ISSN 2347- 6893

How Noscapine metabolise Heme?

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Abstract

Noscapine (NOS) is being used as an antitussive drug for a long time has been recently discovered as a novel tubulin–binding, antiangiogenic anticancer drug that cause cell cycle arrest and induces apoptosis in cancer cells both in vitro as well as in vivo. It is a weak base (pKa~7.8) with 44.69 % absolute bioavailability and half-life of 1.33 h by oral route and requires relatively high doses of NOS (ED_{50}~300 mg/kg bwt) for induction of anticancer activity. In the present study, we conducted experiments, wherein, noscapine was administered according to a dose schedule of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt. Glutathione S-Transferase multigene family plays a critical role in the cellular protection. Toxicity evaluation has yielded that noscapine, do not impinge renal or hepatic toxicity at the dosing regimen (5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 150 mg/kg bwt) undertaken during the course of study. Our results depicted that the antioxidant defense system thereby offering cellular protection.

Keywords: Noscapine, heme metabolism, anti-tumor agent etc.

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Biology

Vol. 2, No. 1

editor@cirworld.com

www.cirworld.com, member.cirworld.com

ISSN 2347- 6893

Introduction

The recent discovery of powerful anticancer properties of noscapine, an isoquinoline alkaloid, is a tribute to this multi-disciplinary approach to cancer therapy. Reports are indicative that Noscapine (NOS) has been used as a cough suppressant in experimental animals including mice, and in humans. [1-5] Reports from studies both in experimental animals and in humans indicate no toxic effects of this drug. Induces conformational change upon binding tubulin and promotes microtubule assembly. Noscapine plays as (i) arrests hela and mouse thymocytes at mitosis; (ii) induces apoptosis in hela and thymocytes cells; (iii) inhibits cell proliferation and; (iv) showed high anti-tumor activity in mice; and (v) eliminate breast tumor by initiating apoptosis in vivo. [1-9]

Toxicological evaluations are commonly used methods for detecting organ effects related to chemical exposure. Disturbance of the balance between the production of reactive oxygen species and antioxidant defences against them produces oxidative stress. [10-22] The antioxidant defence system is sophisticated and adaptive and reduced Glutathione is a central constituent of this system. [21-30] Glutathione detoxifies electrophilic compounds to compete against oxidative stress and to act as cofactor Glutathione S-transferase (GST). The concentration of GSH as free GSH is maintained by GSH reductase (GR) at the expense of cellular NADPH. [31-35]

In our work, we have evaluated the effect of noscapine on oxidative stress and the effect of intraperitoneal administration of noscapine and effect of noscapine at differential dosing regimen on the hepatic and renal GSH and GR activites in Male Wistar rats. We have investigated the organ specific toxicity using serum biochemical parameters. Renal and hepatic short term toxicity was also determined.

Experimental

Experimental animals The present study was approved by the animal care committee of Dr. B. R. Ambedkar center for Biomedical Research, University of Delhi. Male Wista Rats weighing approximately 150g from our laboratory maintained colony were used as experiments; only healthy animals were taken and housed in individual cages having raised wire mesh floors. Animals were housed 3 per cage, standard diet and water. Room temperature was maintained at 25°C±3 with natural daytime and no light after 19h until morning.

Planning of experiment In all experiments, rats were divided into different groups consisting of 2 animals of nearly equal weight, and were treated for 7 and 15 days. Rats were given various doses of noscapine orally, intraperitoneally and sub cutaneous as 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt, 250 mg/kg bwt.

Procedure Mode of sacrifice Animals were fasted 20-24 hours prior to sacrifice. However during fasting period water was available at all times. Animals were anesthetized using chloroform and sacrifice. Blood was drawn from heart, and collected in eppendroff, which were kept for ten minutes at room temperature and then for an hour at 4ºC, and then centrifuged at 800xg for 5 minutes to get serum separated from cellular clot. The animals were immediately dissected to remove their liver tissue, which were washed in ice-cold saline (0.9% NaCl) and extraneous material was removed. Approximately 1g liver tissue was kept for biochemical estimation.

Post mitochondrial supernatant (PMS) preparation Liver was quickly removed, perfuse immediately with ice cold saline (0.9% NaCl) and homogenized in Potter-Elvehjem type glass homogenizer in 0.1M potassium phosphate buffer (pH 7.4) having 0.25M sucrose to give a 20% homogenate. The homogenate was centrifuged at 8000 rpm for 20 minute at 4ºC in an IEC-20 refrigerated centrifuge (rotor No 894) to separate the nuclear debris. The supernatant obtained to 12000 rpm for 30 minutes at 4ºC to obtain post mitochondrial supernatant (PMS), which was used as a source of glutathione s-transferase, catalase and glutathione reductase.

Preparation of microsomal fraction PMS obtained was further centrifuged by ultracentrifuged to 39000rpm for an hour at 4ºC, obtained was taken out and then palate dissolve in homogenizing buffer this is called microsomal fraction.

Biochemical assays

Estimation of Protein 10 µl of tissue homogenate was diluted to 1 ml with water and 5ml alkaline copper sulphate reagent containing sodium carbonate (2%), CuSO4 (1%), and sodium potassium tartrate (2%) was added. After 10 minutes, 0.5ml of Folin's reagent was added, and incubated the mixture for 30 minutes at room temperature, the blue colour developed and the absorbance was read at 660nm. A blank was prepared by using 1 ml of distilled water and analytical reagent. A standard curve was prepared similarly by using Bovine serum albumin (0.1mg/ml) was used as a standard.

Estimation of Catalase activity The assay mixture consisted of 1.95ml phosphate buffer (0.05M, pH 7.0), 1.0ml hydrogen peroxide (0.019M), and0.05ml PMS (10% w/v) in a total volume of 3.0 ml. Changes in absorbance were recorded at 240nm. Catalase activity was calculated in term of nmol H2O2 consumed/min/mg protein.

Estimation of Glutathione reductase activity (GR) The assay system consisted of 1.65ml phosphate buffer (0.1M, pH 7.6). 0.1ml NADPH (0.1mM), 0.1ml EDTA (0.5mM), 0.05ml oxidized glutathione (1mM) and 0.1ml PMS

(10%w/v) in a total volume of 2.0ml. GR activity was quantified at 25 ºC by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidizing/min/mg protein using molar extinction coefficient of $6.22\times10^{-3}M^{-1}cm^{-1}$.

Estimation of Glutathione-S-transferase activity (GST) The reaction mixture consisted of 1.425ml phosphate buffer (0.1M, pH 6.5), 0.2ml reduce glutathione (1mM), 0.025ml CDNB (1-chloro-2,4-dinitrobenzene) 1mM and 0.30ml PMS(10w/v)in a total volume of 2.0ml.The change in absorbance were recorded at 340nm and the enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6\times10^{3}M^{-1}cm^{-1}$.

Estimation of Reduced glutathione activity (GSH) 1.0 ml of post mitochondrial supernatant (PMS) was precipitated with 1.0ml of sulfosalicylic acid (4%). The sample were kept at 4ºC for at least one hour and then subjected to centrifugation at 1200×g for 15 minutes at 4ºC. The assay mixture contained 0.1ml of filtered aliquot, 2.7 ml phosphate buffer (0.1M,pH7.4) and 0.2ml DTNB 5,5'Dithiobis (2-nitro-benzoic acid)(40mg/10ml of phosphate buffer,0.1M, pH7.4) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm in a UV spectrophotometer.

