Neuroscience and Biobehavioral Reviews

Association between chronic psychoactive substances use and systemic inflammation: a systematic review and meta-analysis

--Manuscript Draft--

Manuscript Number:	
Article Type:	Review Article
Keywords:	alcohol, opioids, nicotine; stimulants, cytokines, bioinflammatory markers, chronic psychoactive substance use
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Highlights

- Associations between chronic substances use and inflammation is under studied.
- There is a significant increase in C-Reactive Protein in chronic tobacco users.
- Current data is not enough to derive definitive conclusions.
- Research is needed to assess the inflammatory effects of illicit psychoactive use

Abstract

This systematic review and meta-analysis assess the change in inflammation biomarkers level among chronic psychoactive substance users. To meet the required inclusion criteria, all studies had to describe human participants with an age ≥18y., experiencing chronic psychostimulant (nicotine, amphetamine, cocaine), sedative (benzodiazepine,opioids) and/or cannabinoid use. The comparison group was defined as healthy participants. Studies where included if they reported at least one of the pro/inflammatory biomarkers. Study bias was examined by Funnel plots and heterogeneity by computing the I^2 statistics. Only 21 eligible studies were selected based on 26216 study participants. A small and significant effect size of 0.18mg/L (95% CI:0.10-0.27) was detected in favour of chronic smokers (z=4.33;*P*<0.0001). There was evidence of publication bias for studies measuring IL-6 and IL-10 association with cocaine and IL-6 in association with cannabis. In summary, except for chronic tobacco users, there was no evidence of association between other chronic substances abuse and inflammatory levels. More studies are needed to inform policy and decision makers about the utility of anti-inflammatory based targeted intervention programmes.

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Abstract

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Keywords: alcohol, opioids, nicotine; stimulants, cytokines, bioinflammatory markers, chronic psychoactive substance use.

1. Introduction

Chronic psychoactive substance use is a global escalating public health problem (Degenhardt et al., 2018). The World Health Organization (WHO) defines chronic substance use as the harmful or hazardous use of psychoactive substances (WHO, 2016)). It refers to, but not exclusively, to the chronic use of illicit opioids, cannabinoids and stimulants and licit use of alcohol and nicotine. A recent census estimated that 11.8 million deaths are attributable to chronic psychoactive substance use (Roth et al., 2018). Moreover, 1.5% of global disease burden is attributable to the health consequences of illicit drug and alcohol use (Ritchie, 2019). In a recent systematic review published by the Lancet (Degenhardt et al., 2018), Degenhardt et al. estimated that 31.8 (95% CI: 27.4–36.6) million Disability Adjusted Life Years (DALYs) for 1990-2016 were attributable to chronic drug use. Three-fold higher DALYs (99.2 million (95%CI: 88.3–111.2)) were attributable to chronic alcohol use in 2016 and five-fold higher DAYLYs (148·6 million (95% CI: 134·2–163·1)) were attributable to chronic tobacco use in 2015.

Chronic substance use may also lead to the exacerbation of pre-existing medical conditions, and contributes to the current increasing prevalence/incidence of chronic diseases globally (Schulte and Hser, 2014). Chronic use of alcohol, has been associated to several health risks, including chronic pancreatitis, hepatic and intestinal malignancies cardiovascular and cerebrovascular accidents. Additionally chronic tobacco use has also been associated to an increase in chronic obstructive pulmonary disease (COPD), lung and throat malignancies, and cardiovascular events globally (Laniado-Laborín, 2009).Chronic use of illicit stimulant drugs such as amphetamine and

cocaine has been associated to a higher risk of cerebrovascular accidents (five fold and two fold respectively) (Harris, 2019). Chronic cannabis nonmedical use has been linked to chronic and acute bronchitis, myocardial infarction and an increased risk of lung cancer (WHO, 2016).

The metabolic pathways especially those directly related to inflammatory processes linking chronic substance use and other chronic diseases have not been fully explored (Mitchell et al., 2019). Inflammatory processes are inherently protective against the body invasion from harmful microorganism (Medzhitov, 2010). However, the chronic activation of inflammation (referred also by low grade inflammation) has been clearly linked to chronic diseases such as diabetes and cardiovascular conditions (Tsai et al., 2019). Risk factors to low grade inflammation include age (e.g. mitochondrial dysfunction), unhealthy diet (e.g. high sugar and fat) and stress (physical and emotional). Moreover, chronic conditions are usually linked to high body mass index which is proportional to pro-inflammatory cytokines (Ellulu et al., 2017; Pahwa et al., 2020; Suganami et al., 2016). The physiopathology has been described as a pathological shift from the neutrophils infiltration to macrophage and lymphocytes which are able to produce and release unnecessarily a large amount of cytokines that exacerbates tissue damage (Aoki and Narumiya, 2016; Pahwa et al., 2020). The plausibility of this pathway is more relevant for adult populations for two main reasons: the first is that chronic use of substances peak during adulthood (Schulte and Hser, 2014), the second is that inflammation is tightly regulated during the early stages of life while among adults changes in body composition, energy production and utilization and immune senescence increase the likelihood of inflammatory state (Franceschi and

Campisi, 2014; Kuhlman et al., 2020). Therefore it is unexpected to find any significant association (Kuhlman et al., 2020).

A recent literature review conducted by (Kohno et al., 2019), revealed that chronic use of methamphetamine, cocaine, and alcohol may evoke a neuro-inflammatory response in drug users. This neuro-inflammatory response is characterized by the upregulation of translocator protein (TSPO) by reactive glia cells and activated microglia (Kohno et al., 2019). According to the authors "clinical and preclinical studies have demonstrated a link between immunological cells in blood and activated microglia (Kohno et al., 2019). This up regulatory process has also been related to chronic cannabis use (Rodrigues et al., 2014). Another review conducted by (Piao et al., 2009) suggested that chronic tobacco use alters immune responses through a reduction in (1) antibody-forming cell response in the spleen, (2) decrease in the proliferation of peripheral blood mononuclear cells, regulating lymphocytes, macrophages and (3) affecting the secretion of cytokines and lymphocytes (Piao et al., 2009). However, on the other hand, chronic tobacco use also promotes neuroinflammatory processes by the activation of epithelial and immune cells that release pro-inflammatory factors and promote the recruitment of neutrophils, macrophages, T cells, and dendritic cells (Kohno et al., 2019; Savage et al., 1991; Sopori, 2002; Sopori and Kozak, 1998).

Despite the fact that chronic use of substances such as alcohol, tobacco, cannabis and cocaine might lead to inflammation (including neuro-inflammation) (Imhof et al., 2004; McEvoy et al., 2015; Strzelak et al., 2018), there is as yet no meta-analysis testing the effect of chronic substance use on different inflammatory biomarkers (e.g. C-Reactive Proteins (CRP), Interleukins (e.g. IL-6), Tumor Necrosis Factor (TNF)).

2. Methodology

2.1. Search strategy

A database search was carried out to identify relevant studies conducted from 1st January 2000 to 31 January 2020. Studies written in English, French and Spanish languages were included in the review.

The following databases were selected for this systematic review and meta-analysis: PubMed (via NCBI), Web of science, Cochrane Central Register of Controlled Trials (CENTRAL), CINAHL (via EBSCOhost), SportDiscuss (via EBSCOhost), Academic Search Complete (via EBSCOhost), Health and Psychosocial instruments (via EBSCOhost), PsychINFO (via Proquest), Dissertations (via Proquest), Scopus, Scielo, LILAC, Biosis, CiNii, Medline (via Ovid) and Embase (via Ovid)., A confirmatory search was also conducted on Google Scholar in order to detect possible studies that were not identified through database search., Reference lists from key textbook chapters and from the pooled studies were also scanned to identify relevant cited literature.

