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Assessment of 8-Oxoguanine Glycosylase-1 (OGGI) and Nuclear Factor Kappa-Light Chain Enhancer (NF-κB) P65 Among Substance Abusers in Ado-Ekiti State, Nigeria.

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ABSTRACT
Background/Objectives. Drug misuse involves the unauthorized consumption of substances, wherein the individual ingests significant quantities of drugs or employs techniques that have the potential to endanger their own well-being or that of those in their vicinity. This study seeks to assess the concentrations of 8-Oxoguanine glycosylase (OGG1) and Nuclear Factor Kappa–light-chain-enhancer of activated B cells (NF-κB) p65 among individuals who engage in substance abuse in Ado-Ekiti, Ekiti State. **Method.** A total of 72 subjects comprising of 21 behavioral positive-drug negative subjects, 30 behavioral positive-drug negative and 21 control subjects were recruited for this study. Both NF-κB and OGG1 were estimated using enzyme linked immunosorbent assay (ELISA). The results were presented in tables and chat as mean ± standard deviation. Statistical analysis was done using one way analysis of variance (ANOVA) and Student's t-test using SPSS software. A p-values <0.05 was considered significant. **Results**. The results obtained showed that the mean ± SD of NF-κB in behavioral positive-drug positive, behavioral positive-drug negative and control group was 11.25±1.03, 6.05±2.12 and 5.49±2.93, while OGG1 was 4.93±0.70, 5.59±5.33 and 8.25±1.03 respectively. NF-κB and OGG1 were significantly higher in behavioral positive-drug positive subjects and behavioral positive-drug negative compared to controls (p=0.000), and also when group 1 was compared with group 2 (p=0.000). **Conclusion**. The study concludes that DNA damage is liable due to effect from illicit drug usage. Opioid users had the highest effect on behavioral damage, while tetrahydrocanabinol was the lowest amount of drugs present among the substance abusers.

Key words: Drug abuse, substance abuse, 8-hydroxyguanine, Inflammation mediators

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INTRODUCTION

Substance abuse involves the unauthorized consumption of substances, wherein the individual ingests significant quantities of drugs or employs techniques that have the potential to endanger their own well-being or that of those in their vicinity. This definition includes both physical dependence and psychological dependence (1). Physical dependence caused by prolonged use of a drug refers to an altered physiologic state in which withdrawal symptoms develop when the drug is discontinued. Psychologic dependence refers to a state of intense need to continue taking a drug in the absence of physical dependence. By these definitions, alcohol is a drug that can cause both physical and psychological dependence. Alcohol is considered to be one of several drugs of abuse (2).

Drugs most often associated with this term include: alcohol, amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, hallucinogens, methaqualone and opioids. The exact cause of substance abuse is not clear, but there are two predominant theories: either a genetic predisposition or a habit learned from others, which, if addiction develops, manifests itself as a chronic debilitating disease (3). In 2010, about 5% of people (230 million) used an illicit substance (4). Of these, 27 million have high-risk drug use otherwise known as recurrent drug use causing harm to their health, causing psychological problems, and or causing social problems that put them at risk of those dangers (4). In 2015, substance use disorders resulted in 307,400 deaths, up from 165,000 deaths in 1990 (5). Of these, the highest numbers are from alcohol use disorders at 137,500, opioid use disorders at 122,100 deaths, amphetamine use disorders at 12,200 deaths, and cocaine use disorders at 11,100 (6).

8-Oxoguanine glycosylase (OGG1) is a DNA repair protein involved in the repair of the major product of DNA oxidation, the miscoding base 8-oxoguanine (8-oxo-G) (7). OGG1 is implicated in the first step of the base-excision repair process, removal of the 8-oxo-G damaged base from the DNA duplex, resulting in the creation of an apurinic/apyrimidinic (AP) site (8). Due to the slow AP-lyase reaction kinetics of OGG1, the next step of in vivo AP-site removal may be aided by AP endonuclease (APE1). Polymorphism in the human OGG1 gene is associated with the risk of various cancers such as lung and prostate cancer. OGG1 (8-oxoguanine-DNA glycosylase) is one of the most abundant and well-characterized DNA lesions generated by oxidative stress (9). It has been theorized that about 180 guanines are oxidized to 8-oxoG per mammalian cell per day. 8-oxoG is a miscoding lesion that can cause G:C to T:A or T:A to G:C transversion mutations (10). This lesion accumulates in DNA with age, particularly in the mitochondrial genome, and it has been causally linked to several cancers and neurodegenerative diseases, such as Alzheimer's and Parkinson's. DNA is susceptible to damage by reactive oxygen species, and 8-hydroxydeoxyguanosine (8-OHdG) is probably one of the most abundant DNA lesions formed during oxidative stress (11). Several pathways exist for the removal or repair of this lesion from mammalian DNA. Several polymorphisms in the *hOGG1* gene have been detected in human populations. Investigation was carried out on whether there were differences in increased oxidative stress susceptibility to smoking within the *hOGG1* genotypes (12).

