3.1 Characterization of compounds

3.1.1 Physical properties

Ligands were obtained as precipitates from concentrated ethanolic solution while nickel(II) and copper(II) complexes precipitated upon reflux. Table 1 below shows the physical properties of the synthesized compounds.

Table 1:
Melting point and colours of the ligands and metal complexes.

Compounds	Colour	Melting point (°C)	Percentage yield (%)
$C_{19}H_{16}N_4O$ (*L1*)	Light brown	224.7	76
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	Orange red	>350.0	45
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	Dark green	294.0	42
$C_{19}H_{15}ClN_4O$ (*L2*)	Brownish yellow	132.0	70
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	Brick red	>350.0	42
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	Dark green	300.0	40
$C_{19}H_{15}BrN_4O$ (*L3*)	Light brown	134.7	75
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	Brick red	>350.0	48
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	Dark green	301.0	42

The melting point of L1 is 224.7°C while L2 and L3 melted at 132°C and 134.7°C respectively. It could be due to strong intermolecular hydrogen bond between the hydrogen atom on the nitrogen atom of the indole with the oxygen atom of the imido group. The

intermolecular hydrogen bonding is weakened due to the presence of halogen atoms. On the other hand, compounds with halogen atoms resulted in low melting point because of the withdrawing effect that the halogens hold and this made the hydrogen atom on similar position is less likely to have hydrogen bond with oxygen atom. The melting point of CuL1 shown lower than that observed for CuL2 and CuL3 with a difference of about 5ºC. In the presence of chlorine and bromine atoms however, increases the melting point of CuL2 and CuL3.

3.1.2 Elemental analyses

Table 2:
Percentage of elemental compositions in ligands and complexes.

Compounds	% C		% H		% N	
	Calc.	**Found**	**Calc.**	**Found**	**Calc.**	**Found**
$C_{19}H_{16}N_4O$ (*L1*)	72.15	72.53	5.06	4.91	17.72	17.55
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	66.09	66.28	4.64	4.53	16.23	16.47
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	65.52	65.56	4.60	4.49	16.09	16.22
$C_{19}H_{15}ClN_4O$ (*L2*)	64.96	64.58	4.27	4.27	15.95	15.62
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	60.00	60.39	4.21	3.90	14.74	14.66
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	59.64	59.26	4.19	3.59	14.65	14.89
$C_{19}H_{15}BrN_4O$ (*L3*)	57.87	57.91	3.81	3.66	14.21	13.93
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	53.90	54.30	3.55	3.47	13.24	13.56
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	53.52	53.68	3.76	3.31	13.15	13.32

The calculated values for carbon, hydrogen and nitrogen atomic compositions as shown in Table 2 are in agreement to that of the theoretical values. Experimental values shown for

ligands; L1, L2 and L3 indicated that the ligands were synthesized successfully while for copper(II) and nickel(II) complexes, the values found involved calculation for 1 : 2 molar ratio of metal to ligand. The values obtained correspond to the molecular structure of compounds that had been synthesized. The elemental analyses values also showed that the copper(II) and nickel(II) complexes are of mononuclear complexes.

3.1.3 Interpretation of Infrared (IR) Spectra

The vibrational bands observed for N – H of the ligands; L1, L2 and L3 were at higher frequencies while the same characteristic bands of copper(II) and nickel(II) of the respective ligands were found to appear at lower frequencies (see Table 3). This is apparently seen when L1, L2 and L3 showed their N – H bands at wavenumber above 3400 cm^{-1} but displayed reduction of wavenumber in the range of 3400 – 3900 cm^{-1} as the ligands were bonded to the metal ions (Sreekanth *et al*., 2003).

Table 3:
Characteristic IR bands of L1, L2, L3 and their complexes.

Compounds	**Wavenumber, υ (cm^{-1})**					
	N – H	**C = O**	**C – O**	**C = N**	**M – N**	**M – O**
$C_{19}H_{16}N_4O$ (*L1*)	3423	1645	-	1603	-	-
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	3348	-	1611	1522	431	422
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	3389	-	1611	1530	475	428
$C_{19}H_{15}ClN_4O$ (*L2*)	3414	1661	-	1606	-	-
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	3347	-	1612	1522	483	425
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	3364	-	1604	1571	493	456
$C_{19}H_{15}BrN_4O$ (*L3*)	3401	1664	-	1617	-	-
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	3344	-	1606	1522	476	422
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	3361	-	1600	1533	487	422

Hydrazone compounds may exist as enol or keto tautomeric form in the solid state (Bessy Raj *et al*., 2008). The absorption due to C = O stretching frequency of the ligands was observed at around 1655 cm^{-1}. C = O of an amide is normally present in range of 1640 - 1680 cm^{-1}. However, the bands shown were weak because there is delocalization of electrons between the oxygen atom of the carbonyl group and the nitrogen atom of the imine group (C=N-N-C=O) that changed the oxygen to enolic type which holds a negative charge on it. The characteristic C = O bands disappeared and the new C – O bands were formed which indicated that the enolic oxygen atom has bonded to the metal ions, thus reducing the frequency of vibration of C – O.

The presence of an imine group is seen at a wavenumber lower than that of a carbonyl group generally in range of 1550 – 1640 cm^{-1} (Bessy Raj *et al*., 2008). However, the vibrational bands after complexion with copper(II) and nickel(II) ions gave clear shifting to higher vibrational frequency. This phenomenon can be explained by considering the π back bonding mechanism where electrons from a d-orbital of the metal ion occupy the π^* anti-bonding orbital of the ligand. This makes the bonding of metal-oxygen (M – O) and metal-nitrogen (M – N) are weaker since electrons occupying the π^* state is forbidden.

The wavenumber for imine (C = N) and carbonyl (C = O) of copper(II) and nickel(II) complexes were found to exhibit a small changes than the wavenumber for C = N and C = O obtained for their respective ligands. The vibration frequencies for both C = N and C = O of copper(II) and nickel(II) complexes became slightly weaker therefore the frequencies are observed at lower wavenumber. With this, it is confirmed that both the nitrogen of the imine and the oxygen of the carbonyl are involved in chelation to copper(II) and nickel(II) ions. These bands were found in the spectra of nickel(II) and copper(II)

complexes and were significantly different to those bands seen in the spectra of the ligands. The absorption bands of M – N and M – O could be distinguished where M – N bonds were seen close to 500 cm^{-1} while M – O bonds were spotted near to 400 cm^{-1} (Akitsu *et al*. 2005). However, Philip *et al*. (2004) recorded that υ(Ni – N) is observed at 440 cm^{-1} while Sreekanth *et al*. (2003) mentioned the υ(Cu – N) for the azomethine nitrogen was observed at approximately 400 cm^{-1}. The vibration frequencies of M – N and M – O in Table 3 are in agreement to the vibration frequencies obtained by Akitsu *et al*. (2005). The Cu – N and Ni – N bonds are found approximately near to 500 cm^{-1} while the Cu – O and Ni – O bonds are seen at about 400 – 450 cm^{-1}.

From Table 3, we can see that both M – N and M – O bonds appeared in the lower frequency field. Therefore, the bonds are weaker. Nevertheless, when we compare the vibrational frequency between Cu – N with Ni – N, the bands for Ni – N appeared at higher frequency than the bands for Cu – N. According to the theory of π back bonding, atoms from the ligand that donate electrons to the *d*-orbital of the metal ion do it through the σ bond. Since the metal ion already hold many electrons in its *d*-orbital, it will then return the electrons back to the π* energy state of the ligands. Since copper(II) ion has more electrons than nickel(II) ion, then copper(II) ion often tends to do π back bonding since electrons repulsion in copper(II) ion is greater than in nickel(II) ion. Because of this, bond formed is weak and stretching or vibrational frequency is lowered.

3.1.4 Interpretation of Nuclear Magnetic Resonance (NMR) Spectra

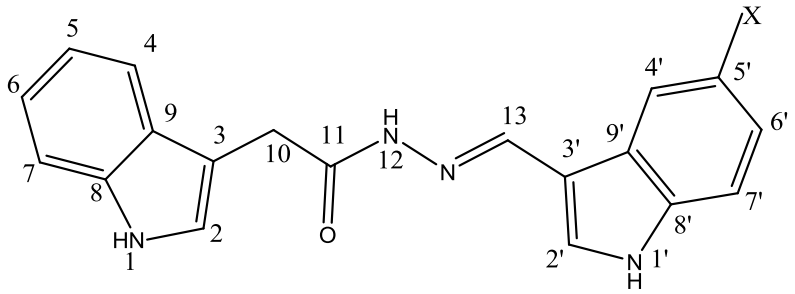


Figure 7: Indole hydrazone derivatives where X is H, Cl and Br.

Table 4 and 5 show the chemical shifts of protons and carbons for L1, L2 and L3. The protons for the ligands were observed at the chemical shifts as presented in Table 4.

Table 4:
Chemical shifts, δ (ppm) in ^{1}H NMR spectra.

Compounds	H1, H1'	H10	H12	H13	H aromatic
$C_{19}H_{16}N_4O$ (*L1*)	10.8 - 11.0	4.1	8.4	11.5	7.0 - 8.2
$C_{19}H_{15}ClN_4O$ (*L2*)	10.8 - 11.3	4.1	8.4	11.7	7.0 - 8.2
$C_{19}H_{15}BrN_4O$ (*L3*)	11.0 - 11.2	4.1	8.4	11.7	7.0 - 8.3

The presence of chlorine and bromine atoms in L2 and L3 respectively did not affect their chemical shifts much. Methylene hydrogen atoms or H10 are found at approximately 4.1 ppm which is more deshielded than the normal methylene group. These protons; where their carbon was directly attached to one aromatic carbon and a carbonyl carbon, were found to be deshielded towards downfield environment and appeared as a singlet due to lack of hydrogen on the adjacent carbons. H13 is shifted to the most downfield environment as compared to others because of the deshielding effect that surrounded it

when C13 was directly double-bonded to a nitrogen atom and also to an aromatic carbon. As for aromatic protons, their peaks were apparently observed at around 7.0 to 8.0 ppm.

Table 5:
Chemical shifts, δ (ppm) in ^{13}C NMR spectra.

Compounds	C10	C11	C13	C aromatic
$C_{19}H_{16}N_4O$ (*L1*)	29.8	172.5	167.0	109.1 - 142.0
$C_{19}H_{15}ClN_4O$ (*L2*)	29.5	172.5	167.1	108.8 - 144.0
$C_{19}H_{15}BrN_4O$ (*L3*)	29.4	172.4	167.0	108.8 - 145.0

C11 of each ligand was observed in the upfield environment in ^{13}C NMR spectra because C11 is deshielded due to direct bonding by a double bond to oxygen and a single bond to nitrogen atoms. However, the peak for imino carbon, C13 was found at slightly downfield region from that found for C11. Aromatic carbons were observed in sp^2 region which is around 100 – 150 ppm while the peak of methylene carbon, C10 was seen at 30 ppm. From this, it is clear that the structures of the ligands were obtained and the desired compound; the Schiff bases were successfully synthesized.

