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Research Article

Urinary 8-hydroxy-2'-deoxyguanosine and Creatinine levels in Confirmed and Suspected Substance abusers in a Nigerian Tertiary Institution

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ABSTRACT

Drug abuse, a patterned use of a drug in which the user consumes the substance in amounts or with methods which are harmful to themselves or others, is a form of substance-related disorder causing health and social problems, violence, suicides, physical dependence or psychological addiction. As drug abuse has been a cause of physiological or biochemical distortion, this work was primarily set to assess Oxidative genotoxicity and neuronal damage in confirmed and suspected substance abusers. This study included 71 subjects -21 were subjects that exhibited behaviors of drug users, tested and confirmed positive for illicit drugs, 30 were subjects that had stopped using drugs but still exhibited the behaviors of drug users, tested and confirmed negative for illicit drug. The remaining 20 subjects that did not have history of drug abuse served as control. Both 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and creatinine were estimated in urine of all subjects. and 8-OHdG was expressed per mg creatinine. The parameters were compared in all groups and gender. Student t-test was used in comparing means, correlation was also done at $p \leq 0.05$. 8OHdG and Protein S100B were significantly higher in both confirmed and suspected substance abusers compared to controls and also when confirmed were compared with suspected substance abusers. DNA and neuronal damage sequel to drug abuse could be as a result of the biochemical, physiological or pathologic conditions characteristic of drug abusers. This finding may help in designing better management of substance abuse.

Keywords: *Neuronal damage, Oxidative DNA damage, Substance abuse.*

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INTRODUCTION

Drug abuse is a patterned use of a drug in which the user consumes the substance in amounts or with methods which are harmful to themselves or others, and it is a form of substance-related disorder (McLellan, 2017). Drugs most often associated with this term include: alcohol, cannabis, barbiturates, benzodiazepines, cocaine, methaqualone, opioids and some substituted amphetamines. Drug abuse became a problem and example is the opioid crisis in the U.S.A. During 2015, there were 42,404 overdose deaths in the United States including 33,091 (63.1%) that involved an opioid. That is an average of 91 opioid overdoses each day. Experts say the United States is in the throes of an opioid epidemic, as more than 2 million of Americans have become dependent on abused prescription, pain pills and street drugs (Schalkoff *et al.,* 2020). Opioids are drugs formulated to replicate the pain reducing properties of opium. They include both legal painkillers like morphine; oxycodone prescribed by doctors for acute or chronic pain, as well as illegal drugs like heroin or illicitly made fentanyl (NIDA, 2021).

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) within a cell causing mutations, which may lead to cancer. Genotoxicity is often confused with mutagenicity; all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic. Genotoxins are mutagens; they can cause mutations. Genotoxins include both radiation and chemical genotoxins (Mohamed e*t al*., 2017). A substance that has the property of genotoxicity is known as a genotoxin. The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations. The permanent, heritable changes can affect either somatic cells of the organism or germ cells to be passed on to future generations (Ren *et al.,* 2017).

Cells prevent expression of the genotoxic mutation by either DNA repair or apoptosis; however, the damage may not always be fixed leading to mutagenesis. To assay for genotoxic molecules, researchers assay for DNA damage in cells exposed to the toxic substrates. This DNA damage can be in the form of single- and double-strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations, and structural and numerical chromosomal aberrations (Alhmoud *et al.,* 2020).

