

ABSTRACT

Multimorbidity of type 2 diabetes, excessive alcohol consumption and HIV infection, and the ultimate antiretroviral therapy are commonplace in our society due to lifestyle choices. Reports indicate that high carbohydrate intake, linked to type 2 diabetes is concurrent with excessive alcohol consumption. The latter is associated with risky sexual behavior that leads to HIV infection and immediate enrollment into combined antiretroviral therapy (cART) programs. The three conditions, diabetes, cART therapy, and alcohol abuse therefore may occur in an individual combinatorial. Additionally, each of these conditions (diabetes, cART therapy, and alcohol abuse), affects cognitive functions. The structure and quantity of hippocampal neurons and their regenerative abilities are crucial for optimal cognitive functions due to their connections to other brain regions. The level of inflammatory cytokines and apoptosis in the hippocampus is equally fundamental. To our knowledge, the impact of this multimorbidity on hippocampal histomorphology and consequent possible functional implications is absent in scientific literature.

Therefore, this study investigated the combined toxicity, effects, or otherwise of alcohol, diabetes, and cART plus their combinations on neurohistological, molecular gene expression of junctional proteins, and neurogenesis parameters of the hippocampus. A total of one hundred and eight HIV naive male adult rats (108) divided into two main groups, diabetic and non-diabetic were used. The diabetic group was further divided into 4 subgroups as follows: (A) diabetic with no treatment (DB), (B) diabetic and cART (DAV), (C) diabetic alcohol (DAL), and (D) diabetic, treated with both alcohol & cART (DAA). The non-diabetic group was further divided into 7 subgroups viz: (E) alcohol and cART treated (AA); (F) received antiretroviral (cART) medication (efavirenz 600mg+ emtricitabine 200mg+ tenofovir 245mg) in gelatine cubes (AV); (G) received 10% alcohol in distilled water v/v *ad libitum* (AL); (H) received plain gelatine cubes (PG); (I) received 20% fructose feed for two weeks and after remained untreated (2FT); (J), received a once off IP injection of citrate buffer (SNC); and (K), untreated controls (NC). The rats were terminated after 90 days, and following perfusion, the brains were harvested, one hemisphere stored at -80°C for immunoassay and PCR while the other hemisphere was fixed in 4% paraformaldehyde for histology and immunohistochemistry analysis.

The diabetic groups (DB, DAV, DAL, and DAA) were observed to be hyperglycaemic, with low glucose tolerance and polydipsia throughout the experiment but these were not observed in the other groups. The pro-inflammatory cytokines were significantly elevated in AL (IL1 α , IL6, and TNF α), AV (IL6 and TNF α), and DAA (IL1 α , and TNF α) groups but reduced in the DB and DAV (IL1 α , IL6, and TNF α) groups. On the other hand, diabetes, cART, and alcohol independently and or in combination significantly reduced antioxidant GPX. An upregulation of antiapoptotic Bcl₂ (DAL, and DAA), pro-apoptotic genes BAX (DB, DAL, DAV, and DAA), and Caspase-3 (DB, DAV, DAL, and DAA) were observed, indicating a central role of Caspase-3 and BAX in diabetes associated hippocampal apoptosis. Additionally, the downregulation of junctional proteins (claudin-5 and occludin) was observed in AV and DB groups only, while hippocampal insulin receptors (INSR and IRS1) were downregulated in AA, and DB-treated groups, but IRS1 was upregulated in the DAL group. Furthermore, a reduction in neuronal number was observed in the CA1 (AL, DB, DAV, DAL, and DAA), CA3 (DAV, DAL, and DAA), and DG (AL, AV, AA, DB, DAV, DAL, DAA) regions of the hippocampus, while the neuronal nuclei size significantly increased in the CA1 and DG hippocampal regions of the AL and DB groups. These histomorphology effects are corroborated by the significant reduction in hippocampal DCX (AL, DB, DAV, DAL, DAA) and Ki67 (AL, AA, DB, DAV, DAL, DAA) expressions in maturing and proliferating neurons. On the other hand, our results highlight the antagonistic interaction of cART and alcohol (AA), on Bcl₂, BAX, Caspase-3 expression, cytokine concentration, neuronal number, and neurogenesis. Additionally, the AA group reveals significant downregulation of junctional proteins and insulin receptors which may induce hippocampal insulin resistance through impaired insulin receptor function. Finally, alcohol and/or cART in diabetes may result in several histological, biochemical, and molecular adverse effects. Furthermore, the significant loss of neurons in the CA3 hippocampal is observed in the DAV, DAL, and DAA, unlike other treated groups. The clinical implication of these findings hinges on possible neural toxicity following a chronic cART regimen amongst diabetic patients who regularly consume alcohol.