3.1.5 Interpretation of UV-Vis Spectra

Charge transfer bands observed were measured within 200 – 1000 nm or 50 000 – 10 000 cm^{-1}. σ – σ* electronic transition occurs less than 200 nm and this electronic transition only possible for molecules containing only single bonds and lack of atoms with unshared electrons. Unsaturated molecules which contain no atoms with unshared electrons will give absorption for electronic transition π – π* while unsaturated molecules with atoms

such as oxygen or nitrogen may undergo electronic transition n – π* other than π – π*. Most *n* – *π** transitions are forbidden therefore are of low intensity (Rao, 1967).

Table 6:
The wavelength of UV-Vis for the ligands and complexes.

Compounds	**λ (nm)**			
	Intraligand		**Charge transfer**	***d - d* transition**
	n – π*	**π – π***		
$C_{19}H_{16}N_4O$ (*L1*)	262	298	-	-
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	268	318	389	599
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	271	337	409	531
$C_{19}H_{15}ClN_4O$ (*L2*)	264	307	-	-
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	268	317	364	581
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	270	344	399	535
$C_{19}H_{15}BrN_4O$ (*L3*)	214	262	-	-
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	268	316	391	565
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	269	347	401	537

The position and intensity of an absorption band of the chromophore in ultraviolet region may sometimes be modified due to presence of the substituent group attached to the basic chromophore structures though the substituent may not give rise to any absorption band. In spite of this, there are also phenomena called auxochromes where the substituents increase the intensity or wavelength. This includes molecules mostly electron-donating groups such as methyl, alkoxy, hydroxyl, amino and also electron-withdrawing group such as halogens. Bathchromic shift does exist in the L2 and L3 where the N → Q transition which is the *π* – *π** electronic transition occurred at longer wavelength (Rao, 1967).

Copper(II) complexes of L1, L2 and L3 were of dark green colour therefore, there are absorption bands that show the presence of the colour which could be seen around 555 – 625 nm. All copper(II) complexes exhibited a *d* - *d* absorption band as a weak shoulder whose maximum absorption are in the visible region at approximately 555 nm and is expected for a square planar chromophore. The weak shoulder also is believed to appear as a result of domination of intense intraligand and charge transfer bands (Sreekanth *et al*. 2003).

It is difficult to resolve three bands because the four lower orbitals are often so close together in energy that individual transfer from the upper d orbital to those lower orbitals cannot be distinguished. For that reason, a single band instead of three bands is observed (Sreekanth *et al*. 2003). For a square planar complex with $d_{x^2-y^2}$ ground state, there are three possible spin allowed transitions which are $^2B_{1g} \rightarrow {}^2A_{1g}$ ($d_{x^2-y^2} \rightarrow d_{z^2}$), $^2B_{1g} \rightarrow {}^2B_{2g}$ ($d_{x^2-y^2} \rightarrow d_{xy}$) and $^2B_{1g} \rightarrow {}^2E_g$ ($d_{x^2-y^2} \rightarrow d_{xz}$, d_{yz}) (Sreekanth *et al*. 2003).

The molecular geometry of the brick red nickel(II) complexes are square planar since the absorbance peaks were found at almost 600 nm. The d - d spectral transition can be assigned to $^1A_{1g} \rightarrow {}^1E_{1g}$, $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ (Philip *et al*. 2004) whose transitions observed approximately between 525 – 555 nm. The weak *d* - *d* transitions of square planar nickel(II) complexes were observed 370 – 430 nm and 430 – 670 nm (Kasumov *et al*., 2005). Intense charge transfer bands obscure the second and third *d* - *d* bands at higher energy region mainly below 450 nm. The bands observed at 570 – 660 nm can be assigned to $^1A_{1g} \rightarrow {}^1A_{2g}$ (Kasumov *et al*. 2005) and this is observed for all nickel(II) complexes of the ligands.

Copper(II) ion is categorized as d^9 ion where it is characteristically stereochemically flexible. Strong field ligands usually yield square planar or six coordinate tetragonal complexes while weak ligands give rise to almost any molecular geometry (Lever, 1984). The spectrum of a nickel(II) species is generally characteristic of a specific stereochemistry, on the other hand is rarely true for copper(II) species (Lever, 1984). The square planar CuN_2O_2 chromophores are well known in Schiff base complexes where the absorption band for its d-d transition usually appear as a broad band from approximately 13 000 – 20 000 cm^{-1} (Lever, 1984) which is from 500 – 770 nm.

Obviously, the characteristic absorption bands for copper(II) and nickel(II) complexes of indole Schiff base compounds were found around 525 – 625 nm, which agreed with the square planar of CuN_2O_2 chromophores (Lever, 1984). Figure 8 until Figure 11 show the UV-Vis spectra of L1 and its Cu(II) and Ni(II) complexes. Other spectra are presented in the appendix.

Figure 8 shows the absorption bands of the ligand, L1. The bands are found below 400 nm which indicates excitation from the $\pi - \pi^*$, $n - \pi^*$ and $\sigma - \sigma^*$. Upon complexation with nickel(II) and copper(II) ions (see Figure 9 and Figure 10), the absorption bands of L1 were shifted to a larger wavelength and a shoulder of *d – d* transition is seen at approximately 600 nm. On the other hand, the intraligand and the charge transfer bands chromophores absorb more UV-Vis and gave very high intensity absorption bands at approximately 200 – 500 nm. The *d – d* transition for CuL1 is enlarged and is shown in Figure 11.

Figure 8:
UV-Vis absorption bands of indolecarboxaldehyde – indolehydrazide
(*L1*)

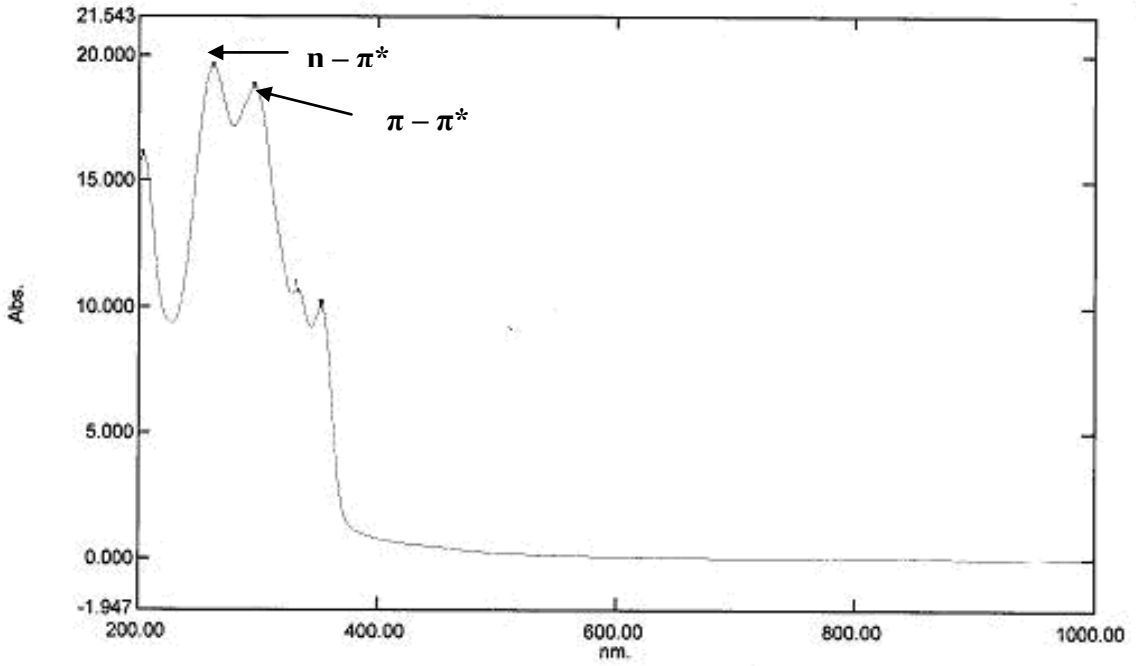


Figure 9:
UV-Vis absorption bands of Ni(indolecarboxaldehyde – indolehydrazide)$_2$
(*NiL1*)

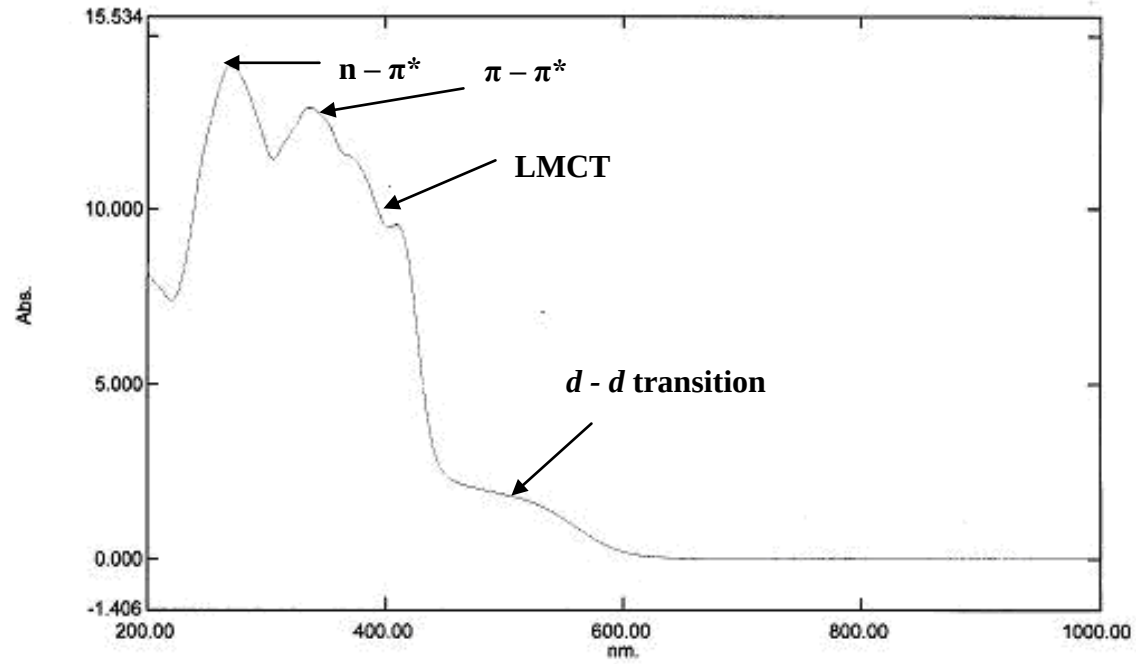


Figure 10:
UV-Vis absorption bands of Cu(indolecarboxaldehyde – indolehydrazide)$_2$ (*CuL1*)