The search terms used are available in appendices (Appendix A) (de Vries et al., 2020; Degenhardt et al., 2008). The PROSPERO registration number is CRD42020192955.

2.2. Inclusion and exclusion criteria

To meet the required inclusion criteria, all studies had to describe human participants with an age of 18 years or older, experiencing chronic psychostimulant (nicotine, amphetamine, cocaine), sedative (benzodiazepine, opioids) and/or cannabinoid use and/or dependency diagnosed operationally by Diagnostic and Statistical Manual of

Mental Disorders (APA, 2013) criteria. The comparison group was defined as healthy participants who never used use licit or illicit psychoactive substances,

Studies where included if they reported at least one of the following standardized pro/inflammatory biomarkers. Biomarkers: C-reactive protein, all types of interleukins, all tumor necrosis factors, sCD40l, APRIL, plasminogen activating inhibitor 1, interferon-gamma, orosomucoid, all types of eotaxin, all types of macrophage inflammatory proteins, Monocyte Chemoattractant Protein 1, Monocyte Chemoattractant Protein 2, chemokines, calcitonin, fractalkine, urokinase plasminogen activator receptor

Case control longitudinal, and/or cross-sectional studies were included. Baseline data were used for longitudinal studies. The exclusion criteria were the following:

Exclusion criteria include:

- Human aged less than 18 years old
- Acute use or intoxication
- Presence of chronic disease
- Presence of communicable disease
- All diagnostic psychiatric and neurological conditions
- Animal studies
- Meta-analysis and reviews
- Studies without basic statistics (only for the meta-analysis)
- All inflammation biomarkers except the retained ones

2.3. Analysis

The proposed review utilized both quantitative and qualitative methods of data analysis.

2.3.1. *Data extraction for meta-analysis*

The primary outcome of the current study consisted in quantifying the effect of chronic substance use on inflammatory biomarkers listed in Table 2.

The review was conducted and documented in line with the PRISMA-P (Moher et al., 2015) and MOOSE (Stroup et al., 2000) checklists. After searching the relevant databases listed in section 2.1, it was possible to pool studies for the meta-analysis testing chronic users of Tobacco, Alcohol, Cocaine, and Cannabis.

Data necessary to compute effect sizes assessing the effect of chronic substance use on inflammatory biomarkers consisted in Means (M) and Standard Deviations (SD) related to metabolic data (levels of inflammatory biomarkers in serum/plasma) obtained from chronic substance users, and in Means (M) and Standard Deviations (SD) related to the same metabolic data obtained from non-substance users controls. These data were therefore extracted from the pooled studies. No raw data were reported. Corresponding authors were contacted If studies fitting the inclusion and exclusion criteria did not report M and SD for relevant metabolic data.

If possible, M and SD were extracted from studies testing the impact of chronic substance use on multiple inflammatory biomarkers. Specifically, it was possible to extract M and SD related to IL-6 and IL-10 biomarkers for Cocaine, and IL-6 for Cannabis. Metabolic data related to CRP levels were extracted for Alcohol and Tobacco.

An attempt was also made to extract and insert as moderators, data related to demographic and health variables known to influence the levels of inflammatory biomarkers. These consisted in age, body mass index, and biological sex. Although, as will be illustrated in the subsequent section, the low number of pooled studies hampered the possibility to test the impact of the above covariates on relevant metabolic data.

2.3.2. *Meta Analysis*

The extracted data were subsequently inserted in the Complimentary Meta-Analysis (CMA) V III software (Borenstein et al., 2013.). A random effect model was selected to conduct meta-analytic calculations instead of a fixed effect model as it was assumed that the pooled studies were not 'identical' (i.e. not displaying the same true effect size) (Higgins et al., 2019). Considering that the pooled studies utilized different immunoassays to measure levels of inflammatory biomarkers in serum/plasma obtained from participants' blood samples, a Standardized Mean Difference (SMD) was selected as a statistical summary measure. According to the 'Cochrane Handbook for Systematic Reviews of Interventions', SMD should be utilized when the studies pooled for a meta-analysis measure the same outcome parameters but employ different measurement/analytical tools (Higgins et al., 2019). Effect sizes were computed utilizing Cohen's benchmark criteria (Cohen, 1988). Particularly, an effect size of 0.8 would have implied a 'large' effect size, an effect size of 0.5 would have implied a 'medium' effect size, and an effect size of 0.2 would have implied a 'small effect size' (Cohen, 1988). Cochran's Q and I^2 tests were utilized to assess heterogeneity between the studies included for the meta-analysis (Higgins et al., 2019).

As introduced in section 2.3.1, data related to age, BMI, and sex were extracted from the pooled studies and inserted in the software to conduct meta-regressions testing the effect of these covariates on levels of inflammatory biomarkers. However, it was not possible to perform any meta-regression as the number of pooled studies for each substance didn't reach 10 (see section 3), that is the minimum number of studies required to perform such analysis (Higgins et al., 2019).To extract data from paper figures we used the WebPlotDigitizer tool (Rohatgi, 2015).

2.3.3. *Publication Bias*

Funnel Plots were inspected to assess the presence of publication bias among the studies pooled for the meta-analysis. Furthermore, Fail Safe-N tests (Rosenthal, 1979) were computed to provide a statistical estimate of publication bias, therefore limiting possible subjective misinterpretation of Funnel Plots' asymmetry, which is likely to occur when there are few studies included in the meta-analysis (Simmonds, 2015).

2.3.4. *Qualitative analysis*

A narrative synthesis methodology (Dixon-Woods et al., 2005) was employed to summarize the findings of studies fitting the inclusion/exclusion criteria but not reporting the statistical data necessary to perform the meta-analytic calculations. Three reviewers (RD, WE, and AAC) identified and pooled key results from each study and provided a descriptive summary of the findings.

2.3.5. *Assessment of study quality*

The quality of included studies was assessed based on the National Institutes of Health (NIH) study quality assessment tools. Scoring system was adopted to characterize study quality : A 'good' study refers to a study presenting low risk of bias (≥7 points), a

‘fair’ study indicates a study presenting an acceptable risk of bias (≥4 points) and a ‘poor’ study indicates methodological defaults (<4 points).

3. Results

The electronic search identified 7553 publications, of them 305 were duplicates (Figure 1). The title and the abstract of 7448 papers were reviewed and only 138 studies were retained at this stage. One hundred and fourteen articles were excluded after reading the text of the retained studies. This led to the retention of 21 articles (13 for the meta-analysis and 8 for the narrative synthesis) which were found to fulfill the inclusion criteria.

The socio-demographic characteristics of the cohort included in each study are displayed in Table 1. Six studies were conducted in Europe (28.5%) (Chalmers et al., 2001; Çolak et al., 2019; Ersche et al., 2014; Fröhlich et al., 2003; Imhof et al., 2004; Martín de Diego and Caro de Miguel, 2006), nine (42.9%) in North America (USA only) (Costello et al., 2013; Ferguson et al., 2019; Keen et al., 2014; Keen and Turner, 2015; King et al., 2017; Levitan et al., 2005; Meier et al., 2019; Mendes et al., 2013; Moreira et al., 2016), three (14.3%) in South America (Levandowski et al., 2016; Neves et al., 2016), three (14.3%) in Asia (Bayazit et al., 2017; Chan et al., 2015). The total number of substance users is equal to 10497 and ranged between 9 and 4661. The number of healthy controls ranged between 17 to 10,999 and overall equal to 15719.