Estimation of Heme Oxygenase Heme oxygenase (HO) is an enzyme that catalyzes the degradation of heme. This results in the degradation of heme and the production of iron, carbon monoxide and biliverdin. The assay system have consisted 90 µl of microsomal as heme oxygenase sample, 150 µl of 0.347 µM NADPH, 50 µl of 0.666 µM hemin, 650 µl of phosphate buffer and 60 µl of biliverdin (cytosolic) fraction. Incubate the following system at 37°C for 15 min., terminate the reaction by keeping in ice and then O.D. should be taken at 455 nm and 520 nm.

Estimation of Cytochrome P450 Cytochrome P450 is an oxidative detoxifying enzyme that catalyzes hydroxylation reaction using NADPH and O2.

$$R\text{-}H + O_2 + NADPH + H^+ \longrightarrow R\text{-}OH + H_2O + NADP + 650$$

Estimation of Serum glutamate pyruvate transaminase, SGPT Principle of UV kinetic method for estimation of ALT:

L–alanine+ α-ketoglutarate ↔ L-glutamate + pyruvate

Pyruvate + NADH +H+ ↔ L- lactate + NAD+

On addition of serum, ALT present in it catalyzes the transamination reaction converting L- alanine (from kit) and α- ketoglutarate (from kit) into L- glutamate and pyruvate. The released pyruvates react with NADH (from kit) in a reaction catalysed by lactate dehydrogenase (LDH) (from kit) to release L- lactate and NAD+. There is a decrease in absorption at 340nm as NADH is converted to NAD+. The rate of decrease in absorption is measured and is proportional to ALT activity in the sample.

Estimation of Serum glutamate oxaloacetate transaminase SGOT:

L-aspartate +α ketoglutarate ⟶ oxaloacetate + L- glutamate

Oxaloacetate +NADH +H ⟶ L- Malate +NAD

On addition of serum, AST present in it catalyzes the transamination reaction converting L- aspartate and α-ketoglutarate into oxaloacetate and L –glutamate. The released oxaloacetate reacts with NADH to release L –malate and NAD+. There is a decrease in absorbance at 340nm as NADH is converted to NAD+. The rate of decrease in absorbance is measured and is proportional to AST activity in the sample.

Estimation of total bilirubin Estimation of total bilirubin is based on the fact that bilirubin reacts with diazotized sulphanilic acid to form an azo compound, the colour of which is measured at 546nm and is proportional to the concentration of bilirubin. For bilirubin the reaction is accelerated by caffeine reagent.

Result & discussion

To protect molecules against toxic radicals and other ROS, cells have developed antioxidant defences that include the enzymes superoxide dismutase (SOD), which destroy toxic peroxides, and small molecules including glutathione. The tripeptide glutathione (GSH), in concert with its reductant NADPH and enzymatic catalysts, can reduce H2O2, lipid peroxidase, disulfides, ascorbate, and free radicals. Glutathione forms conjugates with electrophilic compounds with the help of Glutathione-S-Transferase and Glutathione peroxidases. The product of reaction of GSH with peroxides and disulfides is glutathione disulphide (GSSG) or a GSH adduct of lipid or protein. Glutathione reductase will reduce disulfides using NADPH as a cofactor. A secondary manifestation of cellular free redical stress is the depletion of NADPH needed for GSSG reduction. Cellular transhydrogenases serve to maintain NADH and NADPH in equlibrium; thus, free radical stress can significantly lower the concentration of all reduced pyridine nucleotides in a cell and will affect countless integrated metabolic processes.

ISSN 2347- 6893

Effect on Catalase Activity (Fig. 1)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt increased the activity of catalase by ~1.6 fold as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~23%, ~22%, ~17%, ~11% and ~13% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of catalase by ~27%, ~75%, ~77% and ~64% respectively as compared to control subjects.

A. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 100 mg/kg bwt increased the activity of catalase by ~94%, ~63% and ~3% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~29%, ~26% and ~50% when rats were administrated with the doses of 20 mg/kg bwt, 50 mg/kg bwt and 250 mg/kg bwt respectively.
2. **For 15 days** Administration of NOS at a dose of 5 mg/kg bwt increased the activity of catalase by ~1.1 fold as compared to control. However a marked decreased in the activity of the enzyme was registered by ~47%, ~20% and ~14% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

B. Sub-cutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt and 10 mg/kg bwt increased the activity of catalase by ~22% and ~16% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~32%, ~5%, ~33% and ~46% when rats were administrated with the doses of 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of catalase by ~59%, ~67%, ~44% and ~41% respectively as compared to control subjects.

Effect on Glutathione Reductase Activity (Fig. 2)

A. Oral doses

1. **For 7 days** Administration of NOS at a dose of 5 mg/kg bwt increased the activity of glutathione reductase by ~61% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~80%, ~56%, ~51% and ~4% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively. But there was no remarkable change found in the activity of enzyme when rats were administrated by 50 mg/kg bwt.
2. **For 15 days** Administration of NOS at a dose of 5 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of glutathione reductase by ~41%, ~18% and ~8% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~40% when rats were administrated with the doses of 10 mg/kg bwt.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 20 mg/kg bwt decreased the activity of glutathione reductase by ~94%, ~63% and ~3% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~43%, ~91% and ~1.2 fold when rats were administrated with the doses of 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt increased the activity of glutathione reductase by ~73% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~18%, ~46% and ~11% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

C. Sub-cutaneous doses

1. **For 7 days** Administration of NOS at a dose of 5 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of glutathione reductase by ~3.1 fold, ~2 fold, ~88%, ~99% and ~4.5 fold as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~41% when rats were administrated with the doses of 10 mg/kg bwt.

2. **For 15 days** Administration of NOS at a dose of 5 mg/kg bwt decreased the activity of glutathione reductase by ~65% as compared to control. However, marked increase in activity of enzyme was registered by ~35% and ~8%, when rats were administrated with doses of 10 and 50 mg/kg bwt. But there was no remarkable change found in the activity of enzyme when rats were administrated by 20 mg/kg bwt.

Effect on Glutathione-S-Transferase Activity (Fig. 3)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt decreased the activity of glutathione-S-transferase by ~6% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~1.01 fold, ~21%, ~1.06 fold, ~5.5 fold and ~3.9 fold when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 20 mg/kg bwt decreased the activity of glutathione-S-transferase by ~45%, ~59%, and ~3% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~4% when rats were administrated with the doses of 50 mg/kg bwt respectively.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of glutathione-S-transferase by ~51%, ~58%, ~68%, and ~29% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~4.8 fold and ~5.3 fold when rats were administrated with the doses of 100 mg/kg bwt and 250 mg/kg bwt respectively.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of glutathione-S-transferase by ~3.8 fold, ~2.3 fold, ~1.8 fold and ~3 fold respectively as compared to control subjects.

C. Sub-cutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of glutathione-S-transferase by ~11%, ~49%, ~43%, ~50%, ~2.1 fold and ~2.8 fold respectively as compared to control subjects.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of glutathione-S-transferase by ~1 fold, ~47%, ~91% and ~81% respectively as compared to control subjects.