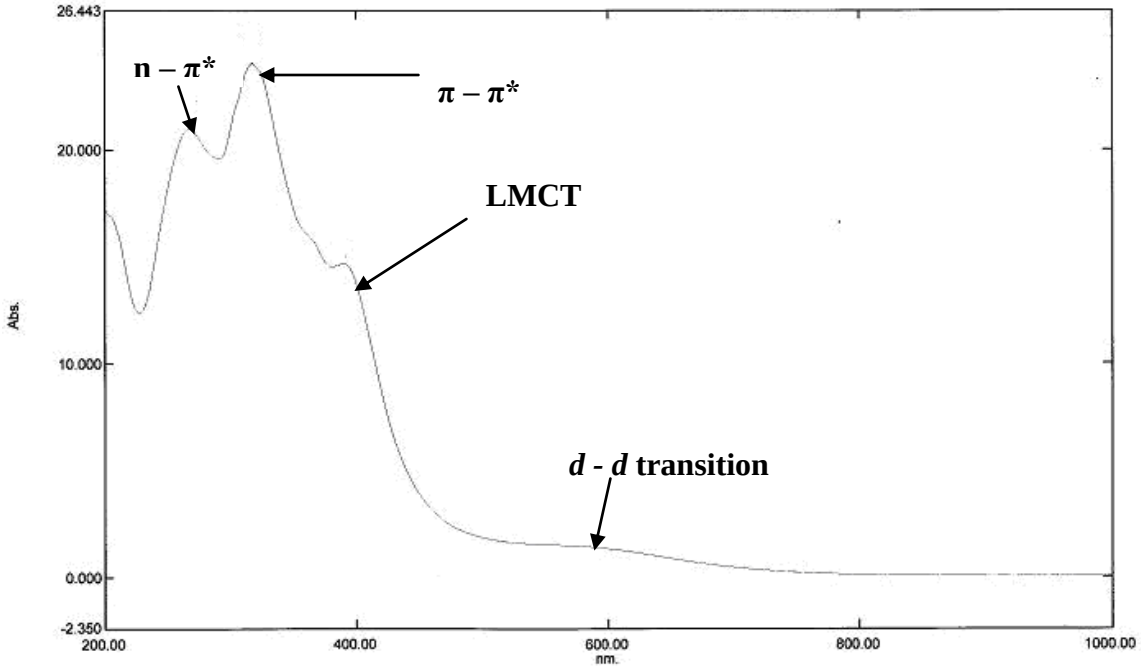
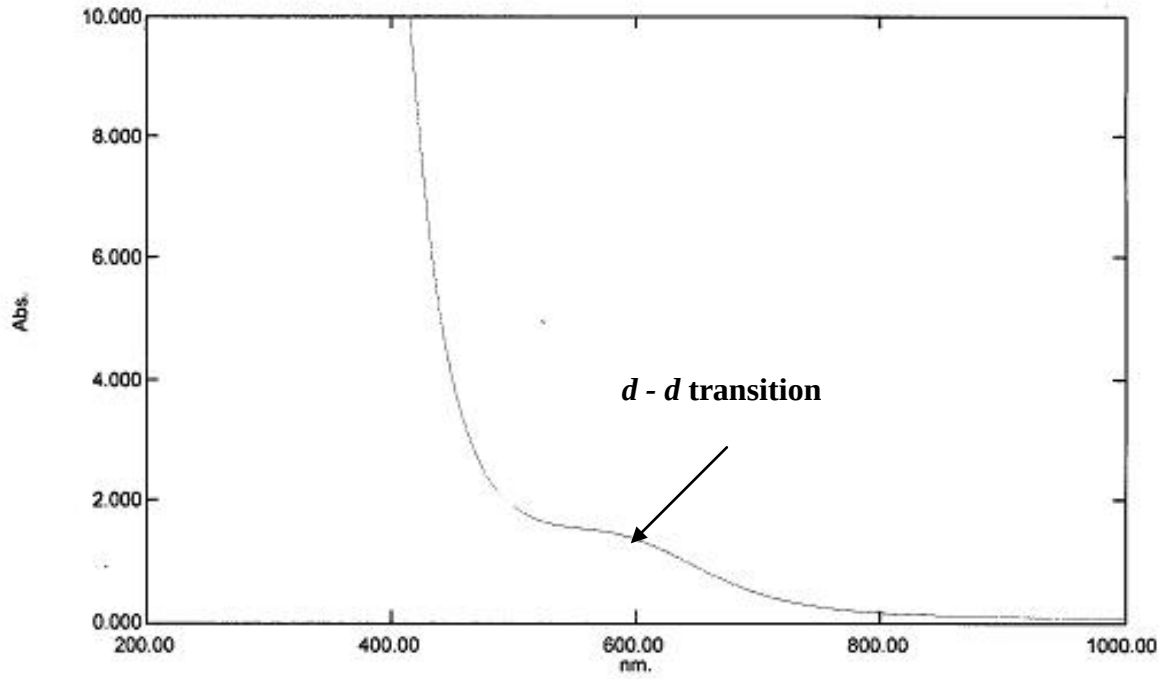


Figure 11:
The *d – d* transition band of Cu(indolecarboxaldehyde – indolehydrazide)$_2$ (*CuL1)*



3.1.6 Magnetic susceptibility

The following mathematical formula was used to calculate the values of effective magnetic moment, μ_{eff} (BM) for all nickel(II) and copper(II) complexes as shown in Table 7. The Pascal's constant of each atom was obtained from Table 12 (see Appendix).

$\chi_m = \chi_g \times MW$ [Equation 3]

$\chi_m^{corr} = \chi_m - \chi_{dia}$ [Equation 4]

$\mu_{eff} = 2.83 (\chi_m^{corr} T)^{1/2} = [n(n + 2)]^{1/2}$ [Equation 5]

Table 7:
Effective magnetic moment of copper(II) and nickel(II) complexes.

Compounds	χ_g x 10^{-5} (cm^3/g)	MW (g/mol)	μ_{eff} (BM)	n
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	0.124	695.55	1.51	0.81
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	0.013	690.69	0	-
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	0.166	764.55	1.83	1.08
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	-0.027	759.69	0	-
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	0.193	853.35	2.07	1.30
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	0.000	848.49	0	-

Table 7 shows that all copper(II) complexes of indole derivatives synthesized have one unpaired electron. Nickel(II) complexes are diamagnetic. The effective magnetic moments, μ_{eff} of mononuclear copper(II) complexes are obtained in the range of 1.4 to 1.9 B.M., for a single unpaired electron system occupying a $d_{x^2-y^2}$ orbital in a ^2D (Akitsu *et al*. 2005).

Figure 12 illustrates the effect of *z*-axis stretching on the e_g and t_{2g} orbitals in an octahedral complex. Orbitals having a *z* component (d_{xz}, d_{yz}, d_z^2) will experience a decrease in electrostatic repulsions from the ligands and will therefore be stabilized (Huheey, 1993). The 'non-*z*' orbitals will be raised in energy and causes the e_g level to split into an upper b_{1g} ($d_{x^2-y^2}$) and a lower a_{1g} (d_z^2) while the t_{2g} is split into a b_{2g} (d_{xy}) and a doubly degenerate e_g (d_{xz}, d_{yz}) (Huheey, 1993).

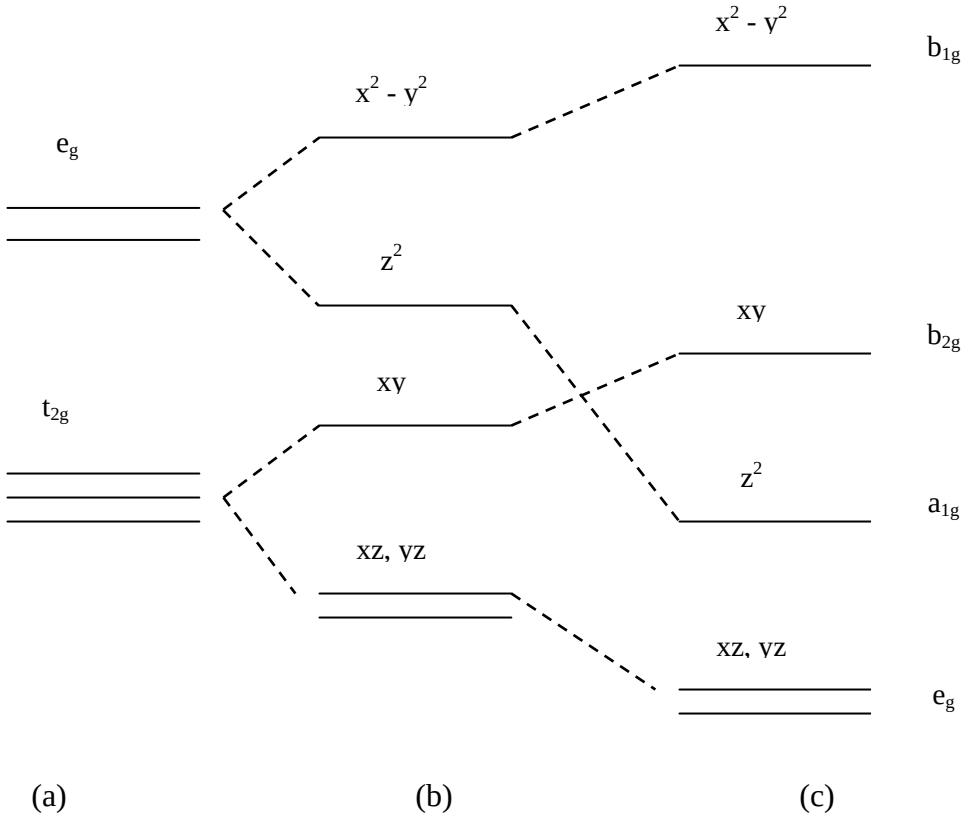


Figure 12: Orbital splitting diagram for an octahedral complex (a), tetragonally distorted complex (b) and the square planar complex (c) (Huheey, 1993).

The square planar geometry is favored by metal ions having a d^8 configuration such as nickel, palladium and platinum ions in the presence of a strong field thus giving low spin complexes with the eight *d* electrons occupying the low energy d_{xz}, d_{yz}, d_z^2 and d_{xy} orbitals while the high-energy $d_{x^2-y^2}$ orbital remains unoccupied. The $d_{x^2-y^2}$ orbital will be raised higher if the surrounding field is stronger (Huheey, 1993). As long as the high level is

unoccupied, the complex will be stabilized because the lower occupied orbitals will drop in energy by a corresponding amount. Since L1, L2 and L3 are strong field ligands, the *d* electrons from the nickel(II) ion occupy the e_g, a_{1g} and b_{2g} states. The electrons of the nickel(II) ion are paired up and resulted in absent of single electron thus, indicating that the nickel(II) complexes are diamagnetic.