Oxidative DNA damage refers to the oxidation of specific bases. It occurs most readily at guanine residues due to the high oxidation potential of this base relative to cytosine, thymine, and adenine. Oxidative DNA damage is a major source of the mutation load in living organisms (Cadet *et al*., 2017). 8-hydroxy-2-deoxyguanosine (8-OHdG) is the most common marker for oxidative DNA damage and can be measured in virtually any species. It is formed and enhanced most often by chemical carcinogens. 8OHdG is formed through the oxidation of guanine at the C8 position in guanine base (Omari & Nasirzadeh, 2021). This oxidative DNA adduct has been detected in different tissues and urine and it is the most commonly used biomarker for oxidative DNA damage as well oxidative stress both in vitro and in vivo (Katerji *et al*., 2019). A variety of environmental agents have been reported to induce elevated levels of 8OHdG, including ionizing radiation; cigarette smoking; metals such as arsenic, iron, and cadmium; and organic chemicals such as carbon tetrachloride and chloroform (Marrocco *et al*., 2017). Elevated levels of 8OHdG have also been detected in some disease conditions, including diabetes, Parkinson's disease, Alzheimer's disease, and chronic hepatitis C infection (Hajam *et al*., 2022). A similar oxidative damage can occur in RNA with the formation of 8-OHG (8-hydroxyyguanosine), which has been implicated in various neurological disorders. DNA damage is an alteration in the chemical structure of DNA, such as a break in a strand of DNA, a base missing from the backbone of DNA, or a chemically changed base as 8-OHdG. DNA damage can occur naturally or via environmental factors (Alhmoud *et al*., 2020). The DNA damage response (DDR) is a complex signal transduction pathway which recognizes when DNA is damaged and influences cellular response to the damage. DNA damage is distinctly different from mutation, although both are types of error in DNA. DNA damage is an abnormal chemical structure in DNA, while a mutation is a change in the sequence of standard base pairs. DNA damages cause major changes in the structure of the genetic material and prevent the replication mechanism from functioning and performing properly (Kohler *et al*., 2016).

Serum protein-100β (S100β) is a 10.4 kDa protein (Sakdejayont *et al*., 2020). S100β is incorporated in the cerebrum through end feet cycles of astrocytes and it has a place with the superfamily of low sub-atomic weight EF-hand type acidic calcium-restricting proteins. This protein is processed in the kidneys as a matter of first importance and afterward released through urine (Zhou *et al*., 2016).

MATERIALS AND METHODS

Study Area: The study was carried out in an institution of higher learning in Ado-ekiti, Ekiti State. Ado-ekiti lies between longitude 7.6124° N latitude 5.2371° E. It has population of 424,340 (2012).

Study Design: A cross-sectional design using a stratified random sampling method was used. Stratification shall be by age, gender and type of drug abused.

Subjects: Group 1 (behavioral positive-drug positive subjects) consisted of 21 subjects that exhibited the behaviors of illicit drug users and have been tested and confirmed positive, In group 2 (behavioral positive-drug negative subjects) were 30 subjects that exhibited the behaviors of illicit drug users and have been tested and confirmed negative. The control: 20 subjects that does not have history of drug abuse serve as control subject. Individuals below the age of 14 were not used in this study.

Sample Collection: Timed or random urine collections was obtained from study subjects in universal bottle and stored at temperatures of -20 degree Celsius for a maximum of 3 days when it was used for the assay of 8-hydroxy-2-deoxyguanosine and creatinine. Urine is collected without added preservatives.

Ethical Clearance: Ethical approval was sought for, from Afe Babalola University Teaching Hospital, Ado Ekiti, Ekiti state. The nature and purpose of research was explained to each participant using an informed consent for literate participants and verbal explanation for illiterate participants. Participants were not forced to answer questions, but at their free will. The participants were assured of confidentiality and voluntary participation and that there is no financial benefit or whatsoever.

Biochemical assays:

8-Hydroxy-2-deoxyguanosine: 8-Hydroxy-2-deoxyguanosine (8OHdG) as a marker of oxidative DNA damage was estimated using standard sandwich enzyme linked immunosorbent assay (ELISA) technology. Briefly, specific 8OHdG polyclonal antibodies pre-coated onto the microwell plates and the enzyme labelled antibody and a serum containing native antigen was mixed to form a sandwich complex. After equilibrium is attained, the antibody bound fraction was separated from unbound antigen by decantation. The density of colour produced is proportional to the concentration of 8OHdG present in the sample captured in the plate. (Hnasko, 2016).

Creatinine: Creatinine was estimated spectrophotometrically using Jaffe assay method. (Murray, 1984) based on the fact that under alkaline conditions creatinine reacts directly with picric ions forming a reddish complex, the absorbance of which can be measured at 520 nm.