Effect on Reduced Glutathione Activity (Fig. 4)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of reduced glutathione by ~20%, ~42%, ~40%, and ~3.7% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~2.9 fold and ~2.6 fold when rats were administrated with the doses of 100 mg/kg bwt and 250 mg/kg bwt.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt increased the activity of reduced glutathione by ~9% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~41%, ~54%, and ~60% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

B. Intraperitoneal doses

1. For 7 days Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of reduced glutathione by ~35%, ~54%, ~47%, and ~4% as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~61% and ~1.8 fold when rats were administrated with the doses of 100 mg/kg bwt and 250 mg/kg bwt.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt increased the activity of reduced glutathione by ~29% respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~56%, ~76%, and ~7% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

C. Subcutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of reduced glutathione by ~78%, ~55%, ~40%, ~39%, ~79% fold and ~55% respectively as compared to control subjects.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt decreased the activity of reduced glutathione by ~69% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~13%, ~44%, and ~1.6 fold when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

Effect on Heme-Oxygenase Activity (Fig. 5)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of heme-oxygenase by ~78%, ~70%, ~38%, ~1.2 fold, ~9.6 fold and ~1.22 fold respectively as compared to control subjects.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of heme-oxygenase by ~1.5 fold, ~58%, ~26% and ~1.6 fold respectively as compared to control subjects.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt increased the activity of heme-oxygenase by ~43%, ~1.9 fold, ~2 fold, ~57% and 1 fold as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~9% when rats were administrated with the doses of 10 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt decreased the activity of heme-oxygenase by ~26% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~73%, ~23%, and ~77% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt.

C. Sub-cutaneous doses

1. **For 7 days** Administration of NOS at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt and 100 mg/kg bwt increased the activity of heme-oxygenase by ~2.3 fold, ~1.7 fold, ~1.4 fold, ~2.8 fold and ~49% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~62% when rats were administrated with the doses of 250 mg/kg bwt.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 20 mg/kg bwt increased the activity of heme-oxygenase by ~13%, ~52% and ~35% respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~34% when rats were administrated with the doses of 50 mg/kg bwt respectively.

Effect on Cytochrome P450 Activity (Fig. 6)

A. Oral doses

1. **For 7 days** Administration of NOS at dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased activity of cytochrome P450 by ~55%, ~45%, ~78%, ~45%, ~2.3 fold and ~42% as compared to control subjects.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt and 250 mg/kg bwt decreased the activity of cytochrome P450 by ~40%, ~14%, ~87%, ~2%, and ~7%, as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~10% when rats were administrated with the doses of 100 mg/kg bwt respectively.

C. Sub-cutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 50 mg/kg bwt and 100 mg/kg bwt decreased the activity of cytochrome P450 by ~39%, ~53%, ~46% and ~55% respectively as compared to control

subjects. However a marked increased in the activity of the enzyme was registered by ~41% and ~3% when rats were administrated with the doses of 20 mg/kg bwt and 250 mg/kg bwt respectively.

Effect on ALT (SGPT) Level (Fig. 7)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of SGPT by ~2 fold, ~6%, ~62% and ~3.1 fold respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~16% and ~47% when rats were administrated with the doses of 20 mg/kg bwt and 50 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of SGPT by ~81%, ~22%, ~78% and ~85% respectively as compared to control subjects.

Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt increased the activity of SGPT by ~50%, ~53%, ~1.7 fold, ~3.8 fold, ~1.9 fold and ~5.4 fold respectively as compared to control subjects.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of SGPT by ~50%, ~22%, ~1.9 fold and ~1.7 fold respectively as compared to control subjects.

B. Subcutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt and 10 mg/kg bwt decreased the activity of SGPT by ~33%, and ~6% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~37%, ~59% ~4.7 fold and ~2.1 fold when rats were administrated with the doses of 20 mg/kg bwt, 50 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of SGPT by ~57%, ~4.3 fold, ~57% and ~57% respectively as compared to control subjects.

Effect on AST (SGOT) Level (Fig. 8)

A. Oral doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt and increased the activity of SGOT by ~1.6 fold, ~8%, and ~23% respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~45%, ~12% and ~35% when rats were administrated with the doses of 10 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt respectively.
2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of SGOT by ~44%, ~1.8 fold, ~2.5 fold and ~1.2 fold respectively as compared to control subjects.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt and increased the activity of SGOT by ~2.3 fold, ~5.8 fold and ~4 fold respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~33%, ~53% and ~35% when rats were administrated with the doses of 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt respectively.
3. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt and 10 mg/kg bwt decreased the activity of SGOT by ~36% and ~54% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~1.1 fold and ~1.8 fold when rats were administrated with the doses of 20 mg/kg bwt and 50 mg/kg bwt respectively.

C. Sub-cutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of SGOT by ~28%, ~14%, ~42% and ~24% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~2.5 fold and ~1.2 fold when rats were administrated with the doses of 100 mg/kg bwt and 250 mg/kg bwt respectively.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 20 mg/kg bwt decreased the activity of SGOT by ~12% ~9% and ~61% respectively as compared to control subjects. However a marked increased in the activity of the enzyme was registered by ~90% when rats were administrated with the doses of 50 mg/kg bwt respectively.

Effect on Total Bilirubin Level (Fig. 9)

A. Oral doses

1. **For 7 days** Administration of NOS at a dose of 5 mg/kg bwt, 10 mg/kg bwt and 50 mg/kg bwt and increased the activity of total bilirubin by ~4.1 fold, ~1.3 fold, and ~33% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~46%, ~55% and ~63% when rats were administrated with the doses of 20 mg/kg bwt, 100 mg/kg bwt and 250 mg/kg bwt.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt decreased the activity of total bilirubin by ~87%, ~80%, ~91% and ~94% respectively as compared to control subjects.

B. Intraperitoneal doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 20 mg/kg bwt, 50 mg/kg bwt and 250 mg/kg bwt increased the activity of total bilirubin by ~1.9 fold, ~29%, ~2 fold and ~77% respectively as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~34% and ~32% when rats were administrated with the doses of 10 mg/kg bwt and 100 mg/kg bwt.

2. For 15 days Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of total bilirubin by ~24%, ~14.3 fold, ~9.1 fold and ~3.2 fold respectively as compared to control subjects.

C. Sub-cutaneous doses

1. **For 7 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 50 mg/kg bwt and 100 mg/kg bwt increased the activity of total bilirubin by ~1.4 fold, ~74%, ~41% and ~14% as compared to control subjects. However a marked decreased in the activity of the enzyme was registered by ~46% and ~22% when rats were administrated with the doses of 20 mg/kg bwt and 250 mg/kg bwt.

2. **For 15 days** Administration of noscapine at a dose of 5 mg/kg bwt, 10 mg/kg bwt, 20 mg/kg bwt and 50 mg/kg bwt increased the activity of total bilirubin by ~4 fold, ~9.8 fold, ~3 fold and ~3.4 fold respectively as compared to control subjects.

Summary

Catalase activity increases at lower dose but decreases at higher doses given orally whereas its activity increases at 5 and 10 mg/kg bwt and the decreases at higher doses when administrated by I.P. and S.C. route. Hence, 5 mg/kg bwt is good as higher doses increase the catalase activity. On comprising the data by route of administration the best route are oral and I.P. Glutathione Reductase activity increases at 5 and 10 mg/kg bwt decreases at higher doses whereas it decreases at doses at 5, 10, 20, and 50 when given I.P. However, when administration subcutaneously. In case of glutathione, S transferase, oral administration decreases at 5 mg/kg bwt and increases significantly at 10, 20, 50, 100 and 200 mg/kg bwt. However, when given I.P. at 5, 10, 20 and 50 mg/kg bwt and increases significantly at 100 and 250 mg/kg bwt subcutaneously administrated GST increases significantly at all doses. Reduced Glutathione, on oral administration RG activity decreases at 5, 10, 20, 50 mg/kgwt whereas it increases at 100 and 250 mg/ kg bwt, it shows the same patterns with I.P. administration, however on subcutaneously administration, it increases at all doses. The elevation of GR, GST and GSH activity can be contemplated to exert a protective role against the cytotoxic or carcinogenic effect of compounds, which are activated by the biotransformation enzymes of the endoplasmic reticulum, by scavenging the electrophilic metabolites. Heme oxygenases, when given orally and subcutaneously, Noscapine showed markedly increases in heme, whereas when given I.P., it decreases at 5 and 10 mg/ kg bwt, and increases significantly at 20, 50, 100 and 250 mg/kg bwt.

