

Mitragyna speciosa  
korth alkaloid extract  
and cytotoxicity  
biology essay



**ASSIGN  
BUSTER**

Nonstandard abbreviations: MSE, Mitragyna speciosa alkaloid extract, DMSO, dimethyl sulfoxide; HEK293, human embryonic kidney cells; HepG2, human hepatocellular carcinoma cells; HCT116, colon carcinoma cells; SH-SY5Y, neuroblastoma cells; A498, renal cell carcinoma; SK-MEL-28, melanoma cells; MEM, Eagle's Medium Essential Medium; FBS, fetus bovine serum; DMEM, dulbecco's modified Eagle's medium; IC50, half maximal inhibitory concentration .

Mitragyna speciosa has great potential to be used as opium substitute and treating opioid abstinence syndrom > Cytotoxicity of the methanolic extract was examined using XTT assay against 6 human cancer cell lines > Tested with 10 concentrations of plant extract from 0. 05 to 300µg/ml > Acute and Chronic in vitro study > Acute dermatotoxicity, chronic and moderate hepatotoxicity, nephrotoxicity and neurotoxicity were reported.

### **Abstract:**

Ethnopharmacological relevance: Kratom, or Mitragyna speciosa Korth, has been reported as a mu- and delta- opiate receptor agonist producing similar effects as those produced by morphine. It has been traditionally utilized as an opium substitute or to alleviate opiate withdrawal symptoms. In spite of its relatively common usage, toxicological data of this plant is still lacking.

Aim: The aim of the study is to determine the acute and chronic cytotoxicity of Mitragyna speciosa Korth alkaloid extract in vitro in various human cancer cell lines.

Methodology: Cells were treated with 0. 05 to 300µg/ml of Mitragyna speciosa Korth alkaloid extract. The effect of the alkaloid extract on

<https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

proliferative responses of the cancer cell lines were assessed by cell proliferation cytotoxicity assay (XTT) after 24, 48 and 72 hours. Untreated cells were used as control.

Results and conclusion: Significant growth inhibitory activity of the alkaloid extract on cancer cell lines were noted. The *Mitragyna speciosa* alkaloid extract showed acute potential toxicity to the skin melanoma cells (SK-MEL-28) after 24 hours incubation with IC<sub>50</sub> value of  $16.15 \pm 1.45 \mu\text{g/ml}$ . Furthermore, moderate chronic hepatotoxicity, nephrotoxicity, neurotoxicity, embryotoxicity and colon toxicity was reported in this study.

Key words: *Mitragyna Speciosa*, cytotoxicity, XTT, cancer cell lines, IC<sub>50</sub>.

## **Introduction**

*Mitragyna speciosa* Kroth (from the Rubiaceae family) is an indigenous plant which is found mainly in the northern Malaysia peninsula and in central and southern parts of Thailand (Suhanya et al., 2009). It is known as “ Biak-biak” and “ Ketum” in Malaysia, and as “ Kratom” in Thailand (Saidin et al., 2008). The major indole-alkaloid constituent found in the *Mitragyna speciosa* is mitragynine, a mu- and delta-opioid receptor agonist. (Juzaili et al., 2010). Studies have shown the psychoactive properties of mitragynine is linked with it's high affinity for opiate receptors, hence indicating it's potential for treating opiate addiction as replacement therapy (Babu et al., 2008).

Traditionally, leaves from this plant have been consumed by the Thai and Malaysian natives mainly to treat diarrhoea, and to produce a stimulant and euphoric effect to combat fatigue and to increase tolerance to the hot sun (Kavita et al., 2008). Furthermore, the leaves have been reported to possess <https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

opium-like properties in which its stimulating effect was found in low dose. High doses of the *Mitragyna speciosa* extract can cause analgesia and hallucination (Suchitra et al., 1998). Due to its unique medicinal properties, kratom has been widely used to treat pain and opium withdrawal symptoms since the nineteenth century. However, addiction and several opioid abstinence syndrome such as irritability, yawning, rhinorrhoea, myalgias, diarrhoea, tremor, nausea, nystagmus, and arthralgia were reported among the chronic users (Kavita et al., 2008). Anorexia, weight loss, skin darkening and psychosis have also been reported (Suwanlert, 1975).

