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DRUGS FOR CHRONIC ALCOHOLISM AND THEIR QUANTITATIVE ESTIMATION

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ABSTRACT

It is well accepted that drinking alcohol regularly for years is toxic to every tissue of our body. After consumption alcohol is metabolized by various enzymes such as alcohol dehyrogenase, catalase and cytochrome P450 into acetaldehyde in the body which leads to intoxication. Metabolic products of alcohol are responsible for the development of various diseases such as liver cirrhosis, trauma, depression etc. An alcohol addict not only keeps his health and day to day life on the linebut also the family welfare's. Considering the wide nature of damage by chronic alcoholism its treatment includes psychological and chemotherapy both. There are several drugs that decreases the alcohol craving it includes acamprosate calcium, fluoxetine, disulfiram, ondansetron and naltrexone hydrochloride. The presented work summaries the details of these drugs including various methods for quantitative estimation of these drugs in the formulations, bulk drugs and biological fluids.

1. Alcohol[1]

Alcohols are hydroxyl group containing compounds. Simple alcohols derived from saturated hydrocarbons, having the general formula $C_nH_{2n+1}OH$. Ethanol is the only alcohol consumed as alcoholic beverage.

After consumption alcohol is metabolized by3 enzymes:

1.1.Catalase: The acetaldehyde is released into the brain after metabolism of alcohol by catalase. Acetaldehyde combines with neurotransmitter to form new compounds known as THIQs (tetrahydroisoquinolines) responsible for alcohol addiction.

1.2.Alcohol dehyrogenase: It metabolizes alcohol in stomach and liver. Hydrogen is released when alcohol dehyrogenase converts alcohol into acetaldehyde which bounds to NAD+ to form NADH.

1.3.Cytochrome P450: It is the third enzyme metabolizes alcohol in liver. Hydrogen is released which bounds to oxygen and NADPH to form water and NADP+.

2. Chronic Alcoholism[2]**:** Chronic alcoholism is a pathological condition resulting from the habitual use of alcohol in excessive amounts. Symptoms of the disease include anorexia, diarrhea, weight loss neurologic and psychiatric disturbances and fatty deterioration of liver leading to cirrhosis.

3. Global situation of chronicalcoholism[3, 4, 5]

3.1. Alcohol consumption is the third largest risk factor for development of various diseases like liver cirrhosis, anemia in developed countries.

3.2. The proportion of disease burden attributable to alcohol use in the developing world is between 2.6% to 9.8% of the total burden for males and 0.5% to 2.0% of the total burden for females.

3.3. Besides the direct toxic effects of intoxication and addiction, alcohol use causes about 20% to 30% of each of esophageal cancer, liver disease, homicide, epileptic seizures, and motor vehicle accidents worldwide.

3.4. In 2004, 35 million deaths and in 2011, 2.4% of daily deaths were caused by chronic alcoholism.

3.5. In 2012, 5.1% of the burden of disease and injury worldwide was attributable to alcohol consumption.

4. Problems associated with chronic alcoholism[6, 7,8,9,10,11]

A person suffering from chronic alcoholism is not only under high health risks but also affecting his social life. Some major problems include:

4.1. Fatty liver, there is an accumulation of fat within the hepatocytes.

4.2. Depression and risk behavior in adolescence.

4.3. High alcohol consumption is a risk factors to fetus leads to spontaneous abortion low birth weight, prematurity and intra-uterine growth retardation in pregnant women.

4.4. Excessive alcohol consumption leads to indirect effect of corticotrophin releasing hormone receptor 1 gene variation on negative emotionality and on right ventrolateral precortex.

4.5. Chronic alcoholism causes differential changes in amygdala and frontal cortex phosphodiesterase 10a expression during acute and protracted withdrawal.

4.6. Excessive amount of alcohol intake leads to childhood adversities and suicide attempts.

4.7. In women chronic alcoholism causes comorbid posttraumatic stress disorders.

4.8. Excessive alcohol use is related with dementia causing atrophic changes, lacunar infarcts, cerebrovascular disease and traumatic brain injury and deep white matter lesions of the brain.