Statistical Analysis

Results obtained were subjected to statistical analysis using SPSS (version 21.0 software, SPSS Inc. Chicago, Illinois, USA). All parameters were expressed as mean ± SD. ANOVA

was the tool of choice in comparing means. Values were statistically significant at $p \leq 0.05$.

RESULTS

Gender and drug positivity distribution of all subjects.: Table 1 shows grouping, gender and drug positivity distribution of all subjects. There was a total of 71 subjects; 21subjects that exhibit the behaviors of drug users and have been tested and confirmed positive (group1), 30 subjects that exhibit the behaviors of drug users and have been tested and confirmed negative (group2) and, the remaining 20 subjects that does not have history of drug abuse serve as control subjects. The project consists of a total of 51 males and 20 females used in this research. Drug abuse is seen to have a higher frequency in males than in females.

Table 1.
Grouping, gender and drug positivity distribution of all subjects.

Variables	Positive drug abuse	Negative	Control
Population (male, female)	21 (15, 6)	30 (21, 9)	20 (15, 5)
Age (years)	19.17 ±0.31	18.88 ±0.96	19.31 ±0.33
Drug positivity			
THC	12		
OPI	7	NA	NA
BZO	2		
Drug positivity by gender			
THC	9 males, 3 females		
OPI	5 males, 2 females	NA	NA
BZO	2 males only		

THC= Tetrahydrocanabinol (Marijuana)
OPI= Opiates
BZO= Benzodiazepines

Frequency of abuse based on drug combinations: Figure 1 shows that frequency of abuse of Marijuana and Opioids (THC & OPI) is highest of all drug combinations followed by Opioid and Benzodiazepines (OPI & BZO) the least frequency of combination occurs in those on Marijuana, opioid and Benzodiazepines (THC, OPI & BZO).

Protein S100B and Urinary 8-hydroxy-2'-deoxyguanosine levels in response to specific drugs of abuse and gender: Abusers positive for THC had the highest levels of both S100Band Urinary 8-hydroxy-2'-deoxyguanosine levels while those positive for Benzodiazepines had the lowest. Those on Opiates having the intermediate S100Band Urinary 8-hydroxy-2'-deoxyguanosine levels. This research also found out that males have higher S100Band Urinary 8-hydroxy-2'-deoxyguanosinethan females (Figures 2 and 3).

Table 2 shows statistical comparison in both S100B and 8OHdG among all 3 groups. Both S100B and 8oHdG were significantly higher in both Group 1 and 2 compared to control and also when group 1 was compared to 2.