Acknowledgements

We are thankful to UGC, New Delhi, India, for providing the spectral, analytical facilities, biological activity and funding, respectively.

Reference:

1. Hartwell LH. and Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science; 1989; 246, 629.
2. Murray A W. Cell cycle checkpoints Curr. Opin. Cell Bio I.; 1994, 6; 872.
3. Bose R, Verheij M, Haimovitz-Friedman A, Scotto K, Fuks Z and Kolesnick R Ceramide synthase mediates daunorubicin-induced apoptosis: An alternative mechanism for generating death signals Cell; 1995; 82; 405.
4. Ye K, Ke V, Keshava N, Shanks J, Kapp JA, Tekmal RR, Petros J and Joshi HC Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells Proc. Natl. Acad. ScL USA; 1998; 95; 1601-06.
5. Ye K, Ke V, Hans EG, David RA, Joshi HC and Kapp JA Noscapine inhibits tumor growth with little toxicity to normal tissues or inhibition of immune responses Cancer Immunol. Immunother.; 2000; 49; 217.
6. Perkin W.H and Robinson Synthesis and resolution of gnoscopine (dl-narcotine) J. Chem. Soc. (London); 1911; 99; 775.
7. Aneja, R,; Dhiman, N.; Idnani, J.; Awasthi, A.; Arora, S.K.; Chandra, R.; Joshi, H.C. Preclinical pharmacokinetics and bioavailability of noscapine, a tubulin–binding anticancer agent. Cancer Chemother Pharmacol. 2007, 60, 831-839.
8. Aneja, R.; Zhou, J.; Vangapandu, S.N.; Zhou, B.; Chandra, R.; Joshi, H.C. Drug-resistant T-lymphoid tumors undergo apoptosis. Blood 2006, 107, 2486-2492.
9. Goodman LS and Gilman A In: "The Pharmacological Basis of Therapeutics" 3rded. MacMillan, NewVork; 1965; 281.
10. Winter CA and Flataker L Antitussive compounds: testing methods and results. J. Pharmacol. Expt. Therp.; 1954; 112; 99.
11. Bickerman HA and Barach A The experimental production of cough in human subjects induced by citric acid aerosols; preliminary studies on the evaluation of antitussive agents. Am. J. Med. Sci; 1954; 228; 156.
12. Ke, Y.; Ye, K.; Grossniklaus, H.E.; Archer, D.R. Joshi, H.C.; Kapp, J.A. Noscapine inhibits tumor growth with little toxicity to normal tissues or inhibition of immune responses. Cancer Immunol. Immunother. 2000, 49, 217–225.
13. Winter CA and Flataker L. Toxicity studies on noscapine. Toxicol. Appl. Pharmacol.; 1961; 3; 96.
14. Zhou, J. and Giannakakou, P. Anticancer agents targeting microtubules for cancer chemotherapy. Curr. Med. Chem. 2005, 5, 65-71.
15. Zhou, J.; Gupta, K.; Aggarwal, S.; Aneja, R.; Chandra, R.; Panda, D.; Joshi, H.C. Brominated derivatives of noscapine are potent microtubule- interfering agents that perturb mitosis and inhibit cell proliferation. Mol. Pharmacol. 2003, 63, 799–807.
16. Bickmann Ha, German E, Cohen Bm And Itkin Se The cough response of healthy human subjects stimulated by citric acid aerosol. II. Evaluation of antitussive agents. Am. J. Med. Bio.; 1957; 234; 191
17. La Barre J, Plisnier H. A propos des propriétés antitussigènes et bronchodilatatrices du chlorhydrate de narcotine. Arch. Int. Pharmacol.; 1959; 119; 205.
18. Zhou, J.; Gupta, K.; Yao, J.; Ye, K.; Panda, D.; Giannakakou, P.; Joshi, H.C. Paclitaxel-resistant human ovarian cancer cells undergo c-Jun NH2-terminal kinase-mediated apoptosis in response to noscapine. J. Biol. Chem. 2002, 277. 39777–39785.
19. Zhou, J.; Liu, M.; Luthra, R.; Jones, J.; Aneja, R.; Chandra, R.; Tekmal, R.R.; Joshi, H.C. EM012, a microtubule-interfering agent, inhibits the progression of multidrug-resistant human ovarian cancer both in cultured cells and in athymic nude mice. Cancer Chemother. Pharmacol. 2005, 55, 461–465.
20. Lasagne L, Owens AH, Shnider BI and Gold GL Toxicity after large doses of noscapine. Cancer Chemother. Res.; 1961; 15; 33.
21. World Health Organisation Wld. Hlth. Org. Tech.Rep. Ser.; 1959; 160; 10.
22. Belville JW, Wallenstein SL, Wald GH, Dowling MD, Hande RW. Effect of noscapine and codeine on the respiratory response to carbon dioxide. Anesthesiology; 1958; 19; 545.
23. Aneja, R.; Vangapandu, S.N.; Lopus, M.; Chandra, R.; Panda, D.; Joshi, H.C. Development of a novel nitro-derivative of noscapine for the potential treatment of drug resistant ovarian cancer and T-cell lymphoma. Mol. Pharmacol. 2006, 69, 1801-1809.
24. Aneja, R.; Zhou, J.; Zhou, B.; Chandra, R.; Joshi, H.C. Treatment of harmone-refractory breast cancer: apoptosis and regression of human tumours implanted in mice. Mol. Cancer Ther. 2006, 5, 2366-2377.

25. Harris EA, Slawson KB Brit. The respiratory effects of therapeutic doses of cyclobarbitone, triclofos and ethchlorvynol. J. Pharmacol. 1965; 24; 214-222.

26. Harris EA Brit. The effects of noscapine and codeine on the ventilatory responses to excess of carbon dioxide and lack of oxygen. J. Pharmacol. 1965; 24; 532-537.

27. Bickerman HA and Barach AL The experimental production of cough in human subjects induced by citric acid aerosols; preliminary studies on the evaluation of antitussive agents. Am. J. Med. Sci.; 1954; 228; 156.

28. Empey EW, Laitinen LA, Young GA, Bye CE and Hughes DTD. Comparison of the antitussive effects of codeine phosphate 20 mg, dextromethorphan 30mg and noscapine 30 mg using citric acid induced cough in normal subjects. Eur. J. Clin. Pharmacol.; 1979; 16; 393-397.

29. Chau TT, Carter FE and Harris L Opiate-induced enhancement of the effects of naloxone on serum luteinizing hormone levels in the male rat: specificity for Mu agonists J. Pharmacol. Exp. Ther.; 1983; 226; 108.

30. Craviso GL and Musacchio JM High-affinity dextromethorphan binding sites in guinea pig brain. I. Initial characterization Mol. Pharmacol.; 1983; 23; 619-628.

31. Balint G and Rabloczky G Antitussive effect of autonomic drugs. Acta Physiol. Acad. Sci. Hung.; 1968; 33; 99-109.

32. Craviso GL and Musacchio JM High-affinity dextromethorphan binding sites in guinea pig brain. II. Competition experiments Mol. Pharmacol.; 1983; 23; 629-640.

33. Karlsson MO, Dahlstrom B and Neil A Characterization of high-affinity binding sites for the antitussive [3H]noscapine in guinea pig brain tissue Eur. J. Pharmacol.; 1988; 145; 195.