3.1. Quantitative analysis

Overall, a total of 15 effect sizes were computed from the pooled studies. Specifically, it was possible to compute five effect sizes for Tobacco (CRP), three for alcohol (CRP), three for cannabis (IL-6), and four for cocaine (two for IL-6, and two for IL-10). These are illustrated in figures 2-6.

Tobacco-CRP (Fig. 2). A small but significant effect size (SMD) of 0.18 mg/L (95%CI: 0.10 to 0.27) was detected in favour of chronic smokers (z=4.33; *P*<0.0001), indicating higher CRP levels in chronic tobacco smokers compared to non-smoker controls. Results from I^2 and Q tests revealed heterogeneity between the pooled studies (Q=13.36, p= 0.01, I^2= 70.06). Inspection of the Funnel Plot (Fig.3) revealed the absence of publication bias, which was confirmed by Fail Safe N test results. Specifically, 171 studies would have been needed to change the effect size from significant to non-significant.

Alcohol-CRP (Fig. 4). A non-significant effect size (SMD) of 0.04 mg/L (95% CI: -0.01 to 0.10) was detected in favour of chronic alcohol drinkers (z=1.54, *P*= 0.12). None of the three included studies reported a significant difference between chronic alcohol drinkers and non-drinkers (*P*=0.13 to 0.76). Results from I^2 and Q tests revealed absence of heterogeneity between the pooled studies (Q=0.09, *P*= 0.95, I^2= 0.00). Inspection of the Funnel plot (Fig.5) revealed the presence of publication bias, which was confirmed by Fail Safe N test results. Specifically, no studies (Fail Safe N=0) would have been needed to change the effect size from non-significant to significant.

Cocaine – IL-6, IL-10 (Fig. 6,7). In relation to IL-6, a non-significant effect size (SMD) of 0.41 µmol/L (95% CI: -1.92 to 2.75) was detected in favour of chronic cocaine users (z=0.34, *P*= 0.72). Both included studies showed a significant association (p<0.01). However, the direction of this association was negative for *Levandowski et al.* (SMD= -0.75 µmol/L (95% CI: -1.25 to -0.26)) and positive for *Moreira et al.* (SMD=1.63 µmol/L (95% CI:0.8 to 2.4)).Results for IL-10 did not report any significant association between chronic cocaine users and healthy controls (SMD= 0.56 µmol/L (95% CI: -8.1 to 9.2)). The three included studies showed a significant association (*P*<0.001). However, *Levandowski et al*. (SMD= 2.02 µmol/L (95% CI:1.4 to 2.6) and Fox et al. reported a positive effect size (SMD=10.4 µmol/L (95% CI:8.4 to 12.4). In contrast, *Moreira et al*. showed a lower IL-10 value among chronic cocaine users with a negative effect size (SMD) of -10.88 µmol/L (95% CI: 8.27 to 13.49).Results of Q and I^2 tests revealed the presence of heterogeneity between the pooled studies for both IL-6 (Q=25.3, *P*<0.001 I^2=96.1) and IL-10 (Q=160.4, *P*<0.001 I^2=98.8). A presence of publication bias was found (Fig. 8).

Cannabis– IL-6 (Fig. 9). Regarding cannabis, a non-significant (z=0.74, P=0.45) effect size (SMD) of 0.34 µmol/L was found in favour of chronic cannabis users compared to controls (95% CI: -0.56 to 1.23). Only *Bayazit et al*. reported significant (*P*<0.001) higher IL-6 among chronic cannabis users (SMD= 1.3 µmol/L (95% CI:0.8 to 1.8). Results from Q and I^2 tests revealed the presence of heterogeneity between the pooled studies (Q=23.52, p=0.00, I^2=92.49). Additionally, the Funnel plot (Fig. 10) showed

clearly the existence of publication bias. This was confirmed Fail Safe N test results as just 1 study would have been required to change the effect size from non-significant to significant.

A summary of the above quantitative analysis is presented in Table 3.

4. Qualitative analysis

4.1. Chronic Tobacco Use

Costello et al.(Costello et al., 2013) conducted a longitudinal study to test the predictive relationship between chronic substance use to different substances (including tobacco), and CRP levels in a sample of 1240 children, adolescents, and young adults with an age range spanning from 9 to 21 yrs. Their results revealed high CRP levels in both chronic tobacco users (Beta 0.91, $P< 0.0001$) and tobacco dependent subjects (Beta 0.89, $P<0.0001$) after adjusting for several confounding factors. These included age, sex, and obesity (Costello et al., 2013). Neves *et al*. (Neves et al., 2016) compared non-smoker controls to chronic smokers in a cross-sectional study. Results did not show a significant difference between both groups for IL-6 ($P=0.074$) and IL-10 ($P=0.220$). Undetectable concentrations of TNF-α were reported in both groups.

4.2. Chronic cocaine use

Fox and al. (Fox et al., 2012) conducted a study to assess cytokine levels in 28 chronic cocaine users and 27 social drinkers. Results showed that chronic cocaine users displayed an elevated immune system inflammatory state, specifically a higher

response of TNF-α when they were exposed to stress. Additionally, chronic cocaine users displayed lower levels of IL-10, an anti-inflammatory cytokine. Likewise, social drinkers showed higher IL-1Ra levels, an anti-inflammatory cytokine that reduces the inflammatory effects of IL-1. Ersche et al. have measured the CRP among cocaine users (n=31, 6.3 mg/L) and found that it is two fold higher in comparison to healthy controls (n=30, 3 ng/L) (Ersche et al., 2014).

4.3. Chronic opium use

Ghazavi and al. (Ghazavi et al., 2013) assessed the link between opium smoking and inflammatory level among adults aged from 20 to 40 years. The substance users were selected to have daily dosage equal or higher to 2 g of opium use per day. A significant higher plasma levels of high-sensitivity C-Reactive Protein (P=0.0001) IFN-γ (P=0.002), IL-10 (P=0.026) and IL-17 (P=0.001) among opium users.

4.4. Chronic heroin use

Chan et al. (Chan et al., 2015), identified that chronic use of opioids (n=34) showed significantly higher levels of IL-1β, IL-6 and IL-8 when compared with healthy control group. Additionally, they also reported that there seems to be a significant correlation between the methadone dosage and both TNF-α and IL-6 levels.

4.5. Chronic cannabinoid use

Keen et al. conducted a cross-sectional study among 77 exclusive Marijuana users and 45 non drugs users. After full adjustment to socio-demographic and physiological

characteristics, non-drug users were 2.73 times more likely to display higher TNF level in comparison to the marijuana users (Keen and Turner, 2015). A large cohort study conducted by *Ferguson and al*., showed that chronic marijuana users (1 year) have significantly lower CRP only in univariate analysis. After adjusting to covariates (age, sex, use of anti-inflammatory medication) the association did not stand (Ferguson et al., 2019).

5. Discussion

The main objective of our study was to examine the change in inflammatory biomarkers level in chronic substance users.

5.1. Key findings

5.1.1. *Chronic tobacco use*

Overall, the current meta-analysis shows that only chronic smoking was significantly associated with a higher CRP level. This might reflect the existence of tissue injuries and vascular inflammation among the chronic smokers (Danesh et al., 1998). There is a longstanding literature about the association between smoking and the occurrence of chronic disorders. It is believed that cigarette smoke increases oxidative stress which may provoke vascular damages and inflammation. Because of this link between smoking and the initiation of inflammatory pathways, many studies were conducted to measure serum CRP concentrations in parallel to smoking status (Ohsawa et al., 2005a) (Costello et al., 2013; de Vries et al., 2020; Degenhardt et al., 2018; Degenhardt et al., 2008; Fox et al., 2012; Gallus et al., 2018; Imhof et al., 2004; McEvoy et al., 2015;

Moher et al., 2015; Neves et al., 2016; Stroup et al., 2000; Strzelak et al., 2018; Wannamethee et al., 2005).