3.1.7 X-ray Crystallography

3.1.7.1 Crystal Structure of 2'-[(1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L1)

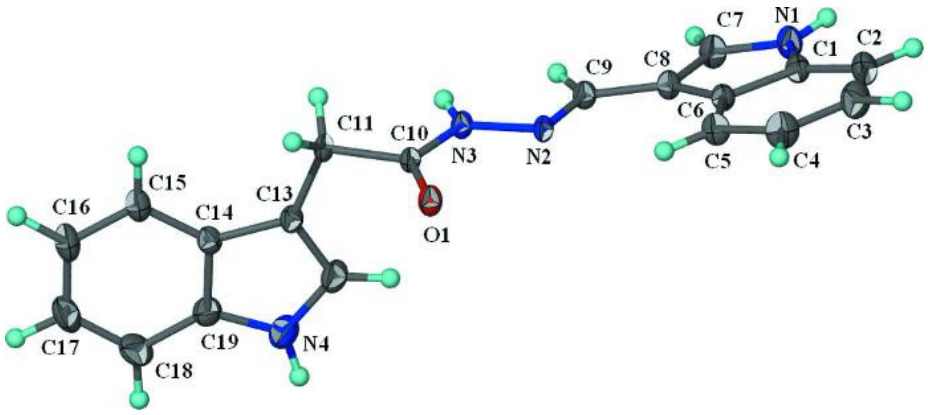


Figure 13: Crystal structure of indolecarboxaldehyde – indolehydrazide (*L1*)

The crystals of L1 are obtained as pale yellow and monoclinic crystals from ethanol. The C=N bond is formed between N2 and C9 where the length of the bond is 1.29Å. The hydrogen attached to N1 is bonded to O1 through an intermolecular hydrogen bond with a bond length of 1.98Å. Besides, there is an intramolecular hydrogen bond between the hydrogen atom attached to N3 with O1. The bond length of the intramolecular hydrogen

bond is 1.97Å. The ligand exists as a keto-enol tautomer because of the delocalization of electrons from O1 to N2 via N3.

3.1.7.2 Crystal Structure of 2'-[(5-chloro-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L2)

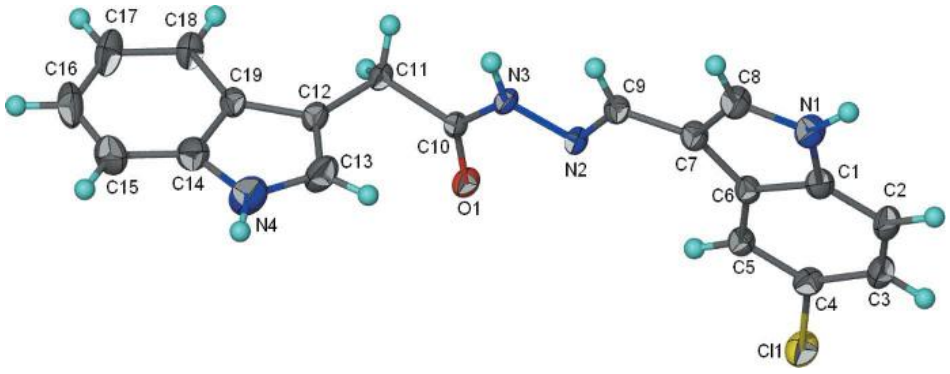


Figure 14: Crystal structure of chloroindolecarboxaldehyde – indolehydrazide (*L2*)

The pale yellow crystal of L2 was obtained from crystallization in ethanol and has a monoclinic shape. The C=N bond is formed between N2 and C9 where the length of the bond is 1.29Å. The hydrogen attached to N1 is bonded to O1 through an intermolecular hydrogen bond with a bond length of 2.02Å, which is slightly longer than the bond length obtained for L1. This is due to the electronegativity effect of chlorine atom that is bonded to C4. Besides, there is an intramolecular hydrogen bond between the hydrogen atom attached to N3 with O1. The bond length of the intramolecular hydrogen bond is 1.96Å. L2 exist as a keto-enol tautomer because of the delocalization of electrons from O1 to N2 via N3.

3.1.7.3 Crystal Structure of 2'-[(5-bromo-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L3)

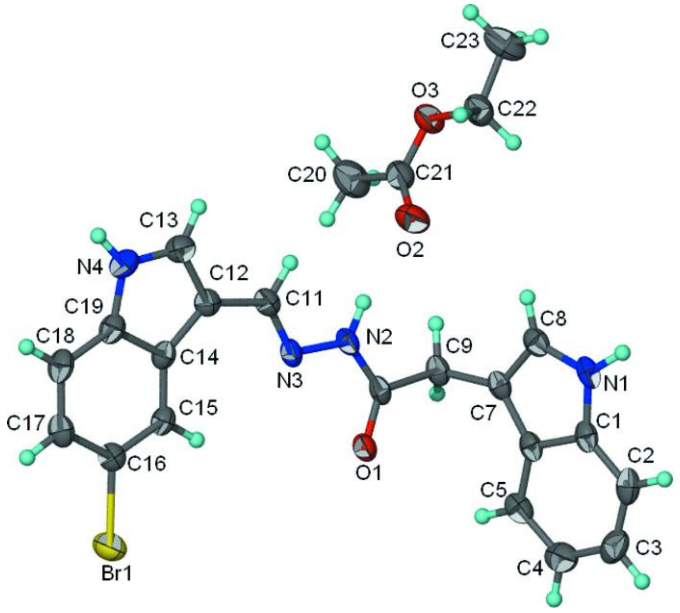


Figure 15: Crystal structure of bromoindolecarboxaldehyde – indolehydrazide (*L3*)

A crystal of L3 is monosolvated with its crystallizing solvent, ethyl acetate and was collected as a colourless plate in a monoclinic system. The C=N bond is formed between N3 and C11 where the length of the bond is 1.28Å. The molecule of L3 is linked to the ethyl acetate solvent molecule through a hydrogen bond of 2.02Å between O2 and hydrogen atom attached to N2. The hydrogen attached to N1 is bonded to O1 through an intermolecular hydrogen bond with a bond length of 2.02Å. Besides, there is an intramolecular hydrogen bond between the hydrogen atom attached to N4 with O1. The bond length of the intramolecular hydrogen bond is 2.00Å. The ligand exist as a keto-enol tautomer because of the delocalization of electrons from O1 to N3 via N2.

3.1.7.4 Crystal Structure of bis{2'-[(1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-κ^{2}N,O}nickelate (NiL1)

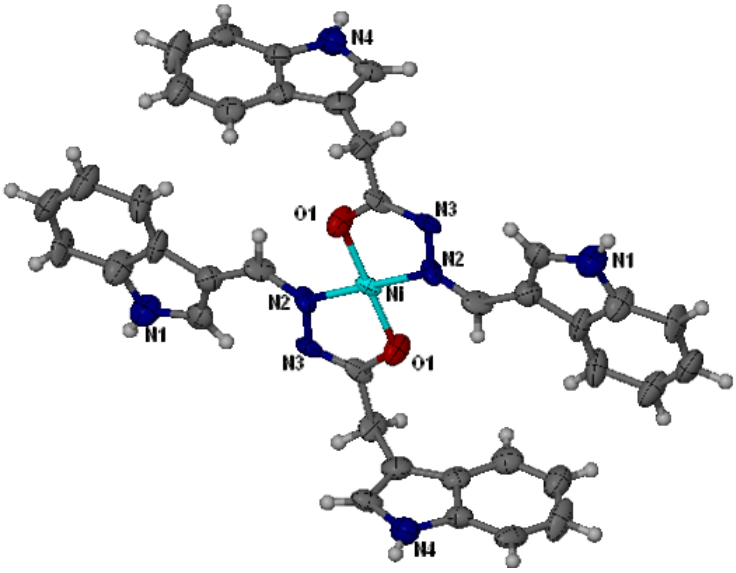


Figure 16: Crystal structure of Ni(indolecarboxaldehyde – indolehydrazide)$_2$ (*NiL1*)

Crystals of NiL1 were obtained from DMF as red, block crystals. The bond length of the imine group (C9=N2) increased to 1.38Å indicating that N2 is now bonded to the nickel(II) ion. In addition, the bond length shown by C10 – O1 is 1.37Å, slightly longer than the bond length of C10 – O1 shown in L1 which is 1.24Å. Therefore, new bonds were formed between the nickel(II) ion with O1 and N2. The square planar geometry of NiL1 is proven by the bond angle of 180° exhibited by each of the O1 – Ni – O1' and N2 – Ni – N2' bonds. The negative charges on both monoanionic ligands were abolished after chelating to the Ni^{2+} ion thus, forming a neutral nickel(II) complex of L1. The negative charges on both monoanionic ligands were abolished after chelating to the Ni^{2+} ion thus, forming a neutral nickel(II) complex of L1. There is an intermolecular hydrogen bond between H4----N3 of

which the bond length is 2.51Å where each cell unit is arranged by stacking the molecules on top of each other in a triclinic system.

3.1.7.5 Crystal Structure of bis{2'-[(5-chloro-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido- κ^2N,O}nickelate (NiL2)

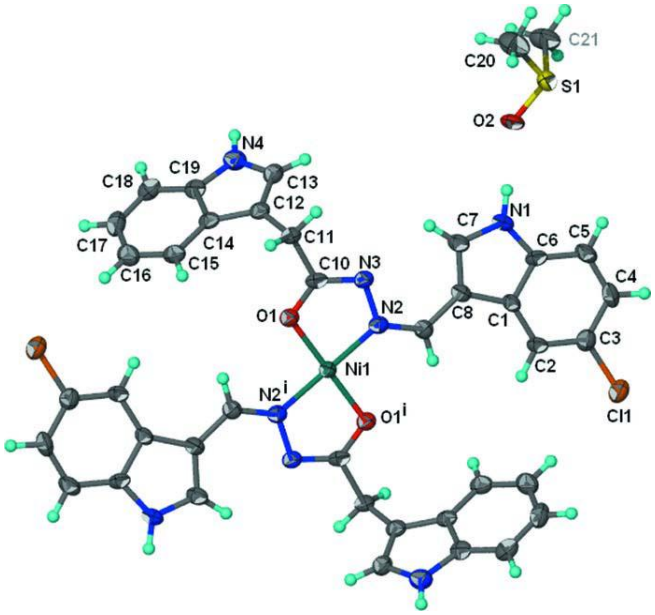


Figure 17: Crystal structure of Ni(chloroindolecarboxaldehyde – indolehydrazide)$_2$ (*NiL2*)

The crystal of NiL2 was built up by a ratio of Ni^{2+} to L2 as 1:2 and was obtained as a monoclinic type of block, red crystal. The bond length of the imine group (C9=N2) increased to 1.30Å indicating that N2 is now bonded to the nickel(II) ion. In addition, the bond length shown by C10 – O1 is 1.30Å, slightly longer than the bond length of C10 – O1 shown in L2 which is 1.24Å. Therefore, new bonds were formed between the nickel(II) ion with O1 and N2. The square planar geometry of NiL2 is proven by the bond angle of 180° exhibited by each of the O1 – Ni – O1' and N2 – Ni – N2' bonds. The negative charges on both monoanionic ligands were abolished after chelating to the Ni^{2+} ion thus,

forming a neutral nickel(II) complex of L2. There are intermolecular hydrogen bonds between H1----O2 and H4----O2 of which the bond lengths are 1.90Å and 2.05Å respectively. The cell units of NiL2 are arranged by stacking the molecules on top of each other in a monoclinic system.