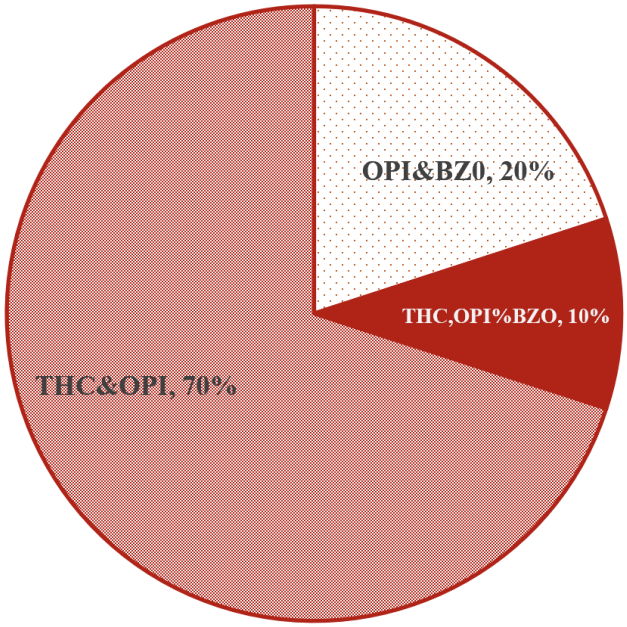

Figure 1.
Frequency of abuse based on drug combinations

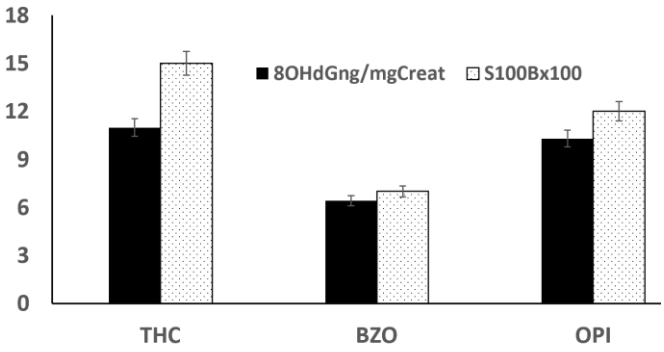

Figure 2;
8-hydroxy-deoxyguanosine:Creatinine ratio and S100B based on drug of abuse.

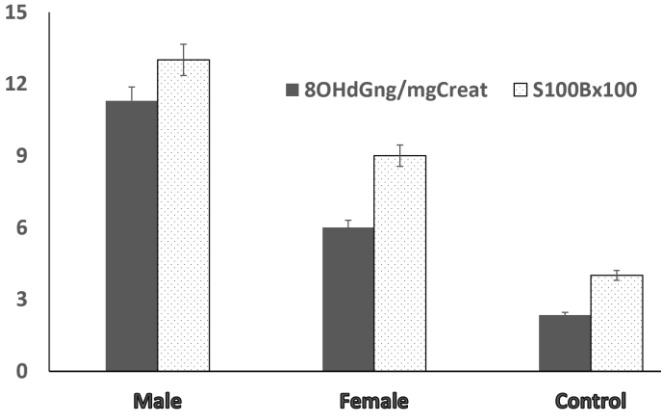

Figure 3;
8-hydroxy-deoxyguanosine:Creatinine ratio and S100B based on gender.

Table 2.
Mean ± SD, and p-value when all groups (Confirmed and Suspected Substance abusers and control) were compared one with another

Variable	Confirmed abusers (Mean ±SD)	Suspected abusers (Mean ±SD)	Control (Mean ±SD)
8OHdG (ng/mg creatinine)	10.20±6.20	5.23±4.37[a]	2.34±1.58[ab]
S100B	0.13+0.04	0.07+0.02[a]	0.03 +0.01[ab]

[a]*=statistically significant at P<0.05 compared to confirmed abusers*
[b]*=statistically significant at P<0.05 compared to suspected abusers*

DISCUSSION

Drug abuse is a patterned use of a drug in which the user consumes the substance in amounts or with methods which are harmful to themselves or others, and it is a form of substance-related disorder (Ksir *et al*., 2002). Drugs most often associated with this term include but not limited to: alcohol, cannabis, barbiturates, benzodiazepines, cocaine, methaqualone, opioids and some substituted amphetamines. Depending on the actual compound, drug abuse including alcohol may be the cause of health and social problems, injuries, unprotected sex, violence, homicides, suicides, physical dependence or psychological addiction (Burke *et al*., 2005). As drug abuse has been a cause of physiological or biochemical distortion (Isralowitz and Richard, 2004). This research was designed to assess DNA damage in young individuals who indulge in substance abuse.

8-hydroxy-deoxyguanosine (8-OHdG) is a sensitive marker of the DNA damage due to hydroxyl radical attack at the C8 position of guanine. This damage, if left unrepaired, has been proposed to contribute to mutagenicity and cancer promotion (Floyd *et al*., 1986). This is the most common marker for oxidative DNA damage and can be measured in virtually any species. It is formed and enhanced most often by chemical carcinogens. The expression for DNA damage is term of 8OHdG ng/ mg urinary creatinine. In this research, there was a higher significant level ($p<0.0001$) of oxidative DNA damage when group 1(behavioral positive-drug positive subject) and group 2 (behavioral positive-drug negative subjects) were compared with control. Furthermore, There was also a significant level ($p <0.05$) of oxidative DNA damage when group 1 (behavioral positive-drug positive subjects) was compared with group 2 (behavioral positive-drug negative subjects). This significantly higher oxidative DNA damage, despite testing negative to drugs of abuse could be a result of insincerity (George *et al*., 1995; Cone *et al*., 1998; Burrows *et al*., 2005) by some or all of these subjects in order to bypass the drug testing. This could further support the notion that high level of DNA damage is a characteristic of drug abuse. The frequency of drug abuse in this research shows that drug abuse is more in males than females. A finding that agrees with the works of Enakpoya. (2009) and Johnston *et al*. (1997) where it was postulated that boys are more likely to engage in drug abuse than girls.