Most of these studies also show a dose-response relationship between CRP levels and smoking intensity and/or duration (Costello et al., 2013; Degenhardt et al., 2008; Gallus et al., 2018; Neves et al., 2016; Wannamethee et al., 2005). Another study (Wannamethee et al., 2005) conducted on older chronic smokers , revealed that there was a significant association between current exposure to tobacco and elevated levels of the inflammatory markers CRP, white cell count and fibrinogen. Furthermore, findings also indicated that smoking cessation resulted in a rapid decrease in haemostatic and inflammatory markers. But this is dependent on early onset of smoking, length of smoking career and number of cigarettes smoked. Similar results regarding the persistent effect of smoking on inflammation and haemostasis have been reported in other studies (Degenhardt et al., 2008; Fox et al., 2012; Yanbaeva et al., 2007b). However, other studies provided conflicting results (Yanbaeva et al., 2007b). The complexity of cytokine-mediated inflammation is described in a study showing that a non-significant increase in CRP levels observed in smokers (Helmersson et al., 2005b). Another study indicated that mean CRP levels were significantly lower in never-smokers compared to current smokers (Wannamethee et al., 2005). The cigarette contains different immunomodulatory toxins, of them the most studied are nicotine, carbon monoxide, acrolein and the Reactive Oxygen Species (ROS) (Lee et al., 2012). Nicotine may exert a control on different cytokines pathway: on the innate immunity (e.g. macrophages) it plays an anti-inflammatory effect by decreasing the production of pro-inflammatory cytokines (e.g. IL-1β, TNF-α) through an interaction with α7 Nicotinic

Acetylcholine Receptor (Wang et al., 2003). The nicotine seems to act without affecting the production of anti-inflammatory cytokines pathway (Wang et al., 2003). Animal models suggest a pro-inflammatory effect of chronic nicotine use , with naïve macrophage cell lines exposure to chronic doses of nicotine seem to raise the TNF level (Lau et al., 2006). ROS (e.g. nitric oxide, superoxide anion) effects carried by the gas phase of smoking and which- effect mostly the upper-respiratory tract are short-lived (Rahman, 2006). The tar may also contribute to the genesis of the ROS through the reduction of oxygen molecules, an action mediated through the semi-quinone radicals (Rahman, 2006). Additionally ROS agents also cause (a) lipid peroxidation which might induce the activation of the inflammation cascade and (b)display a chemotactic effect exacerbating the inflammatory process (Chung, 2005; Hattori et al., 2010). Unfortunately, not enough data was available for the assessment of smoking effect on cytokines, however, the increase of CRP among chronic smokers positively associate our findings to the preponderance of harmful and pro-inflammatory cytokines. At the current time of analysis controversies continue to be reported about the association of smoking and CRP levels (Helmersson et al., 2005a; Ohsawa et al., 2005b; Yanbaeva et al., 2007a). This association might be confounded by several factors such as diet quality, body mass index and lifestyle.

5.1.2. *Chronic alcohol use*

The following meta-analysis did not show a significant association between chronic alcohol consumption and inflammation. While this result was not significant, it is more likely that alcohol may introduce to systemic inflammation. Indeed , the inflammation is believed to be the key mechanism behind the organ and tissue damages due to alcohol

consumption (O'shea et al., 2010). Briefly, the conversion of ethanol to acetate promote the increase of NADH/NAD+ ratio which might in turn speed up the respiratory chain (Cederbaum, 2010; Wu and Cederbaum, 2003a). The following mitochondrial process increases the amount of ROS. The latter may damage the DNA and membrane (lipid peroxidation). The inflammation is believed to be a consequence to the oxidative stress through genesis of the ROS and reactive nitrogen species (Comporti et al., 2010; Wu and Cederbaum, 2003a; Wu and Cederbaum, 2003b). A recent meta-analysis (Adams et al., 2020) focused on identifying abnormal cytokine levels in individuals affected by alcohol use disorder. Their results revealed an abnormal circulating cytokine profile in individuals affected by alcohol use disorder compared to controls. However, individuals affected by chronic diseases were not excluded from the analyses. Another point to raise, is the gut health of chronic alcohol drinkers, as human studies reported an increase of intestine permeability (Leclercq et al., 2012; Leclercq et al., 2014; Maccioni et al., 2020). The disruption in intestine function may lead to activation of the immune response, a pathway mediated by a microbial translocation to the human liver (Hartmann et al., 2015). However, a recent research showed that the increase of intestine permeability was not associated with the microbial translocation (Maccioni et al., 2020).

5.1.3. *Chronic Cannabis use*

In the current meta-analysis, only one study out of three showed a significant increase in the levels of IL-6 among other pro-inflammatory cytokines in patients with cannabis dependency in comparison with healthy controls (Zago et al., 2016). While our results

did not show a significant effect of cannabinoids on IL-6, it is believed that these drugs are protective against inflammation and seems to actively reduce the IL-6 in macrophages (Miller et al., 2011; Mitchell et al., 2019). Despite these findings, the effects of cannabis on the immune system need further investigation.

5.2. Strengths and limitations

The current review presents several strengths, including the exhaustive research terms utilised to identify relevant studies assessing levels of inflammatory biomarkers in chronic substance users. The inclusion/exclusion criteria were stringent as they were selected to remove possible confounders of inflammation such as communicable diseases (e.g. diabetes and neuro-inflammatory diseases (e.g.) diseases and auto-immune diseases (e.g. polyarthritis rheumatoid).

Among the main methodological limitations of the current study there is the few number of retained studies limiting generalised interpretation due to possible low power of the meta analysis. In particular, the limited number of studies pooled for the meta-analysis made it not possible to test for confounding factors that could have a direct effect on the level of pro-inflammatory biomarkers such as: ethnic groups, age, Body Mass Index, waist circumference, visceral obesity, dietary habits, exercise, stress level and lifestyle. We were not able to account for the exposure time and the quantity od substances used. Furthermore, publication bias was present in the quantitative analyses related to cannabis, cocaine, and alcohol., therefore, reducing confidence related to the effect sizes computed from these set of studies (Higgins et al., 2019). A non-detected effect could be partially explained by the technique's discrepancy, which use different

principles and operational features (e.g. standard, time of reaction) of quantification and thus display different sensitivity and cross-reaction with other interferences. Most of included research articles, do not report the precision details related to the used assay especially when it comes to the interleukins and interferons.

5.3. Clinical and Public Health Relevance

The present meta-analysis identified a cross-sectional association between chronic tobacco use and increased inflammation when compared to healthy controls. Inflammation in periphery and brain systems are considered biological markers of aging (Reece, 2007). As a result of telomere shortening in lymphocytes that hypersecrete peripheral pro-inflammatory cytokines, senescent immune cells can lead to a vicious cycle of added inflammation, oxidative stress and subsequent telomere shortening (Effros, 2011). Furthermore, inflammation coupled with increased oxidation may be especially damaging and likely to foster accelerated cell aging (Rawdin et al., 2013). This has both public health consequences as the burden created by chronic diseases are presenting themselves at an earlier age and clinically, where further research is needed to adapt screening tools, risk scores and prognostic models for early identification of at-risk individuals in order to reduce premature morbidity and mortality in this population.