3.1.7.6 Crystal Structure of bis{2'-[(5-bromo-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-κ^2N,O}nickelate (NiL3)

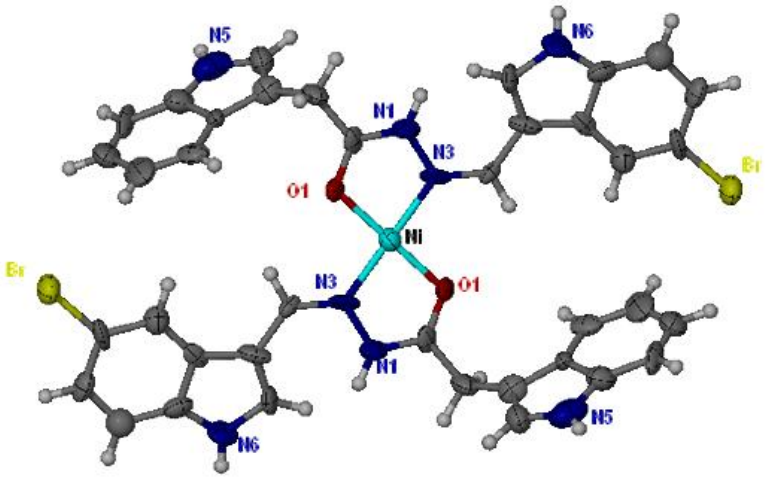


Figure 18: Crystal structure of Ni(II) bromoindolecarboxaldehyde – indolehydrazide (*NiL3*)

A block, red crystal of NiL3 was obtained from crystallization in DMSO and yielded a square planar molecule with nickel(II) ion attached to both O1 and N3 of the ligands. The bond length of the imine group (C11=N3) increased to 1.31Å indicating that N3 is now bonded to the nickel(II) ion. In addition, the bond length shown by C10 – O1 is 1.28Å, slightly longer than the bond length of C10 – O1 shown in L3 which is 1.24Å. Therefore, new bonds were formed between the nickel(II) ion with O1 and N3. The square planar geometry of NiL2 is proven by the bond angle of 180° exhibited by each of the O1 – Ni –

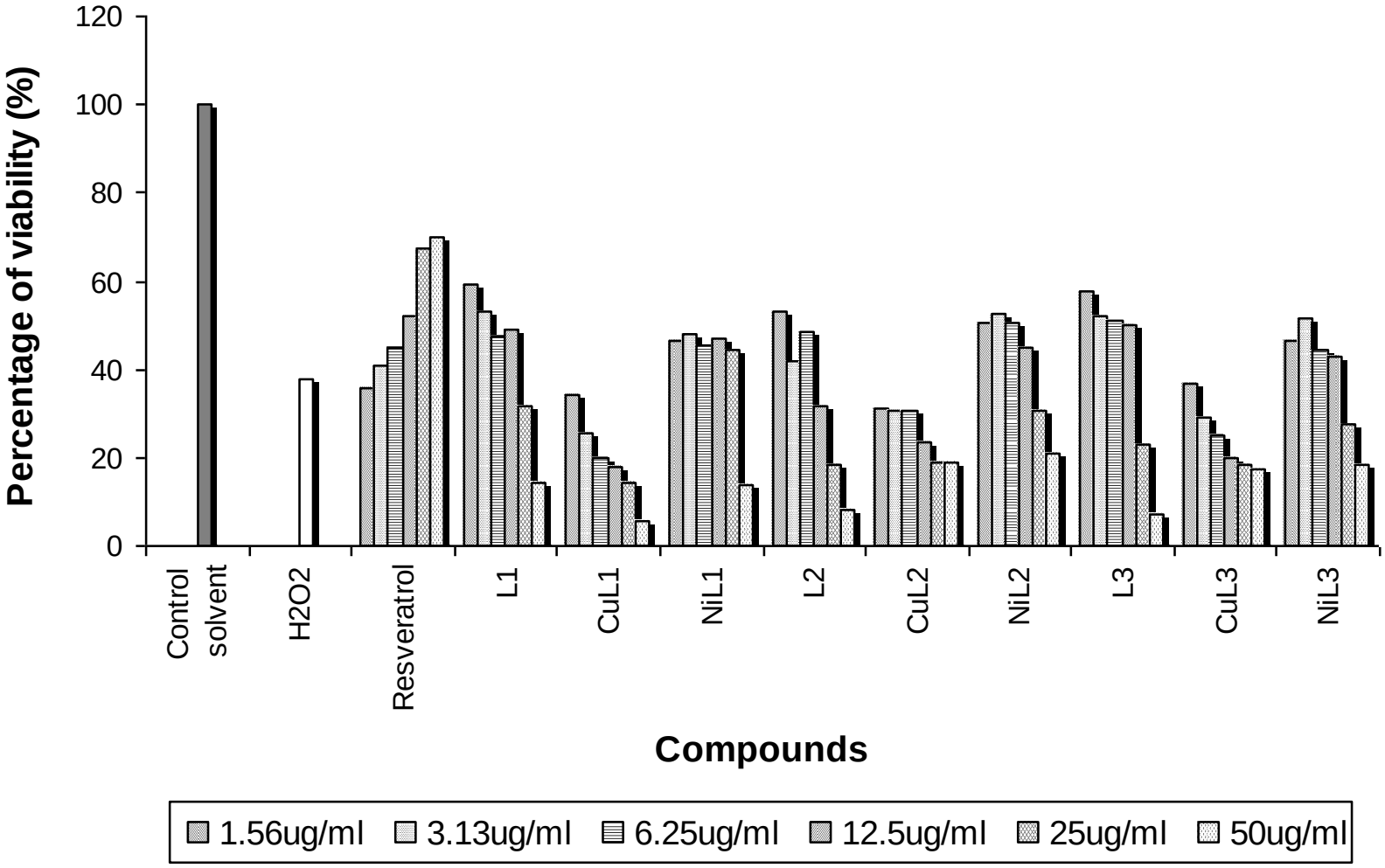
O1' and N3 – Ni – N3' bonds. The negative charges on both monoanionic ligands were abolished after chelating to the Ni^{2+} ion thus, forming a neutral nickel(II) complex of L3. There is an intermolecular hydrogen bond between H5----N1 of which the bond length is 2.54Å where each cell unit is arranged by stacking the molecules on top of each other in a monoclinic system.

3.2 Biological studies

3.2.1 Neuroprotective Screening Test

All test compounds were found to be toxic *in vitro* on NG108-15 cells where they killed the cells at the concentration of 50µg/ml of test compounds. The viability of NG108-15 cells after treated with test compounds was decreased by 20% of the cells treated with 2mM of hydrogen peroxide. The neuroprotective activity of all compounds synthesized protected NG108-15 cells against H_2O_2-induced cell damage in a concentration – dependent manner as shown in Figure 19. L1 protected 14% of the neuron cells while each L2 and L3 protected about 8% of the cells at 50µg/ml. The presence of chlorine and bromine atoms reduces the neuroprotective activity of L1 at this concentration. However, in the presence of copper(II) ions, the neuroprotective effect of L2 and L3 are increased to 18% and 17% respectively but the activity of L1 is reduced to 6% after chelating L1 to copper(II) ion. In this case, the copper(II) ions played an important role in improving the potential of the ligands in protecting NG108-15 cells against H_2O_2-induced cell damage. Nickel(II) ion maintained the activity of L1 but improved much in the neuroprotective activity of L2 and L3.

Figure 19:
The neuroprotective effect of halogenated indole Schiff bases and their copper(II) and nickel(II) complexes.



The viability of NG108-15 cells increased to 21% after treated with 50µg/ml of NiL2 and 18% of the same cell line was protected after treated with the same concentration of NiL3. Although nickel(II) and copper(II) ions affect the activity of the ligands, the viability of NG108-15 cells after treated with all test compounds was found to exhibit low viability compared to the viability of the untreated cells. Resveratrol was also found to protect 70% of the cells at 50µg/ml.

However, the percentage of cell viability increases when the concentrations used to treat NG108-15 cells were reduced. The ligands increased the viability of cells almost in equal percentage at the concentration of 1.56µg/ml. This shows that at the lowest concentration used in this study, the cytotoxicity effect of ligands containing chlorine and bromine atoms could be reduced. Copper(II) of L1, L2 and L3 also increased the neuroprotective activity at 1.56µg/ml to approximately 35% of the viability of cells while nickel(II) complexes of the ligands protected the cells up to 50%. Therefore, H_2O_2-induced NG108-15 cell damage could be reduced or prevented by treating the cells with the halogen substituted indole derivatives and their copper(II) and nickel(II) complexes at the concentration of 1.56µg/ml. In comparison to resveratrol, all test compounds are potentially active as neuroprotectant at a low concentration. On the other hand, the neuroprotective activity shown by resveratrol reduces when the cells were treated with 1.56µg/ml of the compound and decreased the viability of cells to 36%.

Since the test compounds have potential activity in protecting NG108-15 cells against H_2O_2-induced cell damage, the compounds are believed to exhibit as antioxidant compounds due to the ability of the compounds to reduce oxidative stress induced by

hydrogen peroxide. The compounds synthesized might affect in protecting NG108-15 cells by acting as free radical scavengers at the concentration of 1.56µg/ml.

3.2.2 Acute Toxicity Test

Table 8:
Physical observation for abnormalities or mortalities after administration of test compounds.

Compounds	½ hour	1 hour	2 hours	3 hours	24 hours
$C_{19}H_{16}N_4O$ (*L1*)	+	+	+	+	+
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	+	+	+	+	+
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	+	-	-	+	+
$C_{19}H_{15}ClN_4O$ (*L2*)	+	+	+	+	+
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	+	+	+	+	+
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	+	-	-	+	+
$C_{19}H_{15}BrN_4O$ (*L3*)	+	+	+	+	+
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	+	+	+	+	+
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	+	-	-	+	+

\+ Mice were active and did not show sign(s) of abnormalities.
\- Mice were less active.

All mice showed no sign of physical abnormalities or mortality. However, mice received 2g/kg and 5g/kg of nickel(II) complexes started to become inactive after an hour of administration of samples and continue for the next hour. The mice were inactive within 1 to 2 hours after sample administration where their movements became very slow and they preferred to stay in a place at longer time. Their physical appearance was observed to be normal. From the observation, it is believed that nickel(II) complexes of Schiff base of indole derivative compounds had caused adverse toxicity effect for the next two hours but mice came about to be active after the third hours samples were being consumed.

Although all test compounds killed neuron cells *in vitro*, the mice showed no sign of death during experimental period when these compounds were administered into the mice. A compound that is toxic *in vitro* may not be toxic *in vivo* or vice versa. It could be explained in such a way that compounds that enter the body systems would be metabolized thus convert or change their molecular structures into either toxic or non toxic substances (Patrick, 2005). Therefore, test compounds used in this study have shown that they might be non toxic to the mice since there is no mortality observed during the period of observation but may affect the organs or blood if they were to be taken excessively.