34. Kardos J, Blasko G, Kerekes P, Kovas I and Simonyi M Inhibition of [^3H]GABA binding to rat brain synaptic membranes by bicuculline related alkaloids Biochem. Pharmacol.; 1984; 22; 3537.

35. Mourey RJ, Dawson TM, Barrow RK, Enna AE and Synder SH [3H]noscapine binding sites in brain: relationship to indoleamines and the phosphoinositide and adenylyl cyclase messenger systems Mol. Pharmacol.; 1992; 42; 6A.

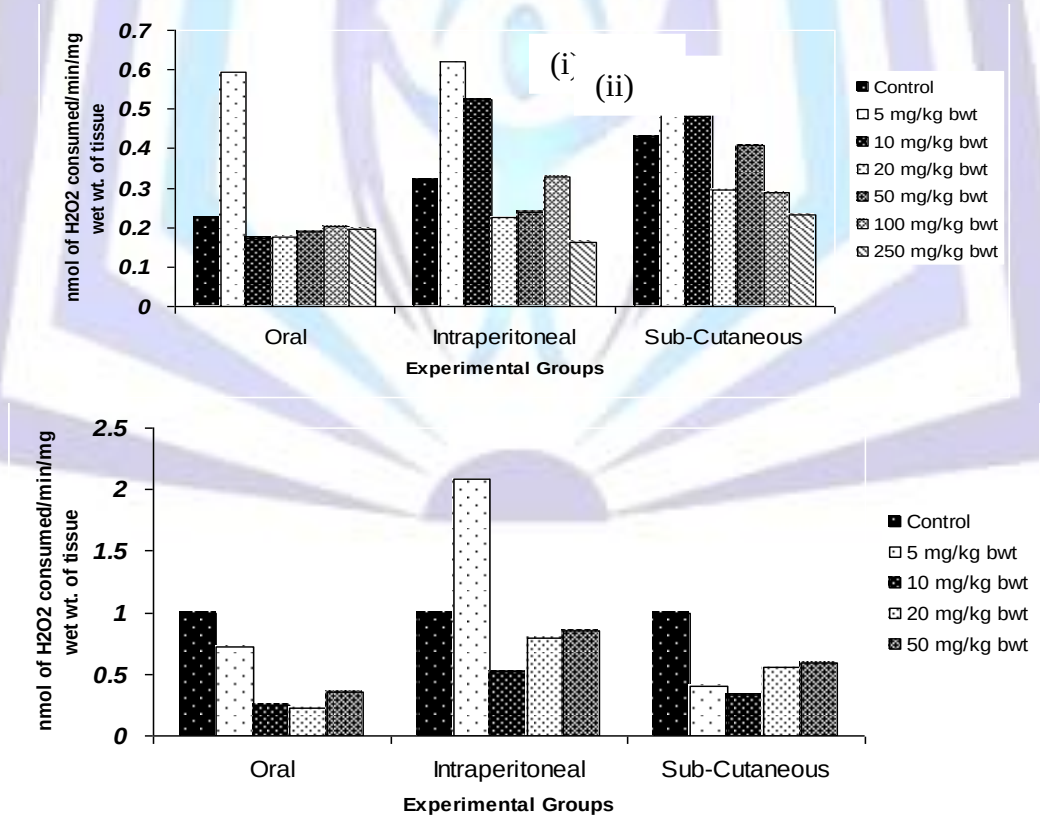

Fig. 1. Catalase activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups

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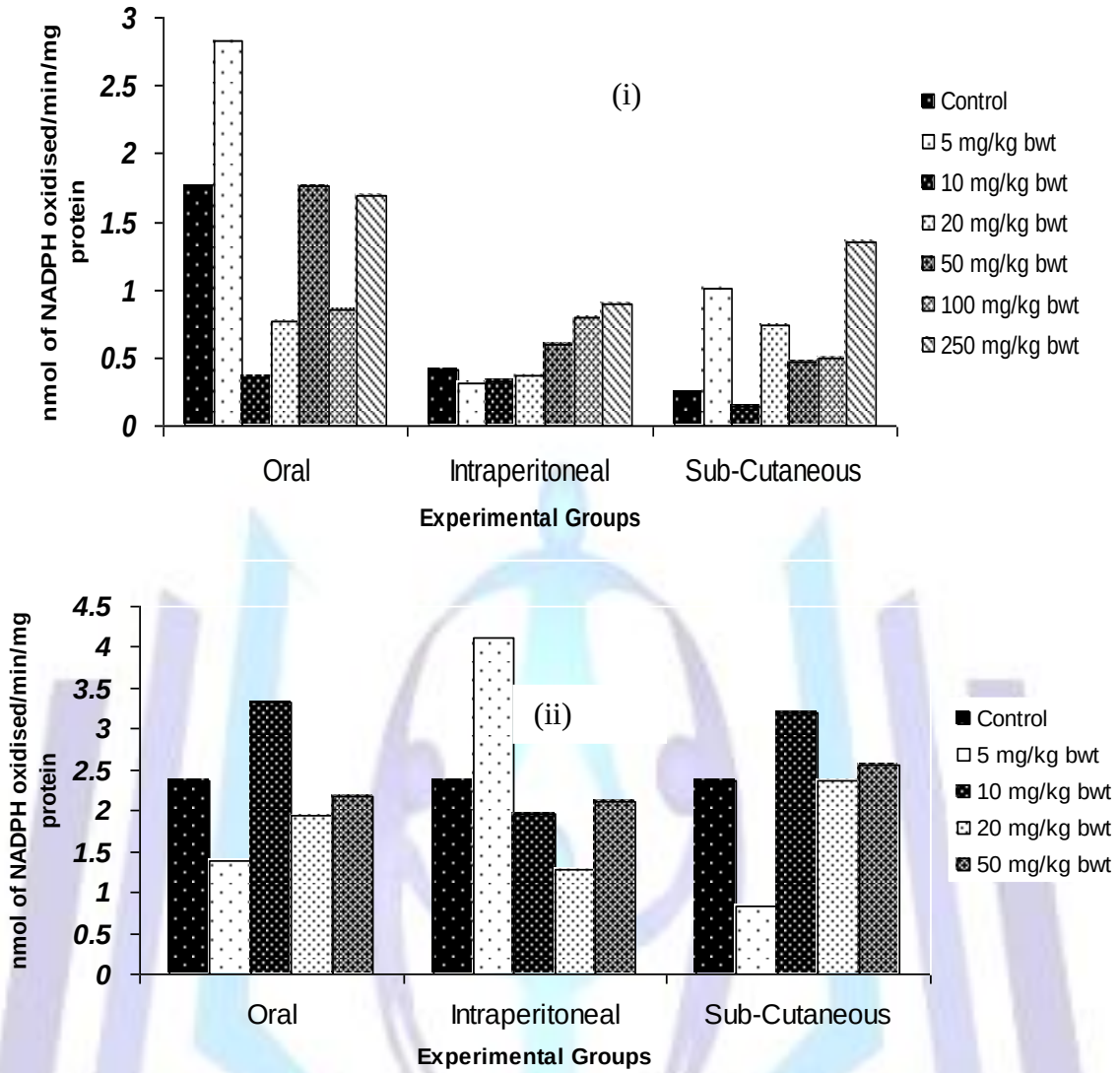
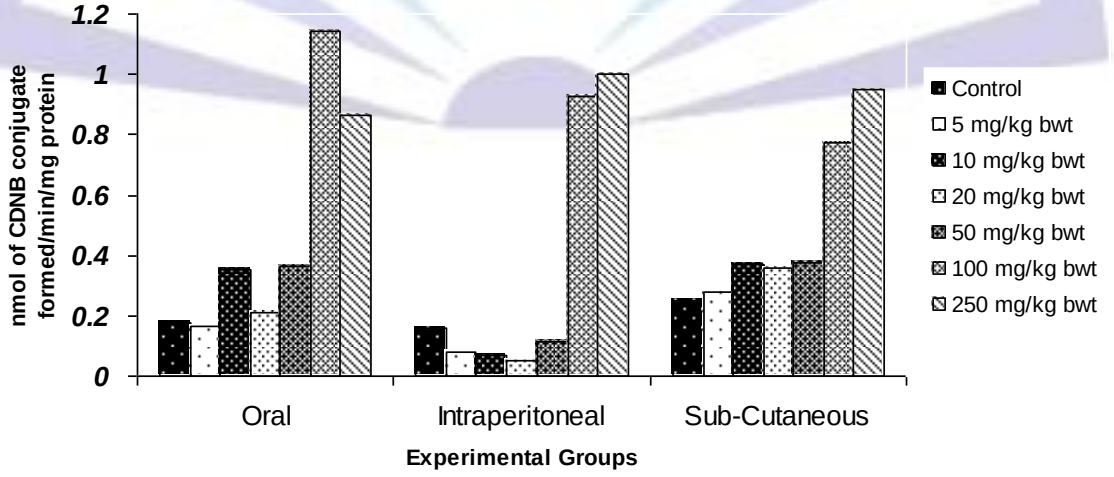