In the context of this systematic review it highlighted the need to pursue methodologically rigorous and well powered studies to identify if such mechanisms are also occurring in other licit and/or illicit psychoactive substances.

Declarations of interest: none.

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Figures captions

Figure 1.QUORUM: Association between chronic psychoactive substances use and systematic inflammation: a systematic review and meta-analysis.

Figure 2. Tobacco-CRP forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

Figure 3. Tobacco-CRP Funnel plot.

Figure 4. Alcohol-CRP forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

Figure 5. Alcohol-CRP Funnel plot.

Figure 6. Cocaine Il-6 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

Figure 7. Cocaine Il-10 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

Figure 8. Cocaine IL-10 Funnel plot.

Figure 9. Cannabis Il-6 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

Figure 10. Cannabis IL-6 Funnel plot.

Table 1. Sociodemographic characteristics of studies included in the systematic review

Number	Study (Country)	Study Quality	Study Design	Substance users			Healthy controls		
				n	Gender	Age in years (SD)	n	Gender	Age in years (SD)
1	Kalk et al. 2017 (UK)		Cross-sectional study	9	100% Males	45.0 (13.0)	20	25% Females	45.0 (7)
2	Colak et al. 2019 (Denmark)	Good	Cross-sectional study	10	–	–	31	–	–
3	Ferguson, Mannes, & Ennis, 2019 (USA)	Good	Longitudinal study	2156	62% Males; 38% Females	–	10999	46.7% Males; 53.3% Females	–
4	Meier et al. 2019 (USA)	Fair	Longitudinal study	25	–	–	55	–	–
5	King et al. 2017 (USA)	Good	Longitudinal study	544	53% Females; 47% Males	49.3 (11.1)	344	56% Females; 44% Males	49.6 (12.4)
6	Bayazit et al. 2017 (Turkey)	Good	Cross-sectional study	34	–	26.0 (9.4)	34	–	26.0 (8.1)
7	Moreira et al. 2016 (Brazil)	Fair	Cross-sectional study	12	75% Males; 25% Females	24.9 (4.8)	24	75% Males; 25% Females	26.29 (4.5)
8	Keen II, Turner, & Callender, 2015 (USA)	Good	Cross-sectional study	46	67% Males; 33% Females	–	77	32% Males; 68% Females	–
9	Keen II, Pereira, & Latimer, 2014 (USA)	Good	Cross-sectional study	46	67% Males; 33% Females	41.3 (11.7)	78	33% Males; 67% Females	46.6 (13.0)
10	Ghazavi et al. 2013 (Iran)	Good	Cross-sectional study	44	100% Males	30.9 (7.3)	44	100% Males	31.0 (5.2)
11	Mendes et al. 2013 (USA)	Fair	Cross-sectional study	15	66% Males; 44% Females	44.3 (1.9)	17	41% Males; 59% Females	37.7 (1.8)
12	Levitan et al. 2005 (USA)	Good	Cross-sectional study	4661	100% Females	52.8 (7.4)	1298	100% Females	52.8 (7.3)
13	Fröhlich et al. 2003 (Germany)	Good	Longitudinal study	1012	39.8% Females; 60.2% Males	–	2055	63.6% Females	–
14	Ersche et al. 2013 (UK)	Good	Cross-sectional study	31	100% Males	36.2 (9.2)	30	100% Males	37.3 (10.4)
15	Imhof et al. 2001 (Germany)	Good	Longitudinal study	42	88% Males; 12% Females	–	178	25% Males; 75% Females	–
16	Neves et al. 2016 (Brazil)	Fair	Cross-sectional study	20	100% Males	34.0 (5.9)	20	100%Males	34.0 (6.4)
17	Costello et al. 2013 (USA)	Good	Longitudinal study	1420	51% Males	–	–	–	–
18	Fox et al. 2012 (USA)	Good	Cross-sectional study	28	57% Females; 43% Males	30.2 (9.4)	27	51% Females; 49% Males	30.2 (9.4)
19	De Diego et al. 2006 (Spain)	Good	Cross-sectional study	200	–	–	344	–	–
20	Chan et al. 2015 (Taiwan)	Good	Cross-sectional study	34	100% Males	40.0(12.0)	20	100% Males	37.9 (2.1)
21	Levandowski et al. 2016 (Brazil)	Good	Cross-sectional study	108	100 %Females	29.0(7.2)	24	100% Females	31.5 (7.5)

Table 2. Fluctuation of the inflammatory biomarkers by chronic substance use

Number	Reference (country)	Substance			Duration of use in years (SD)	Administered dose			Biomarkers				Assay used		
									Cytokines						
		Stimulants	Sedatives	Cannabinoids		Route	Last dose	Dose/intake	Interleukins	Interferons	Tumor Necrosis Factors (TNF)	Acute phase proteins	Technique	CV%	Limit of detection (mg/L)
1	Kalk et al, 2017 (UK)	–	Alcohol	–	–	Drinking	14 days (range 6–29 days)	Mean drinking units/week: 175 (25/day)	↔ IL -1β, IL-6, IL-10, IL-12	↔ IFN γ	–	↔ hs CRP	Cytokines: Luminex human multiplex	–	–
2	Çolak et al, 2019 (Denmark)	Tobacco	–	–	–	Cigarette smoking	–	1) < 15 pack-years 2) 15- 29.9 pack-years 3) ≥ 30 pack years	–	–	–	↑hs CRP (in former and current smokers)	–	–	–
3	Ferguson et al, 2019 (USA)	–	–	Marijuana	- Wave III (past 0.08 year) - Wave IV (past 0.08 year and past 1 year)	–	≥ 1 in the past 30 days	–	–	–	–	↔hs CRP	Enzyme-linked immunosorbent assay	Within assay 8.1% Between assay 11.0%	0.035
4	Meier et al, 2019 (USA)	–	–	Cannabis	3.2 (±4.2)	–	8 hours	–	↑IL-6	–	–	↑hs CRP	IL-6: Enzyme-linked immunosorbent assay Hs CRP: immunoturbidometric assay	-IL-6: 9.1% and 10.2% (intra and interassay) -Hs CRP: 5.5% and 3.0% (intra and interassay)	–
5	King et al. 2018 (USA)	Tobacco	–	–	–	Cigarette Smoking	Current smokers vs. 1-year abstainers	27.3 pack-years	–	–	–	–	Immunoturbidometric assay	–	–
6	Bayazit et al. 2017 (Turkey)	–	–	Cannabis	4.6 (± 0.6)	–	At the time of sampling	–	↑ IL-1β, IL-6, IL-8	–	↑TNF-α		Enzyme-linked immunosorbent assay	–	–
7	Moreira et al. 2016 (USA)	Cocaine	–	–	–	–	–	–	↑IL-6 ↓IL-10	–	–		Enzyme-linked immunosorbent assay	–	–
8	Keen et al 2015 (USA)	–	–	Marijuana	–	–	–	–	↓IL-1α	–	↑TNF		Enzyme-linked immunosorbent assay	–	–
9	Keen et al. 2014 (USA)	–	–	Marijuana	–	–	–	–	↓ IL-6	–	–		Enzyme-linked immunosorbent assay	–	–
10	Ghazavi et al. 2013 (Iran)	–	Opium		<1	Smoking	–	>2 mg/day		–	–	↑ hsCRP	Enzyme-linked immunosorbent assay	–	0.2