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a ubiquitous transcription factor well known for its role in the innate immune response (13). As such, NF-κB is a transcriptional activator of inflammatory mediators such as cytokines. It has recently been demonstrated that alcohol and other drugs of abuse can induce NF-κB activity and cytokine expression in the brain (14). A number of reviews have been published highlighting this effect of alcohol, and have linked increased NF-κB function to neuroimmune-stimulated toxicity (15). Therefore, this research focused on the potentially non-immune functions of NF-κB as possible links between NF-κB and addiction. Besides inflammatory mediators, NF-κB can induce the expression of a different set of gene targets some of which are involved in addictive processes, such as neuropeptides and opioid receptors. Complex behaviors including learning and memory, stress responses, anhedonia and drug reward, and processes that may lie outside the role of NF-κB in the classic neuroimmune response are mediated by NF-κB (13).

As Substance abuse, the excessive use of the habit-forming drugs and the illegal use of drugs or substances which in turn leads to severe addiction and dependence with so many negative effects (16), has a global impact, leading to several health conditions such as brain damage. Additionally, adolescent substance abuse, with its heterogeneity, its complexity and its association with behavioral, physical and mental health problems is of special concern to many; the politician, the economist, clinicians and researchers, families and young people themselves (17). Therefore, this study was carried out to determine the 8- Oxoguanine glycosylase (OGG1) and Nuclear factor Kappa–light-chain-enhancer (NF-κB) p65 in substance abusers (benzodiazepine, tetrahydrocannabinol and opiods) in Ado-Ekiti, Ekiti State.

MATERIALS AND METHODS

Study design

A cross-sectional design using a stratified random sampling method was used. Stratification was by age, gender and therapy.

Area of study

The study was carried out in Afe Babalola University Multi-System Hospital Ado-Ekiti (AMSH) and its surrounding environment. The environment is a teaching hospital located in the western part of Nigeria, Ekiti State. Ado-Ekiti is a city located in South-Western part of Nigeria. AMSH coordinates are 7.6012°N, 5.3018°E. The study involved both out-patients and in-patients at Afe Babalola University Multi-System Hospital Ado-Ekiti, Ekiti State.

Subjects

The subjects studied were divided into three groups of which are;

Group 1 (Confirmed substance abusers); they are those that exhibited characteristics of drug abusers and when tested they are positive for benzodiazepine, tetrahydrocannabinol and opiods.

Group 2 (suspected substance abusers); they are those that exhibited characteristics of drug abusers but when tested they are negative to benzodiazepine, tetrahydrocannabinol and opiods.

Group 3 (Control); they are those that don't exhibit characteristics of drug abusers and when tested they are negative and they also have no history of drug abuse.

Sample size

The minimum size (N) was calculated by single portion formula based

on 6.8% estimated prevalence of drug abusers, with allowance for error of 0.05 at 95% confidence interval (Z) using the formula: $N = Z^2P (1-p) / w^2$ (Araoye, 2004)
Where: Z = confidence Interval at 95,
N= minimum sample size,
W = allowance for error = 0.05
P = estimated prevalence of substance abuse in western Nigeria at 14.4% (5)
Q = 1-p = 1 - 0.144 = 0.856
$N = 1.96^{2*}0.856*0.856 / 0.05^2 = 56.3$

Therefore, to make up for possible losses, a total of 72 Subjects, were recruited for this study and classification was based on gender, age, vascular complication and therapy. The study groups consists of 21 apparently healthy subjects which served as control subjects, 30 newly diagnosed addicted subjects, and 21 substance abusers on therapy.

Inclusion and Exclusion Criteria

Both male and female subjects aged 18 years and above who has abused substances whether on therapy or not were included in this study. Pregnant women, subjects below the age of 18 years and those with underlying health conditions and mental health condition were excluded.

Ethical approval and Informed consent

Ethical approval was obtained from the Health Research Ethics Committee of Afe Babalola Teaching Hospital, Ado-Ekiti, Ekiti State (AB/HREC/22/02/122). Informed consent was sought from each participant before sample collection.

Sample collection

Venous sample of 5ml was collected from the cubital fossa using 22G needle and syringe; the 5ml was dispensed into a plain bottle. The blood was allowed to stand for one hour to clot after which it was centrifuged at 1200rpm for 5 minutes. After centrifugation, the clear serum sample was separated into a separate plain bottle and stored at temperature of -20 degree Celsius. Furthermore, 10ml of Urine sample was collected into sterile universal bottle under supervision for drug testing.