Serum protein-100β (S100β) is a cytoplasmic calcium-binding protein mainly expressed by glia and considered to be a useful biomarker for brain or spinal cord injury (Mercier *et al*., 2013). In this research, S100β was significantly higher in both group 1 and group 2 subjects ($p=0.0169$) when compared with control. It was also significantly higher in group one compared to group 2S100β was also significantly higher in males than in females for group 1 but significantly higher in female than in male for group 2. This finding is in line with the works of (Abdel-Salam *et al*., 2019). in which increased S100β contributes to neuronal damage whereas a decrease in protein by drug abusers will result in decreased extent of neuronal damage. This could be as a result of a direct or indirect threat by some substances on the cells (neurons) of the nervous system (Donato and Heizmann, 2010). Tsai *et al*., had earlier stressed that at cellular or subcellular levels, some substances e.g. morphine and heroine are injurious or lethal to the existence of some cells or cellular components e.g. Nucleus, endoplasmic reticulum (Tsai *et al*., 2019). Thereby triggering the leakage of cellular contents or an initiation of cell lysis or apoptosis (Grotegut *et al*., 2020). This would later increase the concentration of neuronal proteins in body fluids (Michetti *et al*., 2012). It is however a little bit surprising that group 2 individuals (suspected drug abusers but tested negative) also have S100B in significant amounts in their plasma. The likely rationale could be as a result of the fact that substance abusers have invented various means of not being tested positive despite doping. Example of such means are by substitution, Adulteration, and or dilution of samples (Reardon & Creado, 2014). As a result of the findings above, any hypothesis that says neuronal damage is not be consequent to substance abuse is rejected. Consequently, the research question as to whether neuronal damage follows drug abuse can be answered as a Yes. However neuronal damage is most pronounced in those that tested positive for tetrahydrocannabinol (THC) an active or pharmacological component of Indian hemp or marijuana (cannabis sativa).

Abused substances under examination in this research include; Opioids (OPI), marijuana (THC) and, benzodiazepines (BZO). Of all these drugs, marijuana (THC) is most singly abused drug and the DNA damage was more pronounced among those taking it. Marijuana contains THC (tetrahydrocannabinol) and other substances that enter the system which further cause DNA damage. Although THC is a psychoactive drug from the Cannabis plant intended for medical or recreational use (Vij, 2012). Aside THC (tetrahydrocannabinol) which appears to be the cause of the highest DNA damage, there are about 3,500 harmful substances present in marijuana. THC (tetrahydrocannabinol) can last in the blood for more than 2 weeks after it is smoked (Pope *et al*., 2002; Choices, 2016). Its use is widespread among young people, boys are more likely than girls to smoke

or otherwise use marijuana (Johnston *et al*., 2015). Of all drug combinations, DNA damage was most pronounced in individuals where marijuana (THC) and Opioid (OPI) coexist. Marijuana contains THC (tetrahydrocannabinol) and other substances that enter the system causing an increased level of DNA damage. Then when combined with other illicit drugs such as opioid, leads to further elevation of DNA damage. Opioid is derived from opium and include the natural products morphine, codeine. They are any substance that produces morphine-like effects through action on opioid receptors (Offermanns and Stefan., 2008). Opioids are also frequently used non-medically for their euphoric effects or to prevent withdrawal (Lembke and Anna., 2016).