Although, the pharmacological effects of Kratom in human and experimental animals are well established, the doses required to produce toxicity still remain poorly defined. The only toxicological study by Harizal et al. (2010), standardized methanol extract of *Mitragyna speciosa* Korth caused mild nephrotoxicity and severe hepatotoxicity at doses higher than 1000 mg/kg (Harizal et al., 2010). No acute neurotoxicity effects were found in the cortex and hippocampus of the rat (Harizal et al., 2010).

The issue of misuse of the plant by the drug addicts has caused major concerns in Malaysia and Thailand. Consequently, Kratom plant has been listed as a controlled substance in Malaysia, Thailand and Australia. However, in other parts of the world, kratom is currently not strongly regulated. The availability of kratom over the internet has caused significant drug abuse issue, such as self-treatment in opioid withdrawal and in chronic pain (Kavita et al., 2008; Somsorn et al., 2008). The main objective of this study is to investigate the potential acute and chronic in vitro cytotoxicity of

Mitragyna Speciosa Korth alkaloid extracts against different human cancer cell lines in order to estimate its potential toxicity to humans.

## **2. Methodology**

### **2.1 Preparation of Mitragyna speciosa Korth alkaloid extract**

Fresh leaves of Mitragyna Speciosa Korth were collected from the forest in Perlis, Malaysia. Methanol-chloroform extraction method was used to extract the alkaloid compounds. The leaves (5kg) were dried and soaked in methanol for 3 days. The methanol mixture was filtered and the filtrate evaporated using a rotary evaporator. The extraction and evaporation procedure was repeated three times. Following that, the crude methanol extract was re-dissolved in 10% acetic acid and then washed with hexane. The acidic layer was basified to pH 9 using ammonia hydroxide and extracted with chloroform. The collected organic layer was filtered through sodium sulphate anhydrous and the filtrate was extracted using a rotary evaporator to obtain 5.86g of crude alkaloid extract. The alkaloid extract was then identified using Dragendorff test (Juzaili et al., 2010). Lastly, the extract was redissolved in dimethylsulphoxide (DMSO) to form the stock solution, following which, it was filtered using 0.2 $\mu$ m syringe filter before being tested on the different cell lines.

### **2.2 Cell Culture**

The effect of alkaloid extract on cell viability was assessed in a cell culture system using cells from human embryonic kidney cells (HEK293), human hepatocellular carcinoma cells (HepG2), colon carcinoma cells (HCT116), neuroblastoma cells (SH-SY5Y), renal cell carcinoma (A498) and melanoma <https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

cells (SK-MEL-28) which were obtained from American Type Culture Collection (ATCC) and Aurigene Accelerating Discovery Ltd, Bangalore, India. HepG2, A-498, and SK-MEL-28 cell lines were grown in Eagle's Medium Essential Medium (MEM); with 10% fetus bovine serum (FBS) and supplemented with 1% of 100x penicillin-streptomycin antibiotic, 2mM glutamine, 1% non-essential amino acid and 1% sodium pyruvate. Meanwhile, HEK293 cell line was grown in Dulbecco's modified Eagle's medium (DMEM) with high glucose content (4.5g/L). SH-SY5Y cell line was cultured in 1:1 mixture of MEM and Ham's F12 medium. HCT116 cell line was grown in McCoy's 5a medium. The medium for cryopreservation contained 20% FBS and 5-8% DMSO in growth medium. All the cell lines were maintained at 37°C in a 5% CO<sub>2</sub> atmosphere with 95% humidity. At 60-70% confluence, cells were trypsinized and seeded in 96-well plates in respective optimal cell density. Twenty-four hours after the cell seeding, cells were treated with various concentrations of plant extracts.

## **2.3 cell proliferation (XTT) assays**

The cell viability of the cancer cell lines were determined by using cell proliferation XTT assays in which the viable cells were determined by the amount of mitochondrial dehydrogenases released in the living cells. Mitochondrial dehydrogenase of the variable cells cleave the tetrazolium ring of XTT (2, 3-bis[2-Methoxy-4-iodo-5-sulfophenyl]-5-methyltetrazolium-5-carboxyanilide inner salt) yielding orange formazan crystals which can be measured by a spectrophotometer (Scudiero, 1988; Weislow, 1989). An increase or decrease in living cell numbers result in a concomitant change in the amount of formazan formed, indicating the level of cytotoxicity caused

by the plant extract. The cells were seeded into 96 wells plates in respective optimal cell density and incubated for 24 hours to get 70% confluent. Following that, cells were incubated with various concentrations of plant extract (0.05-300µg/ml) for 24 hours, after which, plant extract was removed from each well and then washed with phosphate-buffered saline (PBS). After incubation, for every 100µl of serum free medium, 50µl of XTT solution with phenazine methosulphate (PMS) was added, and incubated for a further 4 hours at 37°C. The absorbance of the samples were checked at 465nm using fluorescence spectrophotometry. Untreated cells were used as control. Cell viability was determined after 24, 48 and 72 hours incubation time and defined as a percentage of cell survival (ratio of absorbance of treated cells to untreated cells). All data were recorded as mean  $\pm$  SEM of triplicate measurements.