Analytical methods

Drug Test: Drug detection in urine (18) and serum (19) was done using immunochromatography based on Nova Test patented kits (Bedford, USA). The drug test is an immunoassay which is based on the principle of competitive binding. Using the rapid drug test diagnosis, the samples were tested for opiates (OPI), tetrahydrocannabinol (THC) and benzodiazepines (BZO). Drug screening tests was carried out using immunochromatographic (lateral flow) assay.

8-Oxoguanine Glycosylase (OGG1): OGG1 was estimated using Enzyme Linked Immunosorbent Assay (ELISA) kit according to manufacturer's instruction (Elabscience, Houston, Texas, USA).

Nuclear factor Kappa–light-chain-enhancer (NF-κB) P65: The Principle is based on standard sandwitch enzymes linked immunosrbent assay (ELISA) technology. NfkB oligos are precoated onto the microwell plates and the antigen is then made to bind followed by an enzyme labelled antibody, resulting in the oligos and the antibodies to form a sandwich complex. After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation. The density of colour produced is proportional to the concentration of analyte present in the sample captured in the plate.

Statistical analysis

The results obtained were presented as mean ± standard deviation. Statistical analysis was carried out using One way analysis of variance (ANOVA) and Student's t-test using Statistical Package for Social Sciences (SPSS) version 24.0. Significant difference was pegged at p-values <0.05.

RESULTS

Figure 1 showed the distribution of all subjects under examination. A total of seventy-two (72) subjects were recruited for this study of which 21 (29.17%) confirmed substance abusers, 30 (41.66%) were suspected substance abusers and 21 (29.17%) does not have history of drug abuse (control).

Figure 2 showed the distribution of substance abuse among subjects according to the type of substances abused. In a total of twenty-one (21) behavioral drug positive subjects, 2 (9.5%) subject were on benzodiazepine (BZO), 4 (19.0%) were on tetrahydrocannabinol (THC), benzodiazepine (BZO) and opioid respectively, 1 (4.8%) was on tetrahydrocannabinol (THC) and benzodiazepine (BZO), 1 (4.8%) was on tetrahydrocannabinol (THC) and opioid (OPI), 10 (47.6%) were on tetrahydrocannabinol (THC) only, and 3 (14.3%) were on opioid (OPI) only. All thirty (30) of behavioral drug negative subjects were Negative to drug test.

Figure 3 showed the distribution of substance abuse based on gender. In a total of twenty–one (21) behavioral positive drug positive subjects who were confirmed regular users of either opiods, tetrahydrocannabinol (THC) and benzodiazepine (BZO), 11 (52.4%) were males and 10 (47.6%) were females.

Table 1 showed the Mean ± Standard Deviation (SD) of Age, NF-κB and OGG1 in behavioral drug positive subjects compared with control. The mean ± SD of NF-κB in confirmed substance abusers and control group was 11.25±1.03 and 5.49±2.93, while OGG1 was 4.93±0.70 and 8.25±1.03 respectively. Age was insignificantly higher (p=0.190) when behavioral positive-drug positive subjects were compared with the control. NF-κB and OGG1 levels were significantly higher (p=0.000), (p=<0.000) in confirmed substance abusers subjects compared with control.

Table 2 showed the Mean ± Standard Deviation of Age, NF-κB and OGG1 in suspected substance abusers and Control. The mean ± SD of NF-κB in suspected substance abusers and control group was 6.05±2.12 and 5.49±2.93, while OGG1 was 5.59±5.33 and 8.25±1.03 respectively. The variation in Age, NF-κB and OGG1 were insignificantly higher (p=0.723), (p=0.431), (p=0.347) in behavioral positive-drug negative subjects compared with control.

Table 3 showed the Mean ± Standard Deviation (SD) of Age, NF-κB and OGG1 in Behavioral positive drug positive subjects compared with Behavioral Positive drug Negative subjects. The Variation in Age and OGG1 were insignificantly higher (p>0.05) in behavioral positive-drug positive subjects compared with the behavioral positive-drug negative subjects. NF-κB was significantly higher (p<0.05) in behavioral positive-drug positive subjects compared with behavioral positive-drug negative subjects.

Table 4 showed the correlation of NF-κB with other parameters in behavioral positive drug positive subjects. The correlation of NF-κB with other parameters of group 1 showed that NF-κB significantly correlates with OGG1 (r = -0.99, p=0.000) and insignificantly correlates with Age (r = 0.897; p=0.114). Table 5 showed the correlation of NF-κB with other parameter

in behavioral positive drug negative subjects. The correlation of NF-κB with other parameters of group 2 showed that NF-κB significantly correlates with OGG1 (r = -0.662, p=0.000) and insignificantly correlates with Age (r =-0.0344, p=0.225).