4.9. It causes thalamic volume deficit contributes to procedural and explicit impairment in HIV infection.

4.10. Heavy drinking can cause anemia, cardiovascular disease and cancer problems.
Chronic alcoholism needs treatment to reduce health risks and craving for alcohol. The treatments for the disease like liver cirrhosis and different types of cancer are poorly developed. This makes the 'risk factor control' a better option. After proper treatment the patient not only improves his physical and mental status but also returns normal to day to day life.

5. Pharmacological actions of alcohol[12, 13, 14, 15,16,17,18]

5.1. Effect on CNS: Ethanol elicits its pharmacological effects by two pathways:

5.1.1. Decreasing the excitatory actions of the neurotransmitter glutamate at the NMDA subtype of glutamate receptor.

5.1.2. Boosting the inhibitory actions of the neurotransmitter gamma-amino butyric acid (GABA) at the $GABA_A$ receptor.

Alcohol interacts with specific neuronal membrane proteins involved in signal transmission leads to changes in neural activity mainly involves two membrane receptors: $GABA_A$and NMDA ion channel receptors. Ethanol enhances the GABA action and antagonizes glutamate action acting as a CNS depressant.

5.2. Effect on CVS system: Heavy alcohol use can raise diastolic and systolic blood pressure due to increased intracellular Ca^{2+} levels with increase in vascular reactivity, stimulation of endothelium to release endothelin and inhibition of endothelium dependent NO production that leads to hypertension.

Alcohol has direct effect on cardiacrhythms. It prolongs the QT interval, ventricular repolarization and sympathetic stimulation. Atrial arrhythmias associated with chronic alcohol use include supraventricular tachycardia, atrial fibrillation and atrial flutter.

5.3.Effect on renal system: Alcohol inhibits release of vasopressin from posterior pituitary gland results in enhanced diuresis with decreased urine output. Persons withdrawn from alcohol exhibit increased vasopressin release and retention of water and dilutional hyponatremia.

5.4.Effect on liver: Ethanol decreases phosphatidylcholine levels in hepatic mitochondria which lead to decrease oxidase activity and oxygen consumption. Cytokines such as TGF (transforming growth factor) and TNF (tumor necrosis factor) are responsible for development of fibrinogensis and fibrosis in liver. Normal liver tissue is replaced by fibrous tissue. Alcohol affects stellate cells in liver, chronic alcohol use is associated with transformation of stellate cells into collage producing myofibroblast like cells resulting in deposition of collagen around terminal hepatic venules causes liver cirrhosis.

6. Modern concept of treatment of alcohol abuse[19, 20, 21, 22]

6.1.Psychological and Motivational enhancement therapy (PMET)

It begins with assumption that the responsibility and capacity for change lies within the patient. The treatment begins by providing knowledge about effects of drinking to the patients. PMET may be one of the most cost effective method of treatment.

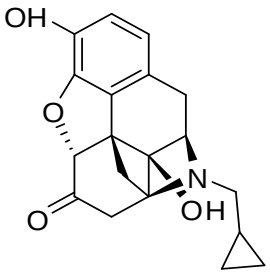
6.2.Couples therapy

The involvement of a nonalcoholic spouse in a treatment program can improve patient participation rates and likelihood that patient will alter drinking behavior after treatment ends.

Behavioral-marital therapy focus on drinking with efforts to strengthen marital relationship through shared activities and teaching of communication and conflict evaluation skills.