### **3. Results**

#### **3.1 Preparation of alkaloid extract**

One kilogram of dried powdered mitragyna speciosa leaves (approximately 5kg of fresh leaves) gave a crude extracts of 105g methanolic extract. From the crude methanolic extract, 5.86g of alkaloid extract was obtained (0.12% yield of fresh leave weight). The amount of mitragynine exist in the alkaloid extract was approxiamately 22-24% (Juzaili et al., 2010)

#### **3.2 Cytotoxic activity of Mitragyna speciosa alkaloid extracts on cancer cell lines**

The results depicted in Table 1, Figure 1 and 2 summarize the cytotoxic effects of the alkaloid extract on HepG2, A498, SK-MEL-28, HEK293 HCT116, and SH-SY5Y cell lines. Table 2 indicated the classification of cytotoxicity for <https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

natural products. Untreated cells were used as control. The IC<sub>50</sub> results (mean ±SEM) are listed as the percentage of cell survival after exposure to different concentrations of MSE and determined at three incubation times. Based on the figure 1 results, XTT assay showed that alkaloid extract caused a significant cytotoxic effect from 0.05 to 300 µg/ml.

### **3. 2. 1 HCT116 cells**

Within 24hrs, there was a clear dose-dependent inhibition of cell proliferation compared to the control (Fig. 2a) and the effect become pronounced at the doses higher than 11µg/ml. By 48 hrs, proliferation of cells treated with lower concentration of alkaloid extract (≈ 33µg/ml) has recovered. However, cell proliferation inhibition remained observed at other extract concentration. Approximately 100% of mortality rate was observed with highest concentration of alkaloid extract, 200 µg/ml. The IC<sub>50</sub> for the cell at 24 and 72 hrs period were 62 and 43µg/ml respectively (Table 1).

### **3. 2. 2 HEK293 cells**

With HEK293 cells, alkaloid extract of *Mitragyna speciosa* caused a dose-dependent inhibition of cell proliferation at all incubation times. As with other cells, cell proliferation recovering was observed at lowest concentration after 48 hrs. At higher doses of extract (> 33µg/ml), cell proliferation was inhibited. After 72 hrs, approximately 100% cells were dead at highest dose, > 100µg/ml (Fig. 2b). The IC<sub>50</sub> value in this cell was estimated as 46 µg/ml in 24 hr and 27 µg/ml in 72 hrs (Table 1).



### **3. 2. 3 SH-SY5Y cells**

With SH-SY5Y cells, low doses of alkaloid extract (1. 23 - 11. 11  $\mu\text{g/ml}$ ) slightly increased the cell proliferation up to 48 hrs (Fig. 2c). However, higher doses ( $> 33\mu\text{g/ml}$ ) inhibited the cell growth substantially within 24 hrs. The IC50 of the cells after 24 and 72 hrs treatment were 66 and  $34\mu\text{g/ml}$ , respectively (Table 1).

### **3. 2. 4 HepG2 cells**

Alkaloid extract treated HepG2 cells had shown the same pattern of cell proliferation inhibition as other cell lines. At concentration higher than  $33\mu\text{g/ml}$ , there was a prominent cell death throughout the experiment (Fig. 2d). Low or no inhibition was found at the two lowest dose. The IC50 for HepG2 cells at 24 and 72 hrs treatment were approximately 49 and  $27\mu\text{g/ml}$ , respectively (Table 1).

### **3. 2. 5 SK-MEL-28 cells**

For SK-MEL-28 cells, acute cytotoxicity was observed within 24 hrs treatment (Fig. 2e). Almost 100% of cell death was reported at 2 highest doses (100 and  $200\mu\text{g/ml}$ ). Low to moderate inhibition was noted at concentration lower than  $33\mu\text{g/ml}$ . Simultaneously, cells were recovered at low concentration of extract. The IC50 for the cells was  $16\mu\text{g/ml}$  approximately at all the time points (Table 1).