Figure 4 showed the NF-κB and OGG1 in behavioral positive drug positive and behavioral positive drug negative subjects with respect to gender. From the results obtained in group 1, OGG1 was higher in males than in females, while in behavioral drug negative subjects, OGG1 was higher in females than in males. In group 1, NF-κB was higher in females than males for drug positive and drug negative subjects respectively. There was significant difference (p<0.05) between the parameters across all groups.

Figure 5 showed the OGG1 in behavioral positive drug positive and behavioral positive drug negative subjects with respect to the type of substances abused. The results obtained showed that NF-κB was higher in all substances abused compared with OGG1.

Figure 6 showed the NF-κB and OGG1 in different substances abused in behavioral positive drug positive subjects with respect to gender. The results obtained showed that BZO in OGG1 was higher in males than females, while BZO in NF-κB was higher in females than males.

Figure 7 showed the NF-κB and OGG1 in different drugs with respect to age and gender. The result obtained showed that BZO drugs in NF-κB was higher within the age range of 19-22 years, while BZO drugs in OGG1 was higher in males than females.

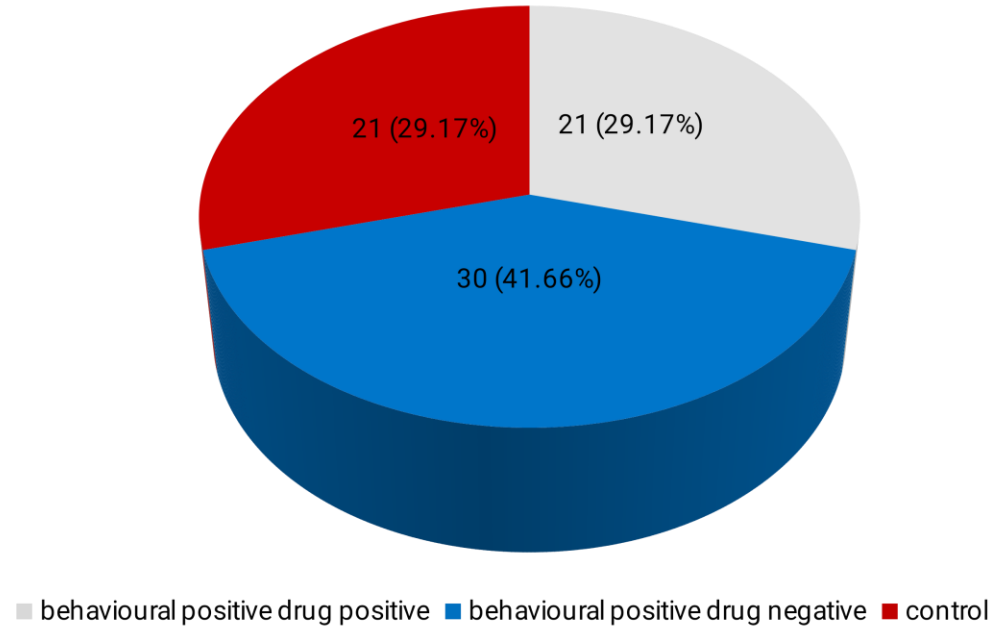

Figure 1: Distribution of all subjects under examination

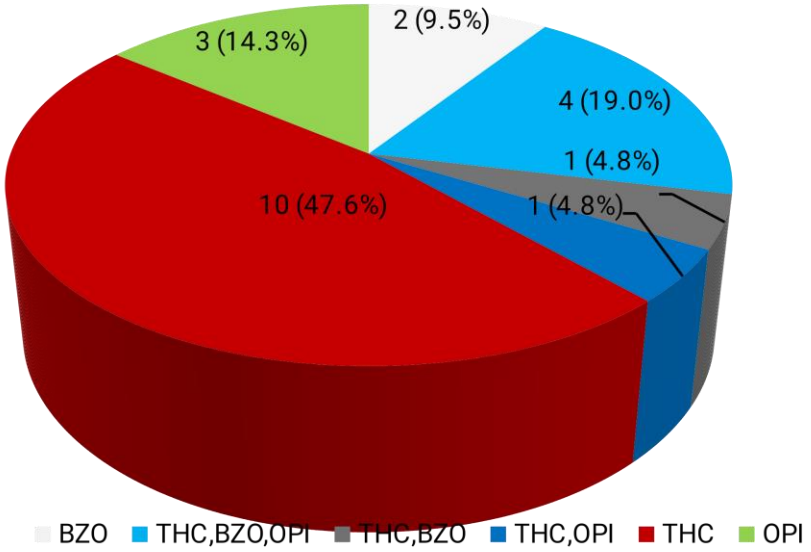

Figure 2: Distribution of substance abuse subjects according to drug use
Key: BZO – Benzodiazaphine, **THC** – Tetrahydrocaanabinol, **OPI** – Opiods