As DNA damage has been known to be a cause of teratogenic and carcinogenic mutations (Hsie *et al*., 1990; Wogan *et al*., 2004.), it may be surprising that cancers and mutations may not be evident in these individuals. This is made possible by a physiological process whereby DNA damage is reversed termed DNA repair. It is only when DNA damage exceeds the rate at which it is repaired that there will be a likelihood of development of cancer and other genetic diseases or abnormalities (Kastan and Bartek., 2004; Hegan *et al*., 2006; Bernstein *et al*., 2008). On the other hand, the inflammation and consequent damage to neurons could be as a result of the deleterious effects of adverse biochemical reaction, disruption of some cellular organelles and or mitochondrial and or nuclear DNA. The overall result is cell death, cytolysis or apoptosis.

In conclusion, the present study shows that DNA and neuronal damage is more pronounced as a result of drug abuse or illicit drug use, subjects who tested positive for THCs (tetrahydrocannabinol) mostly taken in form of cannabis being the drug associated with the highest level of oxidative damage to DNA and neuronal destruction.

REFERENCES

Bernstein H, Payne CM, Bernstein C, Garewal H, Dvorak K (2008). "Cancer and aging as consequences of un-repaired DNA damage". In Kimura H, Suzuki A. New Research on DNA Damage. New York: Nova Science Publishers. pp. 1–47. ISBN 978-1-60456-581-2.Archived from the original on 25 October 2014.

Burke PJ, O'Sullivan J, Vaughan BL (2005). Adolescent substance use: brief interventions by emergency care providers. *Pediatr Emerg Care*. **21** (11):pp.770–776.doi:10.1097/01.pec.0000186435.66838.b3.PMID 16280955.

Burrows DL, Nicolaides A, Rice PJ (2005). Papain: A novel urine adulterant. *J Anal Toxicol*.**29**:pp.275–95.

Choices NHS. (2016). How long does cannabis stay in the body after smoking? – Health questions - NHS Choices. Retrieved 2017-01-09.

Cone E J, Lange R, Darwin WD (1998). In vivo adulteration, excess fluid ingestion causes false-negative marijuana and cocaine urine test results, *J. Anal. Toxicol*. **22**: pp.460

Dizdaroglu, M (1985). Formation of an 8-hydroxyguanine moiety in deoxyribonucleic acid on gamma-irradiation in aqueous solution. Biochemistry **24**, pp. 4476–4481.

Enakpoya, E. (2009). Prevalence of drug abuse among Nigerian adolescents: Implication for Counseling. The Counsellor, 26 (2).

Floyd RA, Watson JJ, Wong PK, Altmiiller DH, Richard RC (1986). Hydroxyl free radical adduct of deoxyguanosine:sensitive detection and mechanisms of formation. *Free Radic Res Commun* **1**: pp.163-72.

George S, Braithwaite RA (1995). An investigation into the extent of possible dilution of specimens received for urinary drugs of abuse screening. *Addiction*. **90:pp**. 967-970.

Hegan DC, Narayanan L, Jirik FR, Edelmann W, Liskay RM, Glazer PM (2006). Differing patterns of genetic instability in mice deficient in the mismatch repair genes Pms2, Mlh1,Msh2, Msh3 and Msh6. *Carcinogenesis*. **27** (12): pp.2402–2408. doi:10.1093/carcin/bgl079.

Hnasko R. (2016): ELISA: Methods and Protocols. In Methods in Molecular Biology. Humana, New York, NY. p21

Hsie,A.W., Xu,Z.D., Yu,Y.J., Sognier,M.A. and Hrelia,P. (1990) Molecular analysis of reactive oxygen-species-induced mammalian gene mutation. *Teratogen. Carcinogen. Mutagen*. **10**. pp. 115–124.

Isralowitz, Richard (2004). Drug use: a reference handbook. Santa Barbara, Calif.: ABC-CLIO.pp. 122–123. ISBN 978-1-57607-708-5.

Johnston L D, P. M. O'Malley and J. G. Bachman. 1997 The Monitoring the Future Study (1975-1997). Monograph, Ann Arbor. University of Michigan. Institute for Social Research.