### **3. 2. 6 A498 cells**

With A498 cells, dose-dependent inhibition of cell proliferation became prominent after  $100 \mu\text{g/ml}$  (Fig 2f). Low or no inhibition of cell proliferation was observed at concentration lower than  $33\mu\text{g/ml}$ . However, there was a

sudden decreasing in cell proliferation after 48 hrs of high dose-treatment (100µg/ml). The IC50 reported in this cells were about 82 and 15µg/ml in 24 and 72 hrs treatment (Table 1).

## **DISCUSSION**

The use of traditional medicines from natural products have become popular all over the world especially in the developing countries. Herbs were always presumed to be safe because of its “ naturality”. However, as quoted famously by Paracelsus, “ All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy”. Self medication among the user without knowing its toxicity and not being aware of the dose that is being consumed is extremely dangerous. On the other hand, most of herbal medicines are given without a proper prescription, high doses or in combination with other medications may cause toxic effects.

Various types of pharmacological benefit from Mitragnyna speciosa had been reported. However, due to its morphine-like properties, it has been aggressively misused among the drug addicts as opium substitute or to alleviate opiate withdrawal symptoms. Several countries such as Malaysia, Thailand, Myammar and Australia have legislated this plant due to its narcotism. Even throught, the potential toxicity of the mitragynyna speciosa is still remain unclear. Therefore, toxicological assessment of this alkaloid extract was investigated in this study. The alkaloid compounds were extracted using the methanol-chloroform extraction method. Previous studies have identified that mitragynine was the most abundant alkaloid found. It has been well established to possess morphine-like effects on in vitro and in vivo studies (Idid et al., 1998; Matsumoto et al., 1996). Other <https://assignbuster.com/mitragynyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

indole alkaloids such as speciofoline, rhychophylline, stipulatine, isomitraphylline and ajmalicine have also been isolated from the leaves as well (Beckett et al., 1965). Besides, there was an interesting finding in which another alkaloid compound, 7-hydroxymitragynine, had been reported to have a stronger analgesic effect and a potent gastrointestinal transit inhibition in the mice when compared to morphine (Matsumoto et al., 2004a, 2006b). Tolerance and morphine-like withdrawal symptoms by 7-hydroxymitragynine and mitragynine have also been reported.

In the present study, the cytotoxic effects of *Mitragyna speciosa* alkaloid extract was evaluated using several human cancer cell lines in vitro. Due to its fast, easy growing and other special mammalian characteristic, cancer cell lines were widely used as primary screening for the in vitro cytotoxicity. For example, HepG2 cells are the transfected cells which showing metabolic activities such as cytochrome P450s metabolism and hydroxylation (Wu et al., 2006). Besides,  $\mu$ - and  $\delta$ - types opiate binding side are presented on SH-SY5Y cells. HEK293 cells are the kidney embryonic cells which consist of many cell characteristics.

In addition, the use of the XTT assay greatly simplifies the procedure for measuring proliferation as compared to the MTT assay, whereby the solubilization step that is present in the MTT assay was avoided. There was a limitation to the high concentrations of *Mitragyna speciosa* alkaloid extract used since extremely high concentrations of *Mitragyna speciosa* interfered with assay fluorescence measurement (Saidin et al., 2008). However, there was a dose dependant toxicity trend seen with the alkaloid extract at the doses of concentration  $\approx$  300 $\mu$ g/ml.

<https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

Regardless of incubation times, In vitro screening of alkaloid extract demonstrated that skin melanoma cell SK-MEL-28 was the most sensitive cell line examined. The IC<sub>50</sub> after 24hrs treatment of SK-MEL-28 was 16.9 µg/ml. Epidemiology study showed that darkening of the skin was found in chronic consumers of Mitragyna speciosa extracts, but the mechanism remains unclear. However, most properly, it was caused by the dark pigment, melanin which is produced by activation of melanocytes in response to the melanocyte-stimulating hormone (MSH) stimulation. MSH is released by the pituitary gland as a response to the mitragynine-stimulating corticotropin-releasing hormone (CRH) release from the hypothalamus.