3.2.3 Ethanol-Induced Gastric Ulcer

3.2.3.1 Gastroprotective Effect of L1 and its Complexes

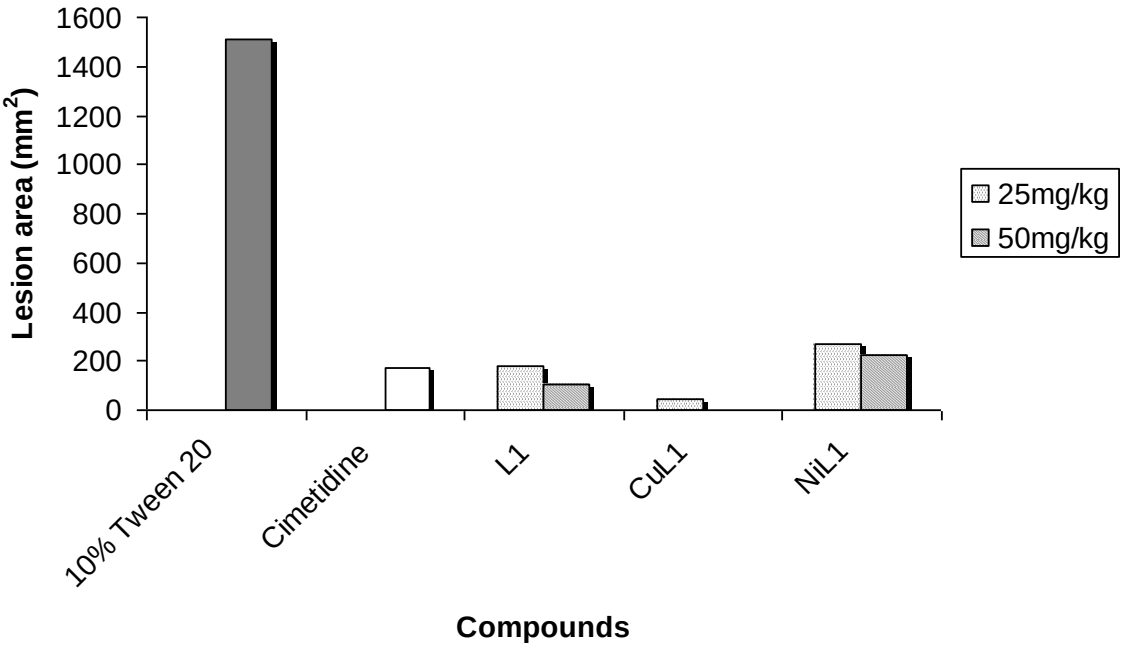


Figure 20: Lesions area on the stomach of a rat after pretreatment with indolecarboxaldehyde – indolehydrazide (*L1*) and its complexes

Figure 20 shows the area of gastric ulcerations after pretreatment with L1 and its complexes. Gastric ulcers area measured on stomachs of the rats that were pretreated with 70mg/kg cimetidine as the standard drug is 168mm^2 while stomachs of the rats that were pretreated with L1 at 25mg/kg and 50mg/kg showed 180mm^2 and 104mm^2 in lesion area. 70mg/kg cimetidine gave the same effect on ethanol-induced gastric ulcers as well as L1 at 25mg/kg. However, the gastroprotective effect of L1 is increased when L1 inhibited the formation of gastric lesions at 50mg/kg and reduced the lesion area by 80mm^2. This shows that L1 is effective when it is used at high dose (50mg/kg). Average mucus weight of stomachs pretreated with 25mg/kg of L1 is 0.35g which is twice lower from the average mucus weight of stomachs pretreated with 50mg/kg which weighed 0.74g. Measurement of pH of gastric juice shows that stomachs pretreated with 25mg/kg and 50mg/kg of L1 have approximate value from 4.5 – 4.8. These values show that L1 did not decrease the acidity of the gastric juice although the compound is potentially active in inhibiting the formation of gastric ulcers.

There is no gastric lesions observed after pretreatment with CuL1 at 50mg/kg but stomachs pretreated with 25mg/kg of CuL1 displayed 46mm^2 of gastric lesion area. However, CuL1 is more active as an anti-ulcer agent since CuL1 inhibited the formation of gastric ulcers effectively than that shown by L1. Copper(II) ion played its role in enhancing the inhibition of ulcers on the stomach and this shows that copper is needed in order to increase the gastroprotective effect of L1. The average mucus weight collected when stomachs were pretreated with 25mg/kg of CuL1 is 0.13g while mucus weight obtained from pretreatment at 50mg/kg is 0.16g. Pretreatment with CuL1 did not increase the production of mucus neither at 25mg/kg nor 50mg/kg. The acidity of gastric juice increased to 2.95 when 50mg/kg of CuL1 is used in pretreatment but there is a steep decrease in pH value to 5.33

when the dose used was 25mg/kg. Rats pretreated with 25mg/kg of CuL1 should exhibit no gastric ulcers since the pH of gastric juice is slightly acidic. Nevertheless, CuL1 works well at 50mg/kg although it actually increased the acidity of the gastric juice at this dose and indicated that the efficacy of CuL1 in inhibiting ulcerations does not depends on the ability in mucus secretion and the acidity of the gastric juice.

NiL1 inhibited the formation of ulcers at both doses where 271mm^2 lesions were observed at 25mg/kg and 228mm^2 of lesions were measured after pretreatment at 50mg/kg. The area of gastric lesions on the stomachs pretreated with NiL1 showed about twice the area found on the stomachs pretreated with cimetidine and L1. It also shows that stomachs pretreated with NiL1 displayed five times more lesion area than that found on the stomachs pretreated with CuL1. Although the gastric lesions areas were larger, the stomach secreted 0.61g and 0.49g of mucus upon pretreatment with 25mg/kg and 50mg/kg of NiL1. The pH values, 5.59 and 7.62 shows that NiL1 reduced the acidity of the gastric juice at both 50mg/kg and 25mg/kg. In this case, the presence of nickel(II) ion enhanced the mucus production and almost neutralizes the pH of gastric juice. However, NiL1 does not inhibit ulcerations as effective as CuL1 and L1. Among the three compounds of this series, CuL1 showed its potency as a good inhibitory agent to inhibit the formation of ethanol-induced gastric ulcer at 50mg/kg followed by L1 and NiL1.

Although there are differences of gastric lesion at different doses, no significance among doses is observed when compared to the vehicle group (10% Tween 20). In addition to that, we can say that the effectiveness of the test compounds is slightly similar to cimetidine in order to prevent ethanol-induced gastric ulceration from occurring. There are also many efforts had been done to determine or to investigate the mechanism of action in protecting

the stomach from ulceration. Damage in mucus defend has been one of the factors in discussion where the existence of mucus barrier on the stomach wall is very important thus, protecting the stomach wall from being eroded by the noxious endogenous agent, hydrochloric acid. So, enhancing mucus barrier may be one of the reasons why ulceration could be prevented. Acidity of the gastric juice plays a role too. If the acidity increases, ulceration area will be increased. However, in this case study, this series of compounds did not show any correlation between the amount of mucus produced and gastric juice acidity but did protect ulceration from forming (Grossman, 1981).

Table 9:
Gross gastric lesion and percentage of inhibition by indole derivatives in ethanol-induced gastric ulcer.

Samples	Doses (mg/kg)	% of inhibition	Gross gastric ulceration area ± S.E.M.	Average mucus weight (g)	Juice pH
$C_{19}H_{16}N_4O$ (*L1*)	50	95	104 ± 6.60	0.35	4.53
	25	83	180 ± 12.2	0.74	4.81
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	50	85	228 ± 5.80	0.49	5.59
	25	82	271 ± 12.1	0.61	7.62
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	50	100	0 ± 0	0.16	2.95
	25	97	46 ± 4.3	0.13	5.33
$C_{19}H_{15}ClN_4O$ (*L2*)	50	96	58 ± 4.9	0.14	4.73
	25	90	151 ± 10.8	0.28	4.60
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	50	73	210 ± 10.8	0.47	6.33
	25	71	439 ± 16.7	0.27	5.22
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	50	90	148 ± 4.10	0.43	4.68
	25	91	131 ± 8.20	0.40	5.45
$C_{19}H_{15}BrN_4O$ (*L3*)	50	96	17 ± 4.8	0.26	4.11
	25	57	221 ± 17.2	0.34	4.84
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	50	75	100 ± 10.8	0.35	3.93
	25	53	848 ± 20.2	0.41	4.65
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	50	95	78 ± 6.8	0.27	4.43
	25	67	500 ± 11.0	0.39	6.43

3.2.3.2 Gastroprotective Effect of L2 and its Complexes

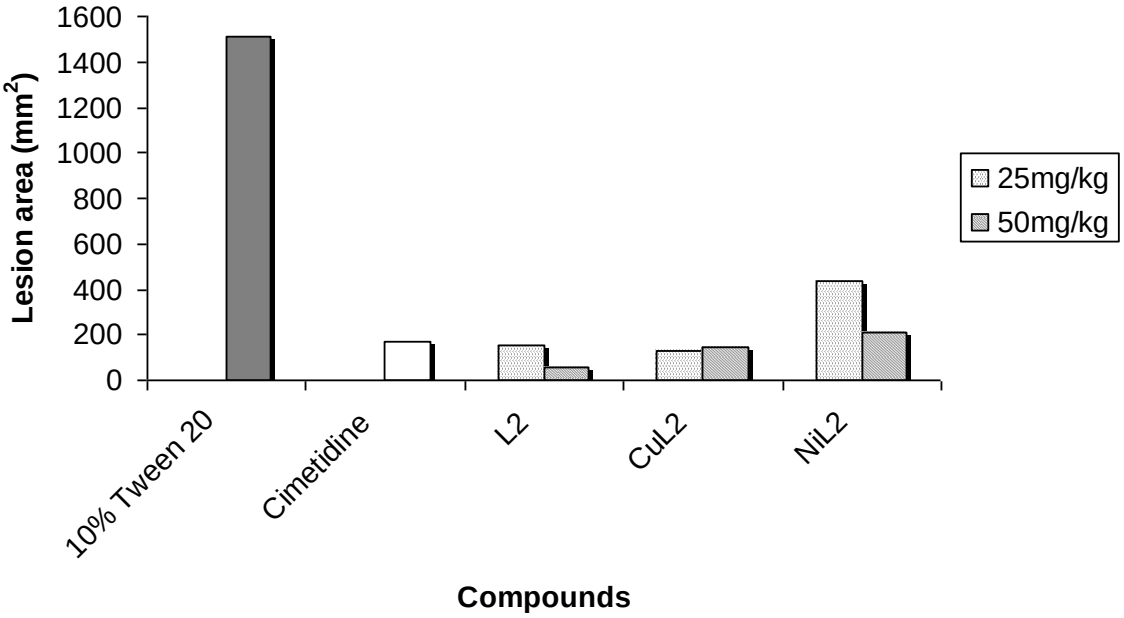


Figure 21: Lesions area on the stomach of a rat after pretreatment with chloroindolecarboxaldehyde – indolehydrazide (*L2*) and its complexes

Figure 21 shows the gastric ulcers area of the stomachs after pretreated with 25mg/kg of chloro-substituted indole derivative (L2) is 151mm^2 and 58mm^2 when the stomachs were pretreated at 50mg/kg. The lesions area shown after pretreatment with 25mg/kg of L2 is almost similar to the lesions area after pretreatment with cimetidine, 168mm^2. The efficacy of L2 in inhibiting the formation of ulcerations is similar to cimetidine but the ulcerations area decreases by 93mm^2 after the stomachs were pretreated with 50mg/kg of L2. The reduction in gastric lesions area at 50mg/kg indicated that this dose is the minimum dose required in order L2 to exhibit gastroprotective effect on ethanol-induced gastric ulcers. Table 9 also shows that gastroprotection activity of L2 at 25mg/kg does not depends much on the mucus weight secreted because the stomachs pretreated with 25mg/kg of L2 secreted 0.28g of mucus, which is twice of the mucus secreted when the stomachs were pretreated

with 50mg/kg of L2. In addition, the pH values for both doses do not contribute much to the reduction of gastric lesions area at high dose (50mg/kg) as the pH values of the gastric juice obtained from pretreatment with 50mg/kg and 25mg/kg are 4.73 and 4.60 respectively. The difference in pH values of gastric juice upon pretreatment with L1 and L2 is not significant.