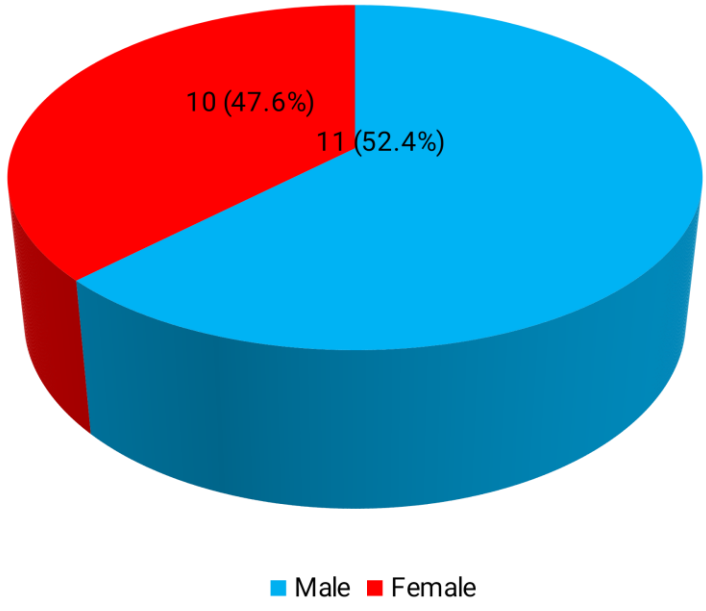

Figure 3: Distribution of substance abuse according to gender
Table 1: Mean ± Standard Deviation (SD) of Age, NF-κB and OGG1 in Confirmed substance abusers subjects compared with control

Group	Confirmed substance abusers (n= 21)	Control (n=21)	p-value
AGE	20.04±1.04	20.47±1.05	0.190
NF-κB	11.25±1.03	5.49±2.93	0.000*
OGG1	4.93±0.70	8.25±1.03	0.000*

***Values are significant at p>0.05**

Table 2: Mean ± Standard Deviation of Age, NF-κB and OGG1 in suspected substance abusers and Control

GROUP	Suspected substance abusers (N=30)	Control (N=21)	p-value
AGE	20.36±1.11	20.47±1.05	0.723
NF-κB	6.05±2.12	5.49±2.93	0.431
OGG1	5.59±5.33	8.25±1.03	0.347

***Values are significant at p>0.05**

Table 3: Mean ± Standard Deviation (SD) of Age, NF-κB and OGG1 in Confirmed substance abusers subjects compared with suspected substance abusers subjects

GROUP	Confirmed substance abusers (N=21)	Suspected substance abusers (N=30)	p-value
AGE	20.04±1.04	20.36±1.11	0.303
NF-κB	11.25±1.03	6.05±2.12	0.000*
OGG1	4.93±0.70	5.59±5.53	0.590