Fig. 2. Glutathione reductase activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups



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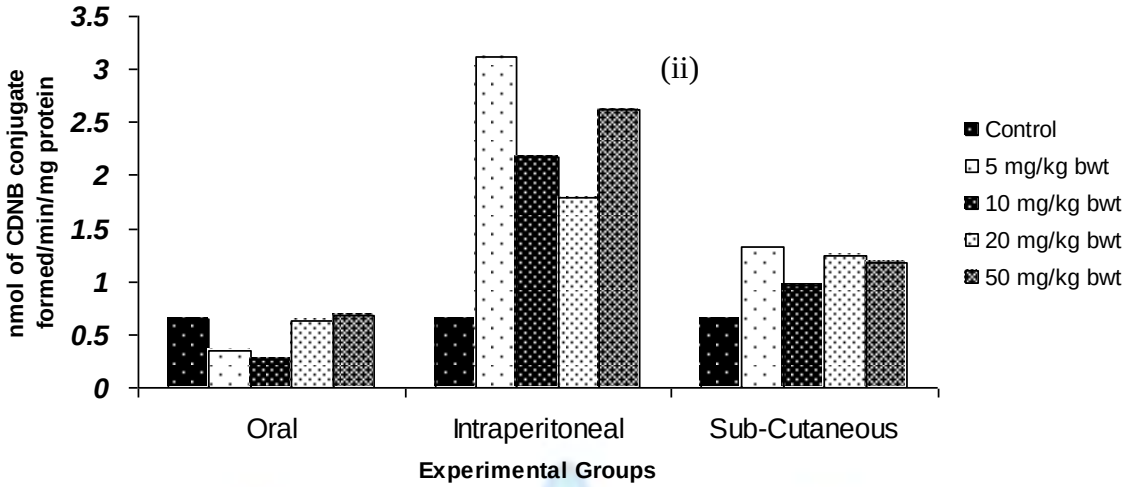

Fig. 3. Glutathione S-transferese activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups

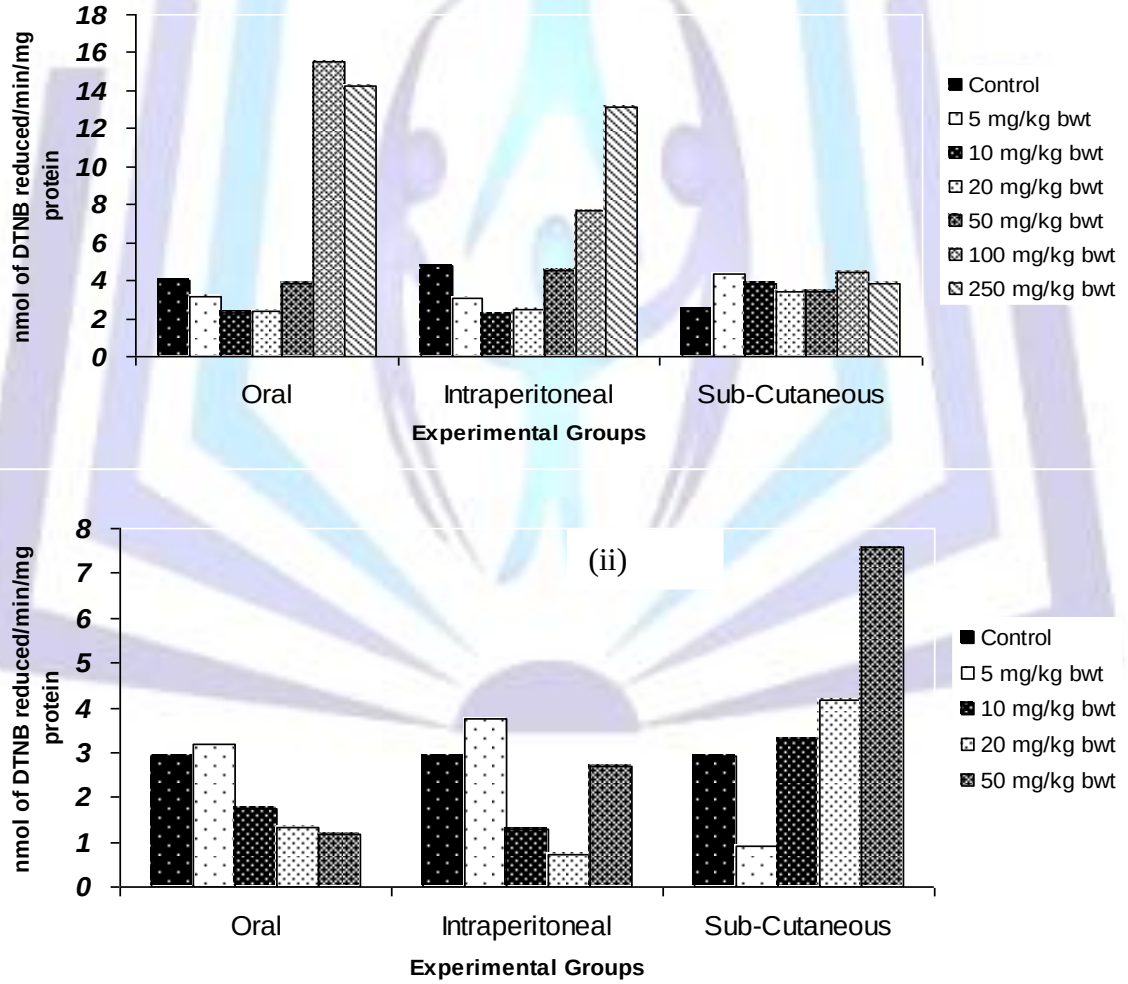

Fig. 4. Reduced Glutathione activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups

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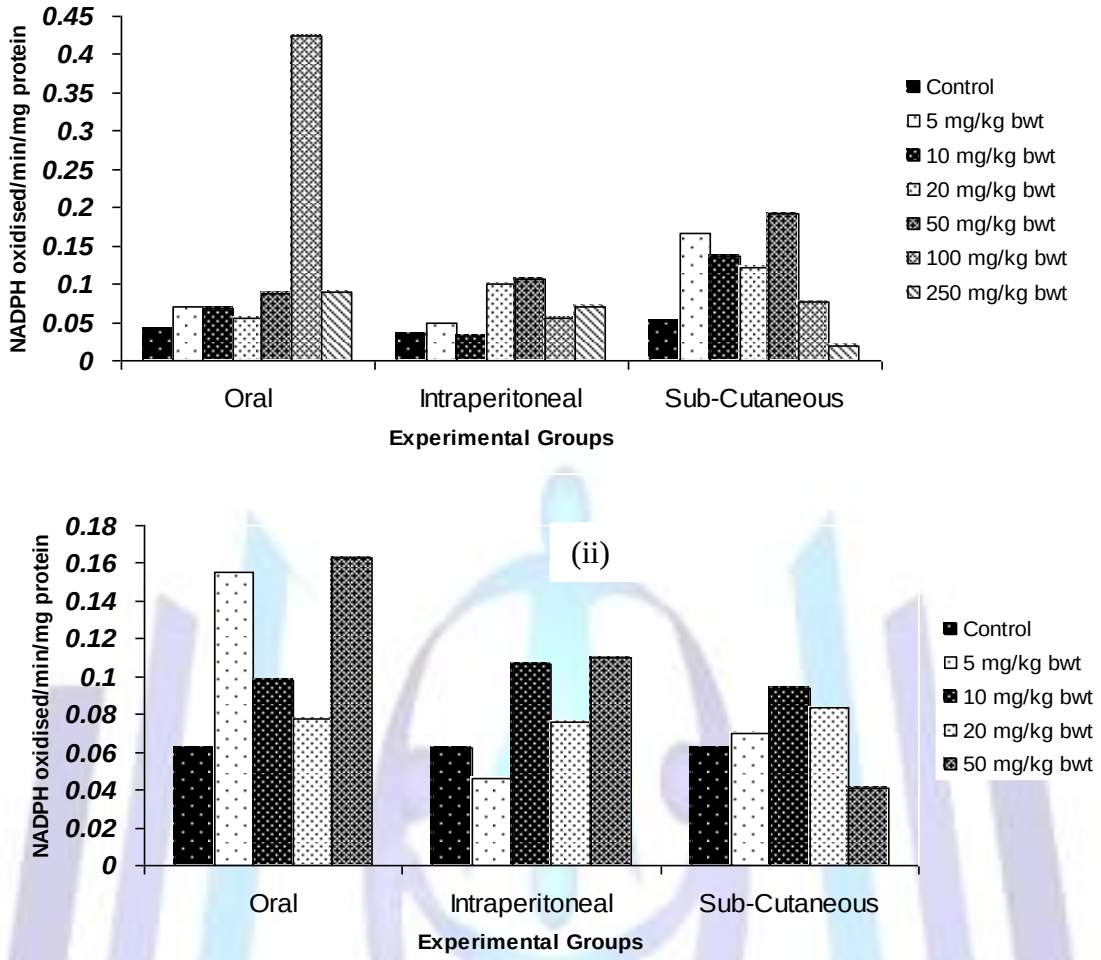

Fig. 5. Heme oxygenase activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups

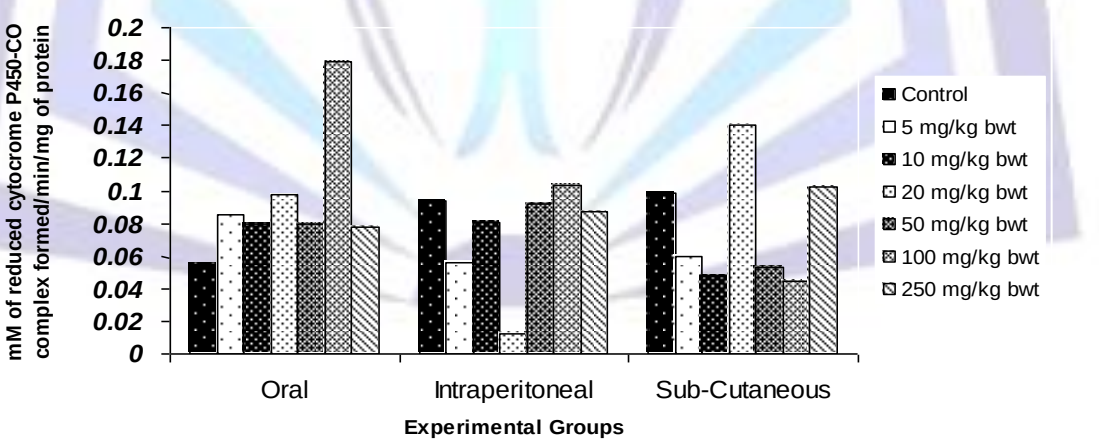

Fig. 6. Cytochrome P450 activity after 7 days of oral, intraperitonal and sub-cutaneous experimental groups

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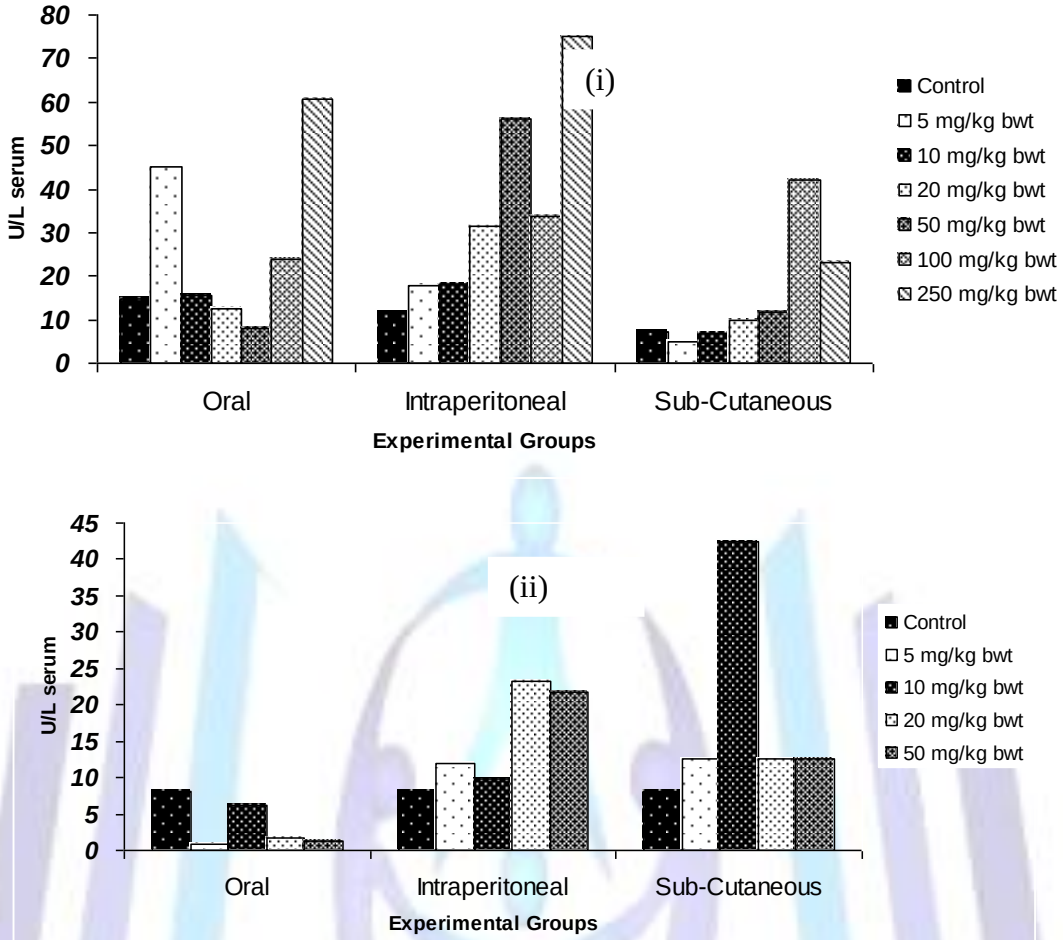
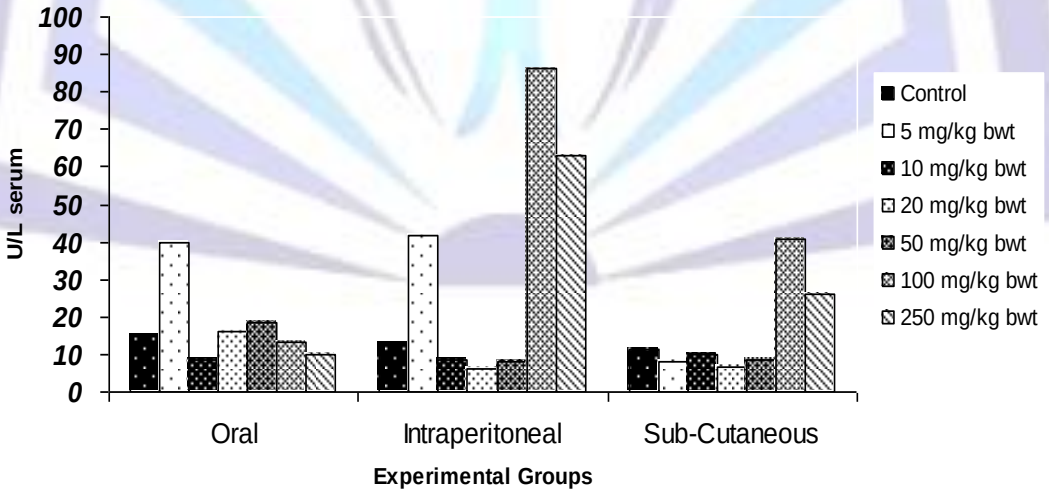