The gastroprotection shown by CuL2 at 25mg/kg and 50mg/kg is almost similar to that shown when the stomachs were pretreated with cimetidine and 25mg/kg of L2. The presence of copper(II) ion did not affect much on the gastric ulcers area thus, higher dose of CuL2 is required in order for the compound to exhibit better effect than cimetidine in reducing gastric lesions area. The secretion of mucus weight does not show significant difference because the mucus produced in pretreatment with 25mg/kg and 50mg/kg were 0.40g and 0.43g respectively. Prevention from ulcerations by CuL2 at both doses tested was not due to the production of mucus. Nevertheless, stomachs pretreated with low dose (25mg/kg) of CuL2 resulted in a decrease in gastric juice acidity, 5.45 while pH of gastric juice for stomachs pretreated with 50mg/kg reduced to 4.68. Copper(II) ion seemed to almost neutralizes the gastric juice at 25mg/kg since the presence of chlorine substituent has already make the gastric juice acidic. On the other hand, if the dose of CuL2 is increased to 50mg/kg, the gastric juice will be acidic and displayed approximate pH value as after pretreated with L2. When 25mg/kg of L2 and CuL2 is used in the pretreatment, it is clear that copper(II) ion is required for the increasing pH value of the gastric juice but when CuL2 is used at 50mg/kg, the compound will exhibit the same effect as that exhibited by L2.

The stomachs pretreated with 25mg/kg of NiL2 shows gastric lesions area of 439mm^2 while stomachs pretreated with 50mg/kg of the same compound exhibits lesions area of 210mm^2. The gastric lesions area is reduced by 229cm^2 when the dose is increased to 50mg/kg. The average mucus weight and pH of gastric juice also increase when 50mg/kg of NiL2 is used to pretreated gastric ulcerations on the stomachs. 0.47g of mucus was produced after pretreatment with 50mg/kg of NiL2 and 0.27g of mucus was secreted upon pretreatment with 25mg/kg of the same compound. Gastric juice of the stomachs pretreated with 50mg/kg of the compound resulted in pH value of 6.33 which is almost neutral while pH value for gastric juice after pretreated with low dose of NiL2 was measured as 5.22. Though L2 made the gastric juice acidic, nickel(II) ion played its role in neutralizing the acidity of the gastric juice. The presence of nickel(II) ion increases the mucus production, reduces the acidity of the gastric juice and prevent the formation of ulcers. Therefore, mucus secretion and pH of gastric juice depend much on the nickel(II) ion. Although CuL2 and NiL2 are square planar, the gastroprotection of CuL2 and NiL2 do not actually influenced by the molecular geometry of the complexes but the metal ion that is bonded to L2.

3.2.3.3 Gastroprotective Effect of L3 and its Complexes

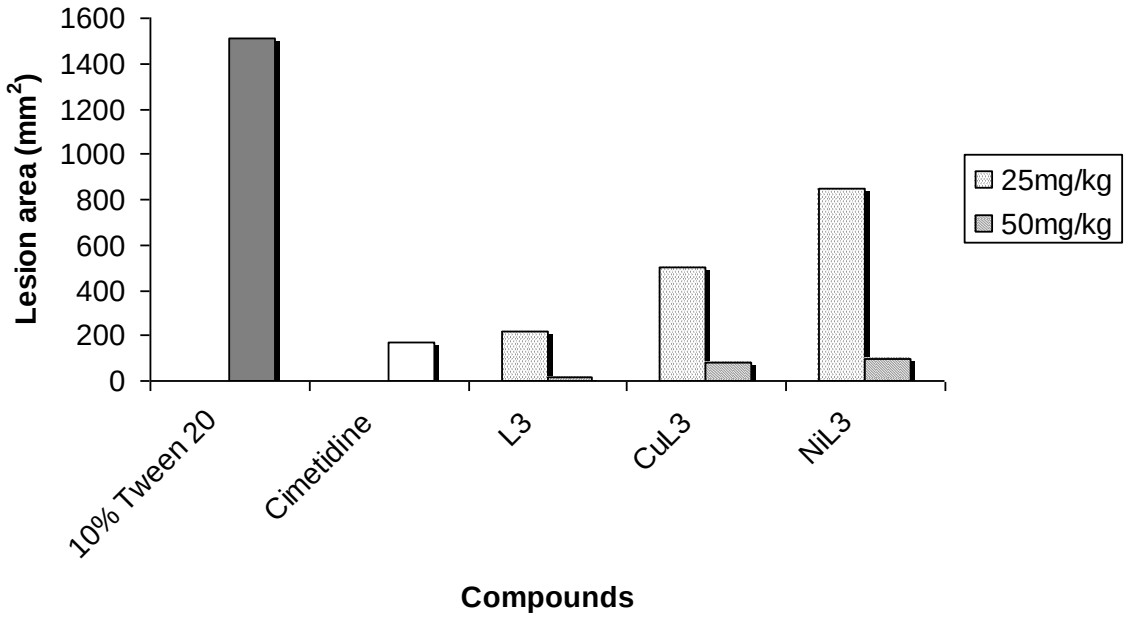


Figure 22: Lesions area on the stomach of a rat after pretreatment with bromoindolecarboxaldehyde – indolehydrazide (*L3*) and its complexes

The gastric lesions area measured when stomachs were pretreated with 25mg/kg of bromo-substituted indole derivatives (L3) is 221mm^2 which is approximately similar to the gastric lesions measured after stomachs were pretreated with cimetidine. A steep decrease in lesions area is observed in Figure 22 when L3 was administered to the rats at 50mg/kg which resulted in lesions area of 17mm^2. The activity of L3 as a gastroprotective agent however, does not influence by the mucus secretion. This is because the mucus obtained after stomachs were pretreated with 25mg/kg (low dose) and 50mg/kg (high dose) are giving almost the same weight. 0.34g of mucus was produced after pretreatment at low dose and 0.26g of mucus was secreted upon pretreatment at high dose. The pH values measured after pretreatment at both doses showed that L3 will result in a slight decrease in the acidity of the gastric juice. Stomachs pretreated with 25mg/kg of L3 resulted in pH

value of 4.84 while pH value obtained after pretreatment at 50mg/kg is 4.11. The presence bromine substituent was likely to increase the acidity of the gastric juice at 50mg/kg rather than in the presence of chlorine substituent (L2) and in the absence of substituent (L1). Hence, L3 is a potential anti-ulcer agent if 25mg/kg of the compound is used in the treatment.

CuL3 decreases the acidity of the gastric juice to 6.43 when 25mg/kg of the compound was administered to the rats but the acidity increases to 4.43 upon administration of the compound at 50mg/kg. The copper(II) ion is therefore responsible in adjusting the level of pH because the pH started to increase when copper(II) is bonded to L3. In order to neutralize the gastric juice, the role of copper(II) ion is very important. The mucus produced after pretreatment with CuL3 is similar to the mucus produced after pretreatment with L3. Table 9 also displayed that the lesions area of stomachs that were pretreated with 25mg/kg is 500mm^2 and the lesions area calculated from stomachs pretreated with 50mg/kg is 78mm^2. This result shows that the ulcerations induced by ethanol could be prevented when 50mg/kg of CuL3 is consumed because at this dose, the pH level of the gastric juice will be increased close to the neutral level. The results also suggested that CuL3 prevent the formation of ulcers through inhibiting the proton pump from secreting too much of acid.

The level of pH obtained after pretreatment with NiL3 resulted in almost similar to the pH level obtained when the stomachs were pretreated with L3. pH 4.65 and pH 3.93 was measured after pretreatment at 25mg/kg and 50mg/kg respectively. This indicates that nickel(II) ion does not change the level of pH and the ion is not required in order to change the level of the pH. Production of mucus also showed no significant difference between the doses tested and suggest that chelating L3 to nickel(II) ion will not trigger the production of

mucus in large quantities. Consumption of 50mg/kg of NiL3 does not prevent gastric ulceration as much as consuming L3 at the same dose. It is believed that when NiL3 is consumed excessively, the mucus of the stomach will be eroded and increases the acidity of gastric juice. These factors will result in damaging the stomach wall. Although NiL3 does not affect much on the mucus secretion and the pH of the gastric juice, NiL3 was found to exhibit gastroprotective effect at 50mg/kg. At this dose, the ulceration area observed was $100mm^2$.

The molecular geometry of CuL3 and NiL3 does not affect in preventing ethanol-induced gastric ulcers. Chelating each copper(II) and nickel(II) ions to L3 resulted in decreasing of activity against gastric ulcerations at 50mg/kg. Absence of metal ion chelating to L3 is a factor that enhances the gastroprotective effect of L3 as an anti-ulcer agent at 50mg/kg but when chelating L3 to the copper(II) and nickel(II) ions, the gastroprotective effects dropped slightly at 50mg/kg. This suggests that having copper(II) and nickel(II) ions is not necessary in order to improve the gastroprotective effect of L3.

3.2.3.4 Percentage Inhibition in Ethanol-Induced Gastric Ulcer

According to the assumptions made by previous researchers, the factors that influenced the formation of gastric ulcer are due to the acidity of the gastric juice and the mucus that protect the stomach wall from being eroded by hydrochloric acid (Yamada, 1999). Damage of defensive mucus barrier and excess acid secretion has been said to cause ulceration. Gastric mucus is a very important protective barrier for gastric mucosa and consists of viscous, elastic, adherent and transparent gel formed by water and glycoprotein where its protective properties depend not only on the structure of the gel but also on the

amount or thickness of the layer covering the mucosal surface (Hiruma-Lima *et.al*, 2006). By reducing the amount of acid secreted and enhancing the production of mucus barrier, ulceration can be cured. Hence, to prevent or to treat ulceration, the pH value of the gastric juice should be increased and the mucus production should be enhanced.