11	Mendes et al. 2013 (USA)	Tobacco	–	–	–	Cigarette smoking	At least 4 hours before laboratory visit	29.6 (± 6.4) pack-years	↔ IL-6	–	–	↔ CRP	IL-6: Enzyme Immunoassay CRP: Immunoturbidimetric asaay	IL-6: 7.8% and 7.2% CRP: < 4.4% (intra-assay) and < 5.7% (between assay)	IL-6: 0.039 CRP: 0.08
12	Ersche et al. 2013 (UK)	Cocaine	–	–	15 (± 7.9)	Intranasal, inhalation, 23% intravenous injection	Last 72 hours	–	–	–	–	↑ CRP	Turbidimetric immunoassay	–	–
13	Levitan et al. 2005 (USA)	–	Alcohol	–	1	Drinking	–	3 categories: 1) 0.01-0.75 unit/day 2) 0.75- 1.5 unit/day 3) > 1.5 unit/day	–	–	–	↓ hs CRP	Turbidimetric immunoassay	7.8%	–
14	Frohlich et al. 2003 (Germany)	Tobacco	– –	– –	11-32	Cigarette smoking	5-23 hours	8.4-16.4 pack-years	– –	– –	– –	↑hsCRP –	Immunoradiometric assay –	12% –	0.05–10 –
15	Imhof et al. 2001 (Germany)	–	Alcohol	–	–	Drinking	Overnight fast	Categorized (drinking unit/day): 1) >0–2.5 2) > 2.5–5 3) >5 – 7.5 4) >7.5 –10 5) >10	–	–	–	↓ CRP (in moderate drinkers (1, 2 and 3) vs. heavy drinkers (4))	Immunoradiometric assay	–	0·05–10
16	Neves et al. 2016 (Brazil)	Tobacco	–	–	16 (7–26)	Cigarette smoking	8-12 hours	–	↔ IL-6, IL-10	–	TNF<LD	–	Enzyme-linked immunosorbent assay	–	10^{-8}
17	Costello et al. 2013 (USA)	Tobacco	Alcohol	Cannabis	0.33	–	–	–	–	–	–	↑CRP	Biotin–streptavidin based immunofluorometric assay	-Within assay: 2.0%, 1.2%, 1.6%, and 1.4% -Between assay: 14.4%, 13.9%, 12.3% and 10.9%	0.010
18	Fox et al. 2012 (USA)	Cocaine	–	–	9.0 ±7.2	–	–	45.9 ± 49.7 g/month	↔ IL-1,IL-10		↑ TNF-α	–	Enzyme-linked immunosorbent assay	–	–

19	De Diego et al. 2006 (Spain)	Tobacco	–	–	19.8	Cigarette smoking	–	12.2 pack-years	–	–	–	↑CRP	Immunoturbidimetry	–	–
20	Chan et al. 2015 (Taiwan)	–	Heroin	–	> 0.08	–	–	–	↑ IL-1β, IL-6, IL-8	–	↑ TNF-α	–	Flow cytometry	≤10%	IL-6 ($2.5\ 10^{-8}$), IL-8 ($3.6\ 10^{-8}$), IL-1β ($7.2\ 10^{-8}$), IL-10 (3.310^{-8}), TNF α ($3.7\ 10^{-8}$)
21	Levandowski et al. (Brazil)	Cocaine	–	–	–	–	4 days	–	↓ IL-2, IL-17, IL-6 ↑ IL-4, IL-10	↓ IFN γ	↓ TNF	–	Flow cytometry	–	–

CV: Coefficient of variation; hs-CRP: high sensitivity C-Reactive Protein; IL-1: Interleukin 1; IL-6: Interleukin 6; IL-8: Interleukin 10; ↑ : Significant increase of biomarker concentration in comparison to control group; ↔: no significant variation of biomarker concentration in comparison to control group; ↓ : Significant decrease of biomarker concentration in comparison to control group

Table 3. Pooled effect sizes for the effect of chronic substance use on inflammatory biomarkers

Substances	Biomarkers	N[1]	Studies[2]	Effect size and 95% confidence interval				Test of null(2tail)		Heterogeneity		Publication bias	
				Effect Size[3]	SE[4]	Lower limit[5]	Upper Limit[6]	Z[7]	P for Z[8]	Q[9]	P for Q[10]	I2[11]	Fail safe N[12]
Tobacco	CRP	12580	5	0.18	0.04	0.10	0.27	4.33	0.00	13.36	0.01	70.06	171
Alcohol	CRP	1325	3	0.04	0.03	-0.01	0.10	1.54	0.12	0.09	0.95	0.00	0
Cocaine	IL-6	67	2	0.41	1.19	-1.92	2.75	0.34	0.72	25.31	<0.0001	96.05	N/A
	IL-10	67	3	0.56	4.41	-8.09	9.21	0.12	0.89	160.5	<0.0001	98.88	18
Cannabis	IL-6	105	3	0.34	0.45	-0.55	1.23	0.74	0.45	23.52	<0.0001	91.49	1

[1]number of chronic substance users; [2]number of pooled studies; [3] Cohen's d effect size; [4] Standard error; [5] Lower limit of the 95% confidence interval for the effect size;
[6] Upper limit of the 95% confidence interval for the effect size; [7] One sample Z Statistic; [8] Probability that Z Statistics is significantly different than 0; [9] Q statistic;
[10] Probability that Q statistics significantly different than 0; [11]*I2* statistic; [12] Classic Fail safe N; CRP : C-reactive protein; IL-6 : Interleukin 6; IL10 : Interleukin 10.

Figures

Figure 1.QUORUM: Association between chronic psychoactive substances use and systematic inflammation: a systematic review and meta-analysis.