*value is significant at $p < 0.05$

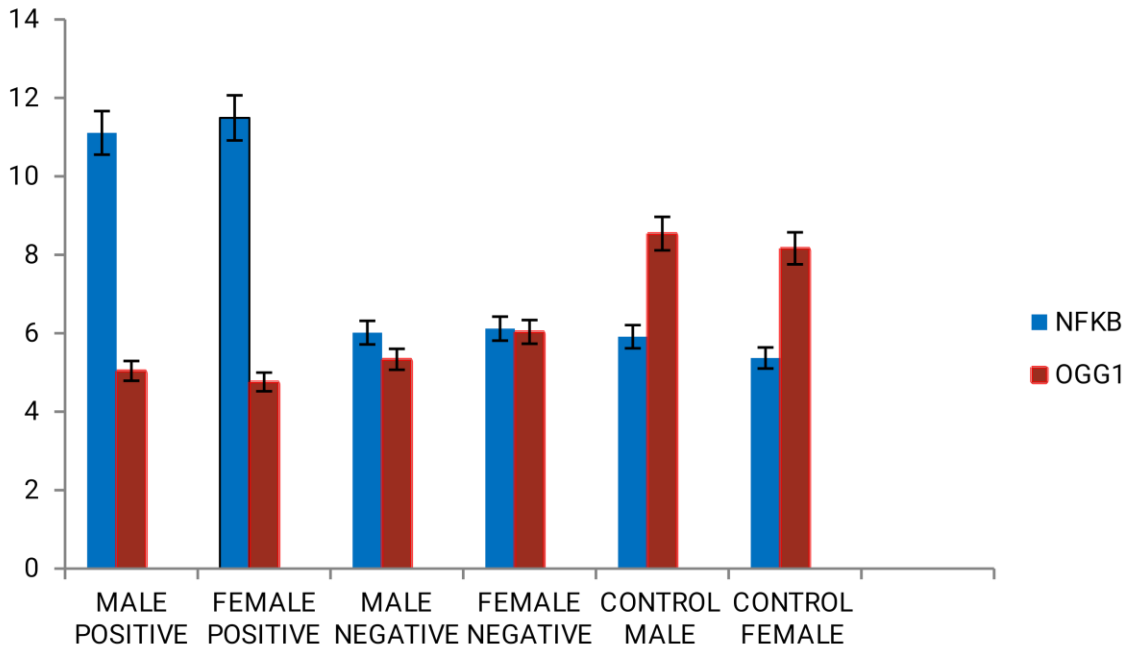

Figure 4: A chart comparing NF-κB and OGG1 in substance abusers with respect to gender.
*Significant increase in parameters when compared
Keys: **NF-κB**: Nuclear factor kappa light chain enhancer for activated B- cells**; OGG1:**8-oxoguanine glycosylase

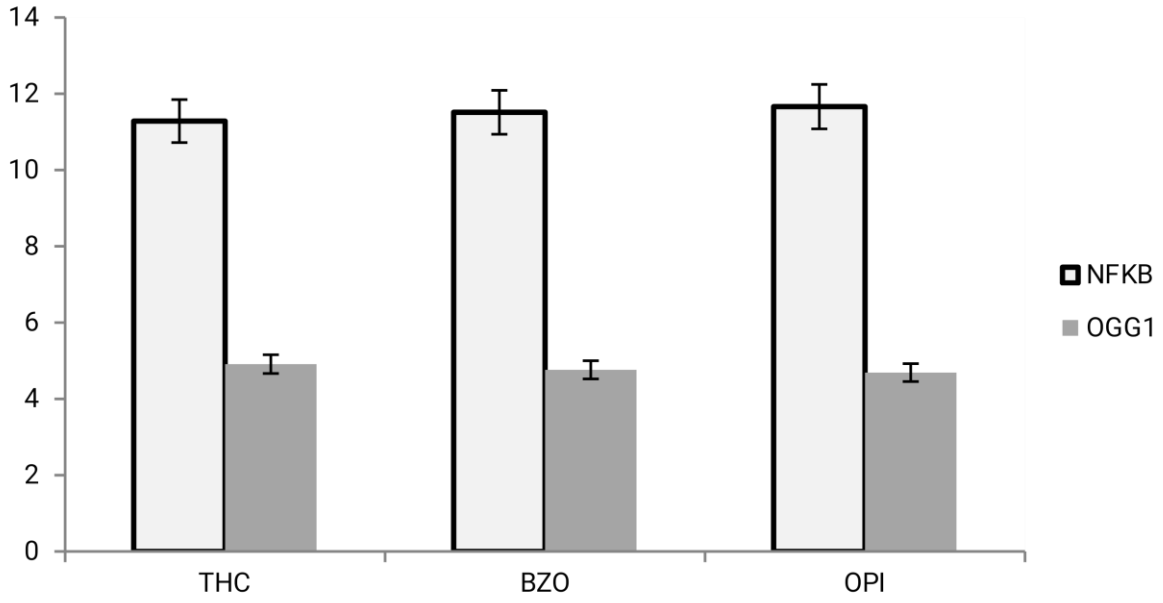

Figure 5. A chart comparing NF-κB and OGG1 in behavioral positive–Drug positive substance abusers in different drugs.
Keys: **THC** – Tetrahydrocanabinol, **BZO** – Benzodiazephine, **OPI** – Opiods