Apart from the acute cytotoxicity effects (24 hr treatment), another major finding in this study was the chronic cytotoxicity effects by Mitragyna speciosa extract as determined by 72 hrs treatment. Moderate cytotoxicity on kidney, liver, colon and nerve cancer cell lines was observed. However, cell proliferation was completely inhibited when treated with the dose of 200 µg/ml. There were some differences in the IC<sub>50</sub> values for the HepG2, HEK293 and SH-SY5Y in this study from those of Saidin et al., (2008). The study of Saidin et al. (2008) showed that mitragynine was most toxic to SH-SY5Y and moderate cytotoxicity to HEK293 and HepG2. The IC<sub>50</sub> results in Saidin's study were relatively higher than the present study. Variation such as the manner of preparation and extraction methods can also contribute to the data variability. Uncontrollable factors such as climate, growth and storage conditions might affect the quality of the substrates as well (Akansha et al., 2008; Hanapi et al., 2010). Besides, the use of relative IC<sub>50</sub> and absolute IC<sub>50</sub> remain controversial among researchers. Relative IC<sub>50</sub> showed the

concentration required to bring the curve down to the point half way of the maximum and minimum plateaus of the curve. Whilst, absolute IC50 is defined as the concentration required to give 50% of inhibition. However, relative IC50 was more commonly used compare to absolute IC50 because the potency of the drug is ignored in the determination of absolute IC50.

Harizal's study showed that oral administration of standardized methanolic extract of *Mitragyna speciosa* resulted in severe hepatotoxicity and mild nephrotoxicity in high doses. This had been proven by histological and biochemical examination of *Mitragyna speciosa* extracts treated liver cells. Kupffer cells, enlargement of nucleus (karyomegaly), and significant elevation of ALT level was found (Harizal et al., 2010). Metabolic activation of xenobiotic was believed to be one of the factors that had caused the hepatotoxicity. Besides, consumption of the the *Mitragyna speciosa* extract by rodents has been found to cause an increase in blood pressure (Harizal et al., 2010). There is a likelihood that the effects produced are due to  $\alpha$ -adrenoceptor antagonists effect, which is the case with yohimbine (Verwaerde et al., 1997). On the other hand, in vivo acute treatment of *Mitragyna speciosa* did not bring any damage in axons and dendrites of the hippocampal neurons (Harizal et al., 2010). High dose of administration among the chronic user are categorized as high risk user because of the drug tolerance and addiction effects.

It has been noticed that mitragynine is structurally similar to yohimbine, an alkaloid with stimulant and aphrodisiac effects which is found naturally in *Pausinystalia yohimbe* (from Rubiaceae family) (Fig. 3). Yohimbine is an  $\alpha$ -adrenoceptor antagonist which has been used to treat idiopathic and <https://assignbuster.com/mitragyna-speciosa-korth-alkaloid-extract-and-cytotoxicity-biology-essay/>

medication-induced erectile disorder (Benjamin et al., 2007). Several similarities in side effects mitragynine and yohimbine such as tremor, irritability, hallucination, dizziness, skin flushing, seizure and renal failure have been reported in the literature (Quinton, 1963; Eric et al., 1989; Lydia et al., 2001). An interesting antinociceptive study had demonstrated that pre-treatment of yohimbine completely blocked the opiate receptor agonist such as morphine (mu-opioid receptor agonist), U-50, 488 (kappa- opioid receptor agonist) and SNC80 (delta- opioid receptor agonist) (Lydia et al., 2001). Based on the chemical structure of yohimbine, ester group and hydroxy group at the C-17 position have the stronger affinity to the  $\alpha$ -2-adrenergic receptor, serotonin and dopamine receptor. Thus, this indicated that yohimbine have high potential to be competitive agonist to the opiate receptor. Minor differences in their chemical structures such as the molecular weight, polarity, indoloquinolizidine structure, total of rings, might given some changes in the pharmacological effects as well.

In conclusion, Mitragyna speciosa alkaloid extract showed most sensitive cytotoxic effect on skin melanoma cells after 24hr of drug treatment. Chronic and moderate cytotoxicity was reported in the human liver, kidney, colon and nerve cancer cells. This finding supports the result of the hepatotoxic and nephrotoxic effects from in vivo study of Harizal et al. (2010). Further studies on other active compounds in the alkaloid extract are necessary in order to identify the most toxic chemical components in the extract. Besides, metabolic activation, especially cytochrome P450 metabolism activation by the extract must be carried out in order to get a better understanding of the mechanism of toxicity.

## **ACKNOWLEDGEMENT**

This research was supported in part by Aurigene Discovery Technologies (M) Sdn. Bhd and University of Malaya Grant PS460/2010A. The work of my colleagues who assisted me in the projects is gratefully acknowledged.