Figure 23 shows the percentage inhibition of test compounds on ethanol-induced gastric ulcers at 25mg/kg and 50mg/kg. The percentage of inhibition was calculated by using Equation 5.

$$\% \text{ inhibition} = \frac{\text{Ulcer area (control)} - \text{Ulcer area (treatment)}}{\text{Ulcer area (control)}} \times 100 \qquad \text{[Equation 5]}$$

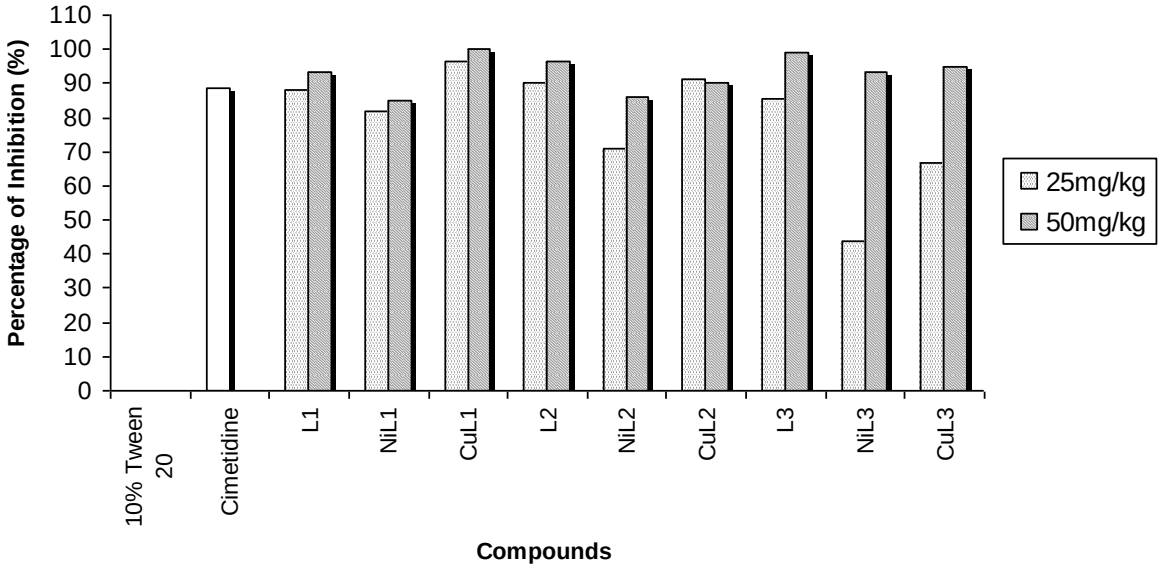


Figure 23: Percentage inhibition of indole derivatives in ethanol-induced gastric ulcer.

Pretreatment with all ligands synthesized inhibited the formation of gastric ulcers in the stomachs excellently. Percentage of inhibitions obtained after pretreatment with L1, L2 and

L3 at 25mg/kg and 50mg/kg were above 80%. Percentage of inhibitions in the formation of ulcers by L1, L2 and L3 at 25mg/kg was 82.5%, 90% and 85.4% respectively. When the stomachs were pretreated with 50mg/kg of L1, L2 and L3, the inhibitions increased to 94.5%, 96.2% and 98.9% respectively. There is no significant difference in percentage of inhibitions since stomachs pretreated with all ligands displayed almost no change between the doses tested. This indicated that consumptions of 25mg/kg of L1, L2 and L3 were already sufficient to protect the stomach wall from gastric ulcers. The presence of chlorine and bromine atoms however, did not contribute much in the inhibition of ulcers thus, indicating that the halogens are not required in order to enhance gastroprotective activity.

Nickel(II) complexes of L1 and L2 did not show significant differences between the doses tested. This is because NiL1 exhibited 82% and 84.9% inhibition of ulcerations after pretreatment with 25mg/kg and 50mg/kg. Besides, NiL2 inhibited ulcerations by 71% at 25mg/kg and 86.1% at 50mg/kg. However, a significant difference in percentage of inhibition was observed when the stomachs were pretreated with 25mg/kg and 50mg/kg of NiL3 resulting in inhibition of 44% and 93.4% respectively. Bromine atoms on the indole rings are actually essential in ensuring the efficacy of the nickel(II) complexes. Nickel(II) ions chelating to L3 increases the gastroprotective activity by 49.4%, which indicating the importance of having bromine atoms and nickel(II) ions in the same structure. Nevertheless, the stomachs that were pretreated with 25mg/kg of L3 and NiL3 exhibited 41.4% difference inhibition of ulcerations hence, indicating that absence of nickel(II) ion would increase the gastroprotective effect of L3 against ethanol-induced gastric ulcers.

Similar trend in inhibition of ulcerations was observed when copper(II) complexes of each ligands were used in pretreatments. CuL1 and CuL2 did not show any significant difference in percentage of inhibition when these complexes were used in pretreatment of the

stomachs at 25mg/kg and 50mg/kg. However, 50mg/kg of CuL3 consumed in the pretreatment resulted in 94.8% of inhibition of ulcerations while pretreatment that was carried out by consumption of 25mg/kg of CuL3 showed a decrease in inhibition to 66.9% with a decrement of inhibition percentage by 27.9%. The percentage of inhibition shown by bromo-substituted indole derivatives and its complexes displayed a clear difference after pretreatment at 25mg/kg was carried out. The copper(II) and nickel(II) ions were actually reducing the activity of L3 as an anti-ulcer agent when the complexes were used at 25mg/kg. Therefore, copper(II) and nickel(II) ions are not essential in maintaining or enhancing the activity of L3 at low dose tested but when the complexes were used at 50mg/kg, the activities of NiL3 and CuL3 in percentage of inhibition almost reach the percentage of inhibition performed by L3.

In accordance to the activities performed by the groups pretreated with L1 and L2, the presence of copper(II) ions in CuL1 and CuL2 demonstrated the same or better gastroprotective effects from their ligands at 25mg/kg. The nickel(II) complexes of L1 and L2 however, exhibited slight reduction in inhibition percentage when pretreatment was performed at 25mg/kg. Overall, substituents and metal ions effects in inhibiting ulcerations on the stomachs are not seen for L1, L2 and the copper(II) and nickel(II) complexes of L1 and L2. Though copper(II) and nickel(II) complexes synthesized are square planar, the molecular geometry did not affect the activities of the compounds. All ligands were seen to act as inhibitory agents on ulcers at low and high doses but their complexes were found to perform at high dose. Only compounds with bromine atoms are dose dependent, where their activities depends on the dose used in the pretreatment while compounds with chlorine atoms and without halogens are not dose dependent.

Since all tested compounds are good as inhibitory agents, they can be used to protect from stomach ulceration to some patients when they take drugs that may cause inflammation and ulceration on the stomach wall such as NSAIDs (aspirin, ibuprofen, naproxen and indomethacin).

3.2.4 Glucose Tolerance Test

3.2.4.1 Percentage of Change in Blood Glucose Level

From the study, all compounds that had been tested on mice for blood glucose lowering effect displayed good results as compared to the negative control group which was pretreated with 5% Tween 80. Average blood glucose levels written in bold as shown in Table 10 resemble glibenclamide since the compounds exhibited hypoglycemic effect similar to that shown by glibenclamide. The risk of hypoglycemia is one of the side effects carries by sulfonylurea classes upon long time use of the drug and this is truly apply for sulfonylurea classes of drugs (Abraham, 2003). Acting as an insulinotropic agent, drugs of sulfonylurea class directly stimulates the secretion of insulin from pancreatic β-cells (Abraham, 2003) which makes up 65 – 80% of the cells in the islets of Langerhans (Galloway, 1988). Sulfonylurea drugs act by closing the membrane-bound ATP-sensitive potassium (KATP) channels on the β-cell thus depolarizes and opens the voltage-gated calcium channels which then triggers exocytosis of insulin (Abraham, 2003).

Table 10:
Average blood glucose levels ± S.E.M. before and after injection of glucose subcutaneously.

Compounds	100 mg/kg		30 mg/kg		10 mg/kg	
	Fasting BGL	**BGL after glucose loading**	**Fasting BGL**	**BGL after glucose loading**	**Fasting BGL**	**BGL after glucose loading**
$C_{19}H_{16}N_4O$ (*L1*)	4.3 ± 0.6	6.5 ± 0.8	5.9 ± 0.4	7.0 ± 0.3	5.4 ± 0.3	5.8 ± 0.6
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	4.7 ± 1.0	4.7 ± 0.6	6.3 ± 0.5	8.2 ± 0.6	6.2 ± 0.4	9.2 ± 2.1
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	5.3 ± 0.8	6.4 ± 0.9	5.0 ± 0.7	6.1 ± 0.7	5.3 ± 0.7	6.3 ± 0.5
$C_{19}H_{15}ClN_4O$ (*L2*)	5.6 ± 0.8	6.3 ± 0.6	5.9 ± 0.6	7.2 ± 0.9	6.3 ± 0.6	8.1 ± 1.2
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	5.3 ± 0.8	6.6 ± 0.8	**5.0 ± 0.6**	**4.7 ± 0.3**	5.6 ± 0.7	7.3 ± 0.7
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	**8.0 ± 0.9**	**6.9 ± 0.2**	5.4 ± 0.5	7.7 ± 0.7	6.7 ± 0.3	7.3 ± 0.3
$C_{19}H_{15}BrN_4O$ (*L3*)	4.5 ± 0.7	6.5 ± 0.5	**7.0 ± 1.0**	**6.7 ± 0.8**	**6.4 ± 0.3**	**5.8 ± 0.3**
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	7.2 ± 0.6	8.7 ± 0.4	7.3 ± 0.5	7.9 ± 0.7	5.8 ± 0.4	5.8 ± 0.3
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	5.1 ± 0.5	7.5 ± 0.7	5.8 ± 0.6	6.6 ± 0.4	5.9 ± 0.3	6.1 ± 0.4
5% Tween 80	Fasting: 4.5 ± 0.4		After: 8.6 ± 0.8			
Glibenclamide	Fasting: 3.5 ± 0.4		After: 2.7 ± 0.3			

**All values are expressed in significant of $p<0.05$.*

The percentage of change in blood glucose level as shown in Table 11, is used to evaluate the anti-hyperglycemic activity of test compounds at 10mg/kg, 30mg/kg and 100mg/kg. Equation 6 is used to calculate the percentage of change in blood glucose level.

$$\text{Percentage of change} = \frac{(BGL_{after} - BGL_{fasting})}{BGL_{fasting}} \times 100 \qquad \text{[Equation 6]}$$

The values of each sample at each dose will be compared to the value of negative control group. The efficacy of a compound in reducing blood glucose level is measured when the

percentage of change in blood glucose level of the group pretreated at a tested concentration is lower than the group pretreated with 5% Tween 80. Zero in percentage of change indicated that the blood glucose level after 90 minutes of glucose loading has reached