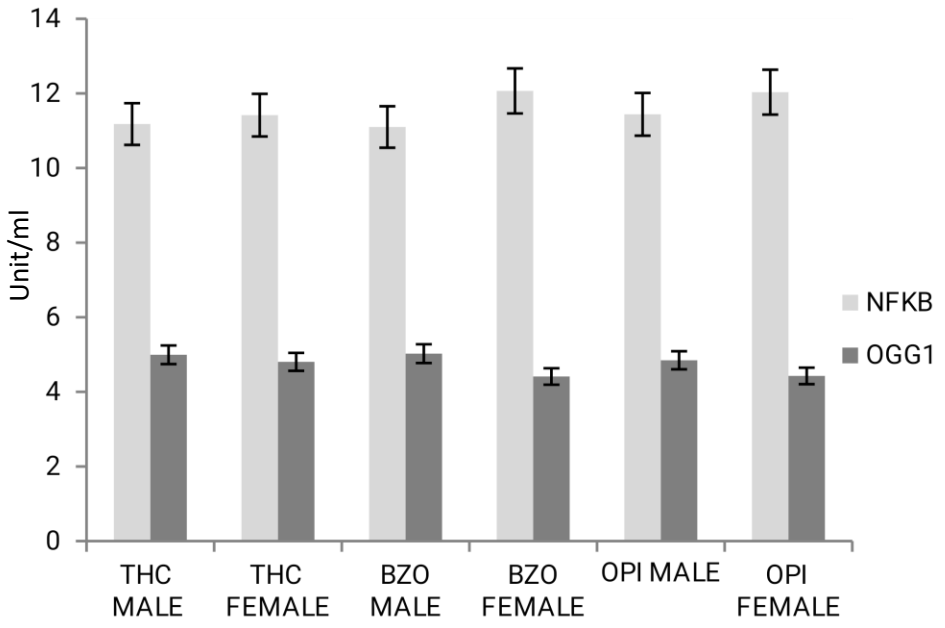

Figure 6: A Chart comparing NF-κB and OGG1 in response to different drug abused in Confirmed substance abusers **with respect to gender**

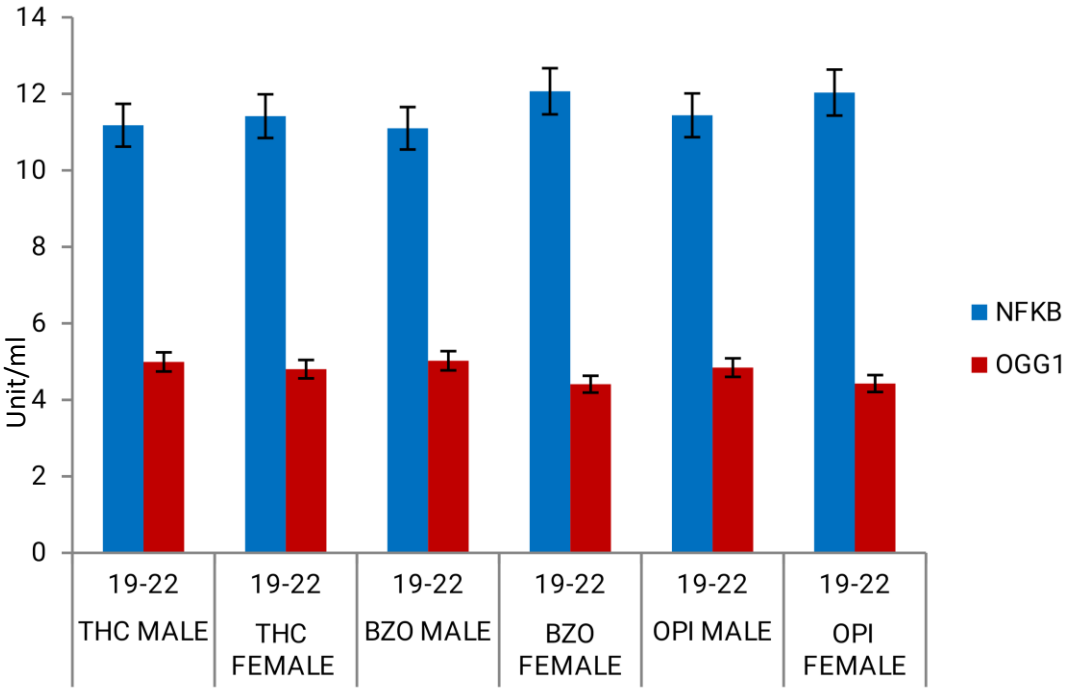

Figure 7: A chart comparing NF-κB and OGG1 in response to different drugs of substance abuser based on age

DISCUSSION

Substance abuse is a common global phenomenon and has invaded the human society as the most important social damage. Substance abuse is a non-adaptive model of drug use, which results in adverse problems and consequences, and includes a set of cognitive, behavioral, and psychological symptoms (20). Substance abuse is a cross-disciplinary topic of research and concern which involves the need to employ concomitantly various theoretical explications and empirical evidence in collaborative efforts to strive for more optimal solutions to limit its contagiousness, and to curb any direct and indirect harm. Substance abuse has been described as a "chronic relapsing disease", with extremely high relapse rates that range from 56.8% to 81.8% (21) therefore; substance abuse usage can lead to severe health complications and increase the risk of mental health state and other side effects. Various examples of drugs abused include: Benzodiazepine, opioid, heroine, marijuana, cocaine, Indian hemp, etc (3). Since, substance abuse has been linked with adverse biochemical reactions and processes, this research was designed to evaluate the levels of NF-κB and OGG1 among confirmed substance abusers and suspected substance abusers in Ado-Ekiti, Ekiti State.