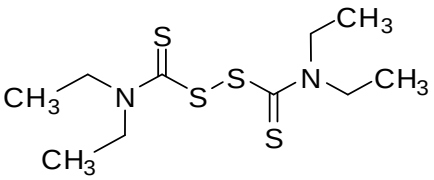
6.3. Chemotherapy of chronic alcoholism

There are several drugs which either interfere with the normal metabolic pathways of alcohol or alter the pharmacological effects of alcohol. This alteration leads to feeling of discomfort and uneasiness in the patient. The chemotherapy of chronic alcoholism involves these drugs to develop aversion for alcohol in the patients. The chemotherapeutic agents for chronic alcoholism act by different mechanism of action and having diversified structure (figure 1)



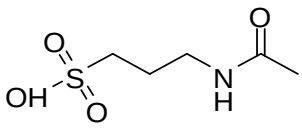
1

Naltrexone



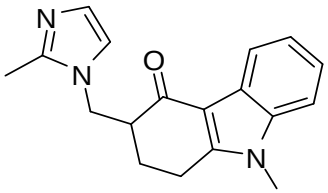
2

Disulfiram



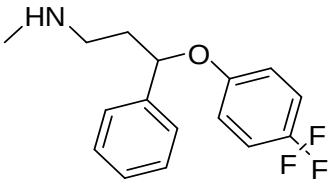
3

Acamprosate



4

Ondansetron



5

Fluoxetine

7. Classification of drugs for chronic alcoholism

The drugs used for treatment of chronic alcoholism can be classified according to their mode of action.

A. Opioid receptor antagonist: Naltrexone

B. Aldehyde Dehydrogenase inhibitor: Disulfiram

C. GABA analogues: Acamprosate

D. Serotonin receptor antagonist: Ondansetron

E. Serotonin reuptake inhibitors: Fluoxetine

7.1.Opioid receptor antagonist

7.1.1. Naltrexone[23, 24, 25, 26]

Naltrexone reduces relapse rate after abstinence in multiple clinical studies. It reduces heavy drinking when used by people who continue drinking and is less effective in patients after abstinence.

7.1.1.1. IUPAC name

17-(cyclopropylmethyl)-4,5α-epoxy- 3, 14-dihydroxymorphinan-6-one

7.1.1.2 Marketed preparations

It is marketed under the name of Revia, Depade, Vivitrol and Relistor.

7.1.1.3. Mechanism of action

Naltrexone is an opioid receptor antagonist. It causes blockage of μ- opioid receptors reduces reinforcing effects of alcohol leading to decreased feelings of intoxication and cravings.

7.1.1.4. Opioid dependence

Naltrexone overcomes opioid addiction by blocking the drug euphoric effect. It has little effect on opioid cravings.

7.1.1.5. Rapid opioid detoxification

Naltrexone is used for rapid detoxification induces opioid receptor blockade in state of impaired consciousness.

7.1.1.5. Side effects of naltrexone

Naltrexone causes nausea, headache, anxiety, sedation, abdominal cramping, liver damage, respiratory depression and death.

7.1.1.6. Contraindications

Naltrexone (50mg per day) should not be used by persons with acute hepatitis or liver failureor those with recent opioid use (7-10 days).

7.1.1.7. Dose

Recommended dose of Vivitrol is 380mg is delivered intramuscularly once a month. 50mg/day should be administering for several months.

7.2.Aldehyde Dehydrogenase inhibitor

7.2.1. Disulfiram[27, 28, 29, 30]

Disulfiram does not have any effect on relapse rate and hence does not reduce alcohol cravings but produce uneasiness and discomfort in patients after alcohol consumption.

7.2.2.1. IUPAC name

1, 1', 1'', 1'''-[disulfanediylbis (carbonothioylnitrilo)] tetra ethane

7.2.2.2. Marketed preparation

It is marketed under the name of Anabuse and Antabuse.

7.2.2.3. Mechanism of action

At normal metabolism, alcohol is broken down in the liver by enzyme alcohol dehyrogenase to acetaldehyde, which is converted by enzyme acetaldehyde dehyrogenase to acetic acid. Disulfiram blocks this reaction at intermediate stage by blocking acetaldehyde dehyrogenase. After alcohol intake under the influence of disulfiram, concentration of acetaldehyde in blood may be five to ten times higher than during metabolism of same amount of alcohol alone. This produces immediate and severe negative reaction to alcohol intake.

7.2.2.4. Side effects of Disulfiram

Side effects include flushing of skin, accelerated heart rate, shortness of breath, nausea, vomiting, headache, visual disturbance, mental confusion and circulatory collapse.