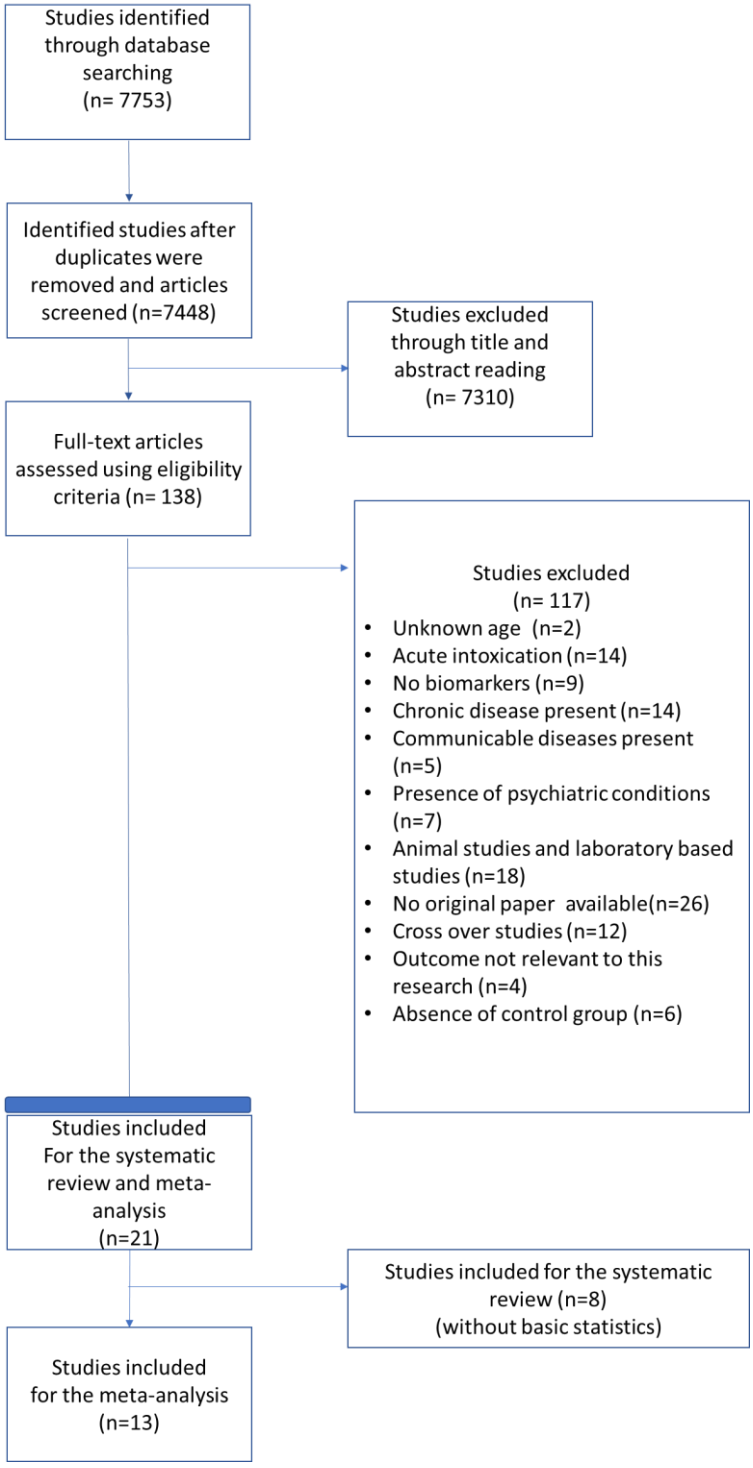

Figure 2. Tobacco-CRP forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

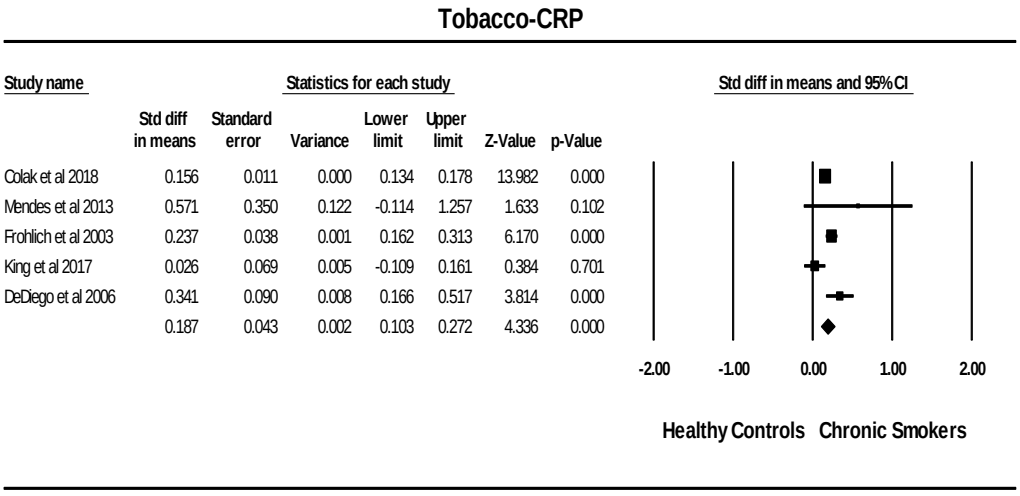

Figure 3. Tobacco-CRP Funnel plot.

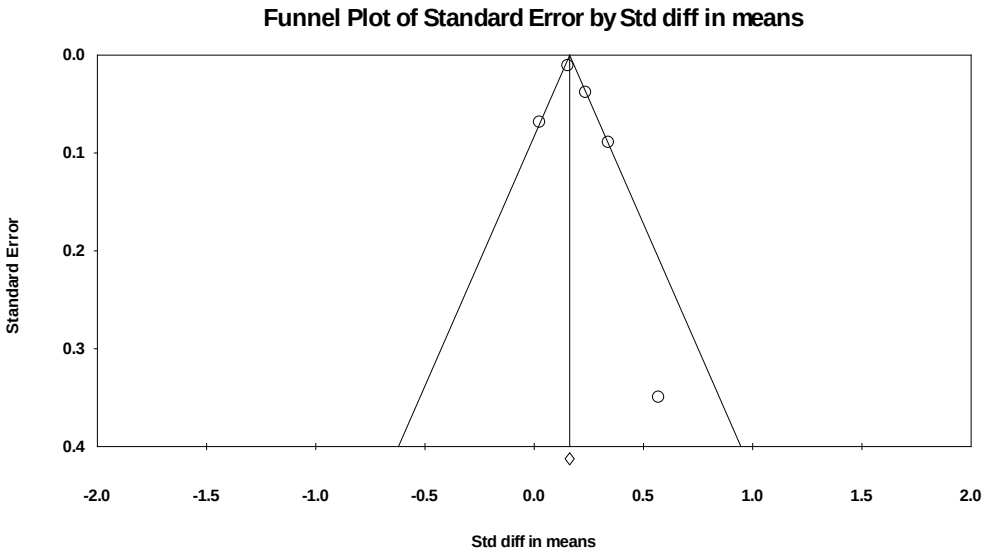

Figure 4. Alcohol-CRP forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

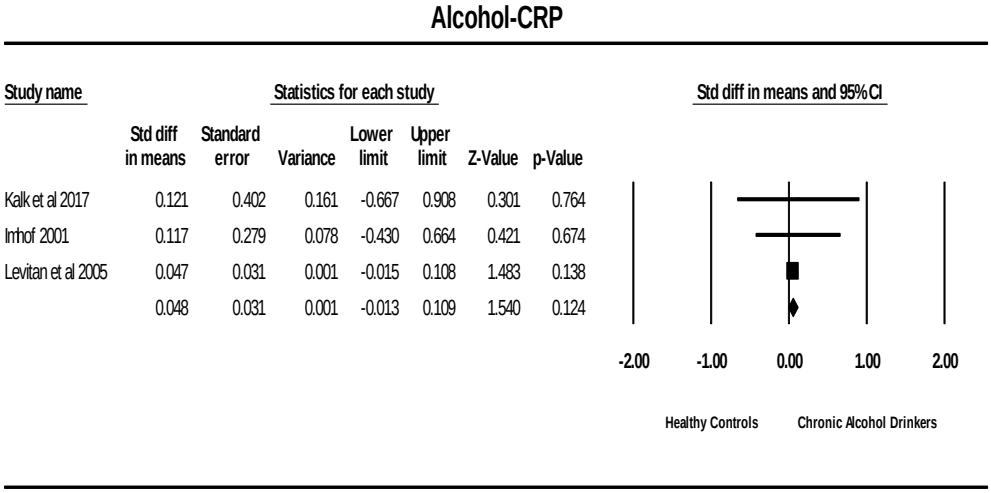

Figure 5. Alcohol-CRP Funnel plot.