In this research, NF-κB level was higher in subjects compared with control. Furthermore, NF-κB was significantly ($p=0.0001$) higher in group 1 individuals (behavioral positive and drug positive) when compared with both group 2 (behavioral positive and drug negative) and control subjects respectively. This finding is similar to previous research (13) who reported that NF-κB can induce the expression of a diverse set of gene targets besides inflammatory mediators, some of which are involved in addictive processes, such as opioid receptors and neuropeptides. NF-κB mediates complex behaviors including learning and memory, stress responses, anhedonia and drug reward, processes that may lie outside the role of NF-κB in the classic neuroimmune response (13). Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a ubiquitous transcription factor with varied roles within the mammalian cell (22). While best known for its regulation of inflammation and innate immunity, NF-κB has a wide range of gene targets and can influence complex behaviors such as learning and memory, addiction and depression. This highlights these diverse functions and draw links between specific gene targets and the development of addictive-like behaviors (23). Another mechanism by which NF-κB could influence drug reward and drug seeking is via its role in the behavioral and physiological processes involved in memory formation. Associations between drug properties and certain environments or associated stimuli are mechanisms that can drive continued drug seeking and relapse in drug addicts.

In this study, OGG1 was significantly ($p<0.05$) higher in group 1 and 2 subjects compared with control. OGG1 is a DNA repair gene which encodes the enzyme responsible for the excision of 8-oxoguanine, a mutagenic base byproduct which occurs as a result of exposure to reactive oxygen. The action of this enzyme includes lyase activity for chain cleavage. Alternative splicing of the C-terminal region of this gene classifies splice variants into two major groups, type 1 and type 2, depending on the last exon of the sequence. In this study, OGG1 activity was higher in group 1 subjects when compared with both group 2 and control ($p= 0.0001$) and was

insignificantly lower in group 2 subjects when compared with control. This finding is similar to previous study by Miglani *et al*. (24) and Karsten (25). Karsten (25) demonstrated a mechanism of action of the OGG1 inhibitor TH5487, which prevents the assembly of pro-inflammatory transcription factors and mitigates acute airway infection in mouse models of pneumonia. Hence, she propose both enzymes to be promising novel targets to treat inflammation and suggest that redox and DNA repair pathways could be useful targets for future immunomodulating therapies.

The illicit drugs under examination in this research include; Opioid (OPI), Tetrahydrocaanabinol (THC) and benzodiazepines (BZO). THC is a psychoactive drug from the cannabis plant intended for medical or recreational use. THC can last in the blood for more than 2 weeks after it is smoked (26). Opioid is derived from opium and include the natural products of morphine and codeine. They are any substance that produces morphine like effects through action on opioid receptors. Opioid are also frequently used non-medically for their euphoric effects or to prevent withdrawal (27). Benzodiazepines are a class of medications that slow down activity in the brain and nervous system. In this study, 9.5% of the subjects were on benzodiazepine only, 19.0% were on tetrahydrocannabinol, benzodiazepine and opioid, 4.8% were on tetrahydrocannabinol and benzodiazepine, 4.8% were on tetrahydrocannabinol and opioid, 47.6% were on tetrahydrocannabinol only, and 14.3% were on opioid only. Taking opioids in combination with other central nervous system depressants—like benzodiazepines, alcohol, or xylazine—increases the risk of life-threatening overdose. In 2021, nearly 14% of overdose deaths involving opioids also involved benzodiazepines, a type of prescription sedative commonly prescribed for anxiety or to help with insomnia (28). Benzodiazepines work to calm or sedate a person, by raising the level of the inhibitory neurotransmitter GABA in the brain. Research by Bachhuber *et al.* (29) and Sun *et al.* (30) showed that people who use opioids and benzodiazepines concurrently are at higher risk of visiting the emergency department, being admitted to a hospital for a drug-related emergency, and dying of drug overdose. One limitation of this study was that only three substances of abuse (opioid, tetrahydrocaanabinol and benzodiazepines) were studied. Future study should consider including other substances of abuse not captured in this study to make a generalized statement.

CONCLUSION

The study concludes that DNA damage and NF-κB activation is likely to be precipitated by illicit drugs. Opioid (OPI) was the most abused substance, while Tetrahydrocaanabinol (THC) had the lowest percentage of drugs present among the substance abusers. Similarly, opiods was the highest in NF-κB biomarkers, while THC was the highest in OGG1 biomarkers. As drug abuse has become a social addiction, we recommend that there should be enforcement of strict laws and regulations on drug misuse to avoid drug of abuse sold to younger individuals. The addition of counseling activities and education on effect of drugs should be introduced into the school activities. Medications and management of withdrawal symptoms should be provided to help drug abusers in the case of relapses.

Conflict of Interest: None declared.

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