7.2.2.5. Contraindications

It is not recommended in patients with peripheral neuropathy, seizures, cirrhosis with portal hypertension. Hepatotoxicity is rare but causes fatal adverse effects.

7.2.2.6. Dose

Disulfiram is given in a dosage of 250mg per day with a maximum dosage of 500mg per day.

7.3. GABA analogues:

7.3.1. Acamprosate[31, 32, 33, 34]

7.3.1.1. IUPAC name

3-Acetamidopropane-1-sulfonic acid

7.3.1.2. Marketed preparation

Marketed in the form of Acamprosate D-3 calcium salt and Acamprosate D-6 calcium salt

7.3.1.3. Mechanism of action

7.3.1.4. Effect on NMDA receptor

Alcohol inhibits the activity of N-methyl-D-Aspartate receptor. Chronic alcohol consumption leads to overproduction of these receptors causes symptoms of delirium tremens, excitotoxic neuronal death. Withdrawal from alcohol induces release of neurotransmitter glutamate, which activates NMDA receptor. Acamprosate reduces release of glutamate.

7.3.1.5. Neuroprotective effects

Acamprosate is neuroprotective; it protects neurons from damage and death caused by effects of alcohol and causes neurotoxicity. Acamprosate protects cultured cells from ischemia and reduces release of glutamate.

7.3.1.6. Side effects

Side effects include headache, nausea, diarrhea, flatulence.

7.3.1.7. Contraindications

Acamprosate is removed by kidneys so minimum dose should be administer to patients suffering from impaired kidney diseases (creatinine clearance between 30ml/min and 50ml/min). It is also contraindicated in those patients suffering from strong allergic reaction to acamprosate calcium.

7.3.1.8. Dose

Acamprosate should be administered at a dose of 333mg enteric coated tablets.

7.4. Serotonin receptor antagonist

7.4.1. Ondansetron[35, 36, 37, 38]

7.4.1.1. IUPAC name

9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl) methyl]-2, 3-dihydro-1*H*-carbazol-4(9*H*)-one

7.4.1.2. Marketed preparation

Ondansetron marketed as Zofran.

7.4.1.3. Mechanism of Action

Ondansetron inhibits 5-HT_3 receptors in CNS including postrema, nucleus tractus solitarious and amygdala and through 5-HT_3 receptor antagonism. It inhibits dopamine release cell firing in nucleus accumbens. In peripheral nervous system, it inhibits 5-HT_3 receptors, blocking depolarization of vagal afferent nerves and myentric neurons leading to attenuation of 5-HT_3 receptor mediated nociceptive response.An imbalance between two chemical messengers in the brain that is serotonin and dopamine generates craving for alcohol. Ondansetron blocks serotonin receptor which decreases alcohol-induced dopamine release, resulting in decrease in alcohol drinking behavior.

7.4.1.4. Side effects

Constipation, dizziness and headache are the most commonly reported side effects. It is broken down by hepatic CytochromeP_{450}system and also has effect on metabolism of other drugs. Ototoxicity has been reported if injected quickly**.**

7.4.1.5. Contraindications

Ondansetron is a well-tolerated drug with few side effects. Constipation, dizziness, and headache are the most commonly reported side effects associated with its use.

7.4.1.6. Dose

The maximum recommended dose for patients with severe liver function impairment is 8mg/day. Single doses of injectable ondansetron should not exceed 16mg at one time.

7.5. Serotonin reuptake inhibitors

7.5.1. Fluoxetine[39, 40, 41]

7.5.1.1. IUPAC name

N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine

7.5.1.2. Marketed preparation

Marketed preparations are Prozac and Sarafem.

7.5.1.3. Mechanism of action

Fluoxetine is a selective serotonin reuptake inhibitor and selective brain steroidogenic stimulant and does not inhibit norepinephrine and dopamine reuptake. Large doses causes increase of brain concentration of dopamine and nor epinephrine mediated by $5HT_{2A}$ and $5HT_{2C}$ receptors leads to antidepressant action of Fluoxetine.