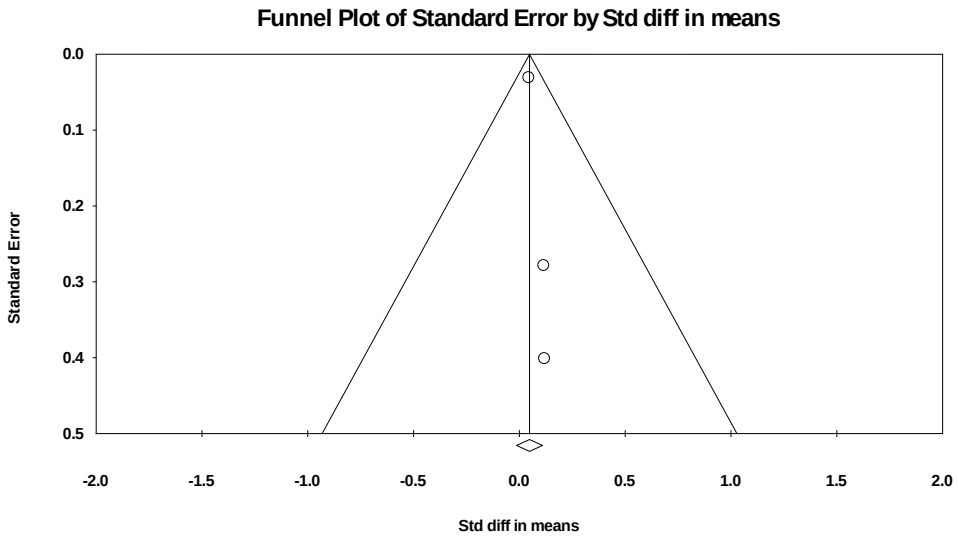

Figure 6. Cocaine Il-6 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

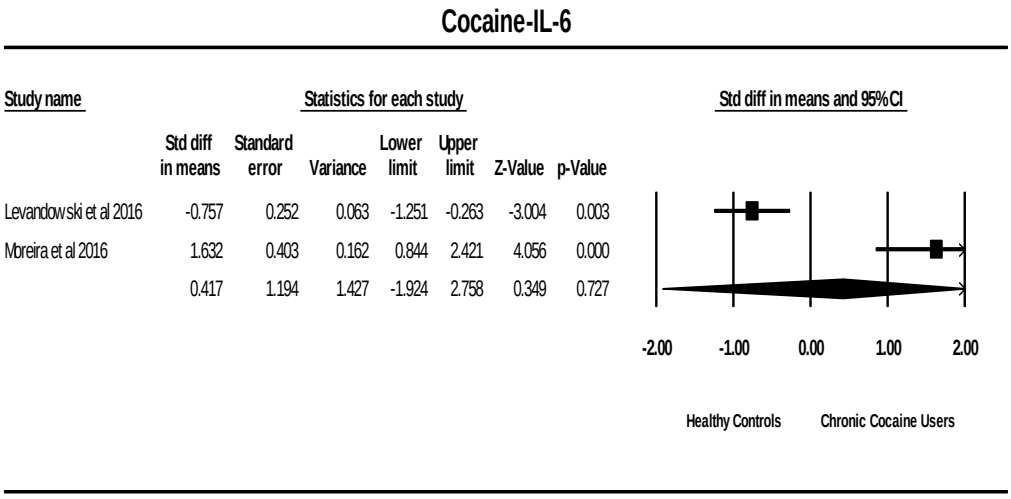

Figure 7. Cocaine Il-10 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

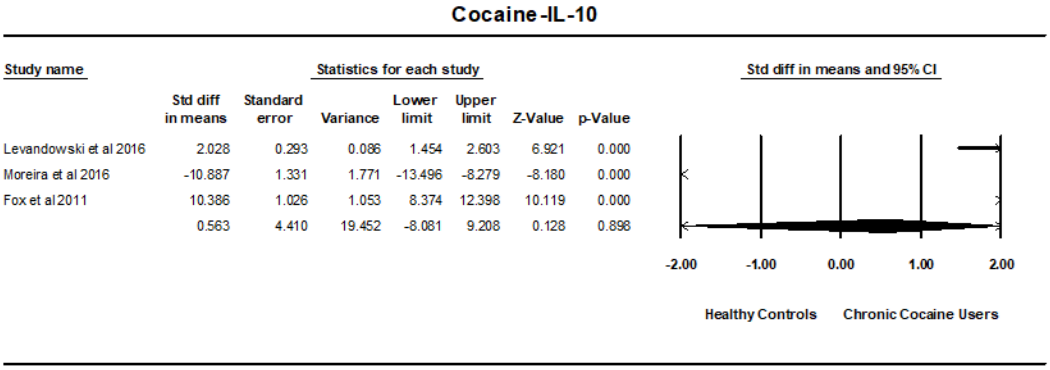

Figure 8. Cocaine IL-10 Funnel plot.

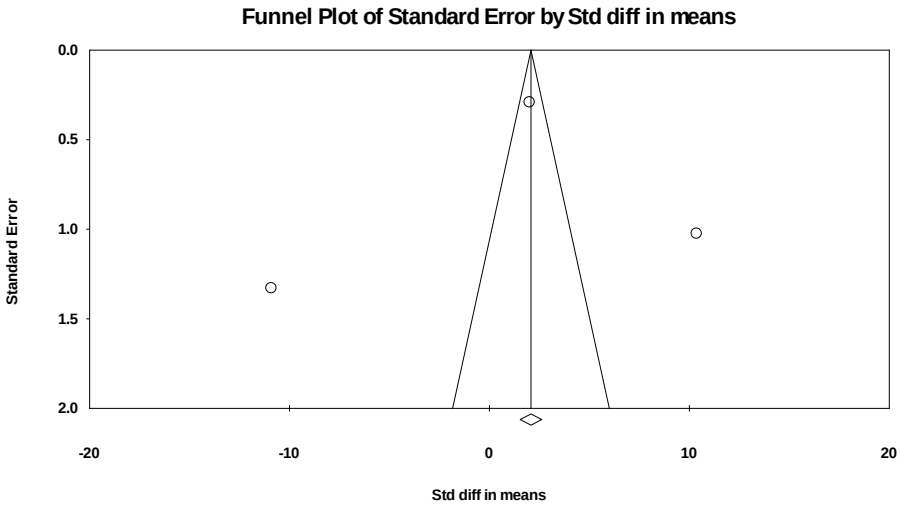

Figure 9. Cannabis Il-6 forest plot (std diff= standard difference; Z value=one sample z statistics; p value= probability that Z statistics is significantly different than 0; Lower limit= lower limit of the 95% confidence interval for the effect size; Upper limit= upper limit of the 95% confidence interval for the effect size). Filled squares represent the mean difference derived from the studies analyzed. Horizontal bars represent 95% confidence intervals. Filled rhombus show the overall, combined mean difference. CI: Confidence interval.

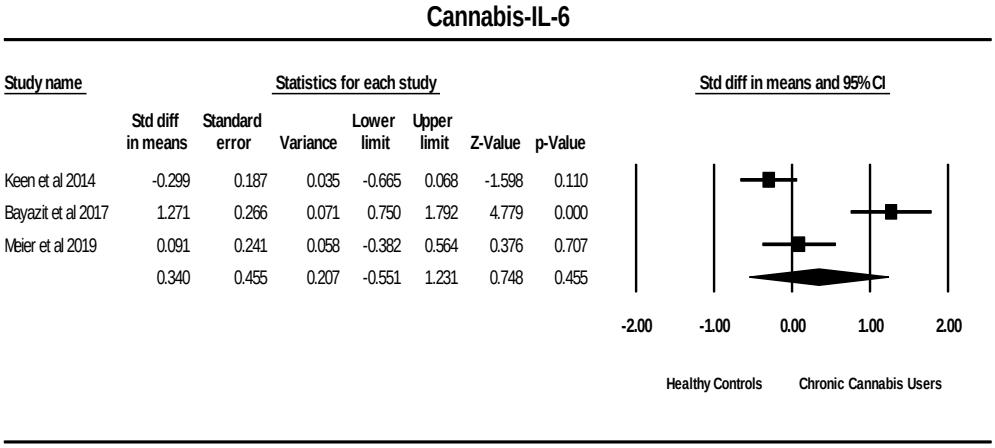

Figure 10. Cannabis IL-6 Funnel plot.