Johnston L, O'Malley P, Miech R, Bachman J, Schulenberg J. (2015): Monitoring the Future National Survey Results on Drug Use: 1975-2015: Overview: Key Findings on Adolescent Drug Use. Ann Arbor, MI: Institute for Social Research, The University of Michigan; 2015.

Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts II LJ, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP,Barrett JC (2005a). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radic. Biol. Med*. **38**, pp. 698–710.

Kakimoto M, Inoguchi T, Sonta T, Yu HY, Imamura M, Etoh T, Hashimoto T, Nawata H (2002). Accumulation of 8-hydroxy-2′-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats. Diabetes **51**, pp.1588–1595.

Kasai H, Tanooka H, Nishimura S (1984). Formation of 8-hydroxyguanine residues in DNA by X-irradiation. *Gann* **75**, pp. 1037–1039.

Kasprzak KS (2002). Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis. *Free Radic. Biol. Med*. **32**, pp. 958–967.

Kastan, M.B, Bartek, J (2004). Cell-cycle checkpoints and cancer. *Nature*, **432**. pp.316–323.

Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, Sato Y, Fujikawa K, Takahashi M, Takayama T, Ikeda T, Niitsu Y (2001). Normalization of

elevated hepatic 8-hydroxy-2′-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res*. **61**, pp. 8697–8702.

Köhler, Kerstin, Ferreira, Pedro, Pfander, Boris, Boos, Dominik (2016). The Initiation of DNA Replication in Eukaryotes. *Springer, Cham*. pp. 443–460. doi: 10.1007/978-3-319-24696-3_22. ISBN 9783319246949.

Kolle, Susanne (2012). Genotoxicity and Carcinogenicity. BASF the Chemical Company. Retrieved 2013-03-16.

Ksir, Oakley Ray, Charles (2002). *Drugs, society, and human behavior* (9th ed.). Boston [u.a.]: McGraw-Hill. ISBN 0072319631.

Lembke, Anna (2016). Drug Dealer, MD: How Doctors Were Duped, Patients Got Hooked, and Why It's So Hard to Stop. Johns Hopkins University Press. ISBN 978-1421421407.

Lu A L, Li X, Gu Y, Wright PM, Chang DY (2001). Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem. Biophys*. **35**, pp.141–170.

Moreira PI, Honda K, Liu Q, Santos MS, Oliveira CR, Aliev G, Nunomura A, Zhu X, Smith MA, Perry G (2005). Oxidative stress: the old enemy in Alzheimer's disease pathophysiology. *Curr. Alzheimer Res*. **2**, pp. 403–408.

Offermanns, Stefan (2008). Encyclopedia of Molecular Pharmacology. 1 (2 ed.). Springer Science & Business Media. pp. 903. ISBN 9783540389163

Owen AD, Schapira AH, Jenner P, Marsden CD (1996). Oxidative stress and Parkinson's disease. *Ann. NY Acad. Sci*. **786**, pp. 217–223.

Pope HG, Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Todd D (2002). Cognitive measures in long-term cannabis users. *J Clin Pharmacol*.**42**:pp. 415–475.

Vij (2012). Textbook Of Forensic Medicine And Toxicology: Principles And Practice. Elsevier India. pp. 672. ISBN 978-81-312-1129-8.

Wogan GN,Hecht SS, Felton JS, Conney AH, Loeb LA (2004). Environmental and chemical carcinogenesis. *Semin. Cancer Biol*. **14**, pp. 473–486.

Sakdejayont, S., Pruphetkaew, N., Chongphattararot, P., Nanphan, P., & Sathirapanya, P. (2020). Serum S100β as a predictor of severity and outcomes for mixed subtype acute ischaemic stroke. *Singapore Medical Journal.* 206-211.

Zhou, S., Bao, J., Wang, Y., & Pan, S. (2016). S100β as a biomarker for differential diagnosis of intracerebral hemorrhage and ischemic stroke. *Neurological Research.* **38**(4):327-332.

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