The inhibition of serotonin reuptake increases the concentration of serotonin in body leads to elevation in mood and impulse stability, reducing desire for alcohol consumption.

7.5.1.4. Side effects

Side effects includes abnormal dreams, abnormal ejaculation, anorexia, anxiety, diarrhea, dry mouth, dyspepsia, flu syndrome, impotence, insomnia, nausea, nervousness, sweating, rashes, tremor, vasodilatation and itchiness.

7.5.1.5. Contraindications

Should not be given to patients on MAO inhibitors such as phenelzine and tranylcypromine.

7.5.1.6. Dose

50-80 mg of Fluoxetine should be administered for treatment of chronic alcoholism.

8.Method of estimation of different drugs used in chronic alcoholism

8.1. Method for estimation of Acamprosate Calcium[42, 43, 44, 45, 46, 47, 48, 49, 50]

Besson *et al.*, (1998) have developed a rapid, accurate, precise, and economical spectrophotometric method for determination of acamprosate calcium in beagle dog plasma using UV spectrophotometer. Acidified plasma samples were extracted with phosphate acetate buffer. Phosphate buffer potassium dihydrogen phosphate buffer having pH 6.8 (concentration 3M) and distilled water was used as a vehicle for method development. Standard stock solution was made with a concentration of 100 µg ml. Solutions were scanned in UV range 200-400 nm. Acamprosate calcium shows absorbance maxima at 217 nm. Working standard solution with concentration from 10-50 µg/ml was prepared from stock solution. The calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. Curve showed linearity with correlation coefficient (r^2) 0.989 at 217 nm. The validated method was successfully used to analyze plasma samples for application in pharmacokinetic studies and effectively employed for quality control analysis of acamprosate calcium in bulk and in tablet dosage form.

Rhee YS (2008) developed a selective and sensitive method employing high-performance liquid chromatography and validated it for the determination of acamprosate calcium in human plasma. Acidified plasma samples were extracted with buffer solution which was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and pH was adjusted to 3.0 using 30% v/v of orhtophosphoric acid in water. The chromatographic

separation was performed on a reverse phase C18 column, with mobile phase acetonitrile and buffer in ratio 70:30 v/v at a flow rate of 1 ml/per minute at a wavelength of 210 nm. The precision was exemplified by relative standard deviation of 0.149%. A good linear relationship was observed for drug between concentration ranges from 75 to 225 µg/ml.

Williams SH *et al*., (2005) selective, precise, accurate and sensitive method employing high-performance liquid chromatography was developed and validated for determination of acamprosate calcium in its tablet dosage form, human plasma, dog plasma and urine. Acidified samples were extracted with buffer solution (which was prepared by adding 2.3 g of disodium hydrogen orthophosphate anhydrous and 1.75 g of potassium dihydrogen phosphate in 1000 ml water and pH was adjusted to 7.00±0.05 by orthophosphoric acid) and methanol in ratio 90:10 v/v. The chromatographic separation was performed on a reverse phase C18 column, Ace Phenyl (250×4.6 mm; 5 µ) at a wavelength of 217 nm at a flow rate of 1 ml/minute. The precision was exemplified by relative standard deviation of less than 0.51%. A good linear relationship was observed for drug having correlation coefficient 0.999.

8.2. Methods for estimation of Naltrexone: [51, 52, 53]

Clydewyn M. Anthony (2008) developed a precise method for determination of naltrexone in tablet dosage form by high-performance liquid chromatography. For this purpose two buffer solutions were used. First one was prepared by dissolving 1.08 g of octane sulfonate and 23.8 g of sodium acetate in 800 ml of water. 1ml of triethylamine and 200 ml of methanol was added to it and pH was adjusted to 6.5±0.1. Second solution was prepared by dissolving 1.08g of octane sulfonate and 23.8g of sodium acetate in 400ml of water. Flow of variable mixture of solution A and B was applied. Zorbax C18 column 3.9 mm×15 cm was used. Wavelength was set 280 nm. The relative standard deviation was not more than 2% and tailing factor was not more than 4.