Fig. 7. Serum Glutathione pyruvate tramsaminase activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups



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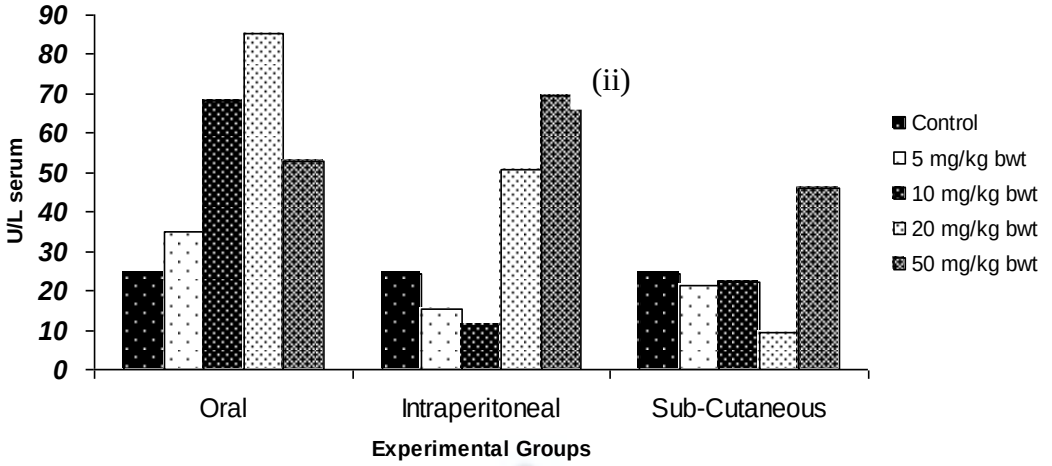

Fig. 8. Serum Glutathione oxaloacetate transaminase activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups

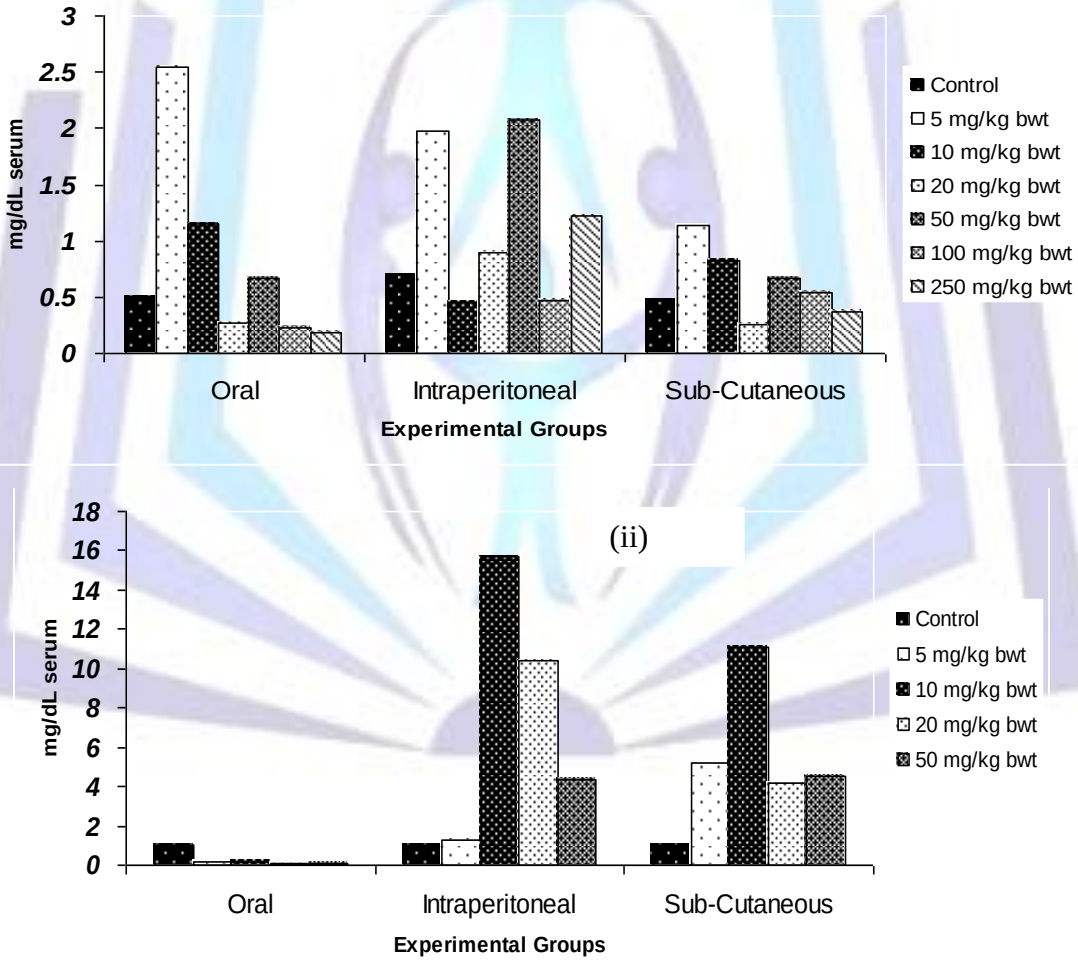

Fig. 9. Total billrubin activity after (i) 7 and (ii) 15 days of oral, intraperitonal and sub-cutaneous experimental groups