 1888–1895,

28. Wedge, S. R., Porteous, J. K., and Newlands, E. S. (1997). Effect of single and multiple administration of an O6 -benzylguanine/temozolomide combination: an evaluation in a human melanoma xenograft model. Cancer Chemother. Pharmacol., 40: 266–272,

29. Zhang, J., M. F. Stevens, and T. D. Bradshaw. (2012). Temozolomide: mechanisms of action, repair and resistance. Curr. Mol. Pharmacol. 5:102–114.