Srikalyani *et al.*, (2013) developed a simple, specific and accurate high performance liquid chromatographic method for analysis of naltrexone hydrochloride in bulk and dosage forms. Phenomenex C18 column (250×4.6 mm,5 µm) with mobile phase containing 0.05% v/v triethylamine in water(pH 6.5) and acetonitrile (45:55% v/v) in water was used and examined at 215 nm wavelength. The retention time was 3.4 min showed a good linearity in concentration range of 40-200 µg/ml with a correlation coefficient of 0.999.

Neil *et al.*, (2007) A simple, precise, rapid and reproducible reversed phase high performance liquid chromatographic method has been developed for the quantitative estimation of naltrexone hydrochloride in bulk drug and its formulation dosage forms using column Oyster(250×4.6 m,5 µm). Mobile phase consist of combination of ammonium acetate buffer (pH 5.8) and acetonitrile in ratio 60:40 v/v and was pumped at a flow rate of 1.0 ml/min. The injection volume was 10 µl. The detection was carried out at 220 nm and calibration curve was linear in the range of 12-36 µg/ml. The method was validated statistically for its linearity, precision, accuracy. The intra and inter-day variation was found to be less than 1%. Due to its simplicity, rapidness, high precision and accuracy the proposed HPLC method may be used for determining naltrexone in the bulk drug sample and its pharmaceutical formulations.

8.3. Method for estimation of Disulfiram: [54, 55, 56, 57]

Rockville M.D (2004) developed a simple, specific and sensitive high-performance liquid chromatography method for determination of disulfiram in pharmaceutical preparations. Separation of disulfiram was achieved on an Ace C18 column (250×4.6 mms, 5 µm) using UV detection with λ 275 nm. The mobile phase consisted of methanol-water (80:20 v/v). The analysis was performed in less than 5minute at a flow rate of 1.5 ml/min. Calibration curve was linear over concentration range of 0.25-7.5 µg/ml. Intra and inter day precision values for disulfiram were less than from 4.62 and accuracy was better than 4%. Mean recovery of disulfiram was 99.9% for pharmaceutical preparations. The limits of detection and quantification were 0.10 and 0.25 µg/ml. Hence method was successfully applied for quality control of commercial disulfiram dosage form to quantify the drug and to check formulation content uniformity.

8.4. Method for estimation of Fluoxetine: [58, 59]

Wong *et al.*, (2005) carry out analytical method development and validation of fluoxetine in bulk drug and pharmaceutical dosage form. Method is carried out with UV spectroscopy. The developed method can be used for estimation of Fluoxetine in pharmaceutical dosage form. λ_{max} was determined at 226 and 258 nm. Accuracy shows percentage recovery in the range of 97-102%w/w. Relative standard deviation value was not greater than 2%. Correlation coefficient was between 0.997 and 0.825.

Sejal *et al.*, (2009) developed a simple, accurate method for estimation of fluoxetine in pharmaceutical preparations by using high performance liquid chromatography. Mobile phase

used was acetonitrile: methanol: 0.032 M ammonium acetate buffer (45:05:50 v/v/v) at a flow rate of 1.5 ml/min. Separation was done at a wavelength of 235 nm. Accuracy was 101% for Fluoxetine hydrochloride.

8.5. Method for estimation of Ondansetron: [60, 61]

Zalak *et al.*, (2012) developed a simple, precise and rapid reverse phase high performance liquid chromatography method validated for estimation of ondansetron in bulk drug and in a synthetic mixture. The analysis was carried out using Hypersil ODS C18 (250×4.6 mm, 5 µm). The separation was carried out using mobile phase containing buffer adjusted to pH 3.6 and acetonitrile (40:60 v/v) at a flow rate of 1.0 ml/min at a wavelength of 210 nm. The drug was resolved on the stationary phase and retention time was around 4.092 minutes. Hence the method was validated and shown to be linear for Ondansetron. The correlation coefficient of ondansetron was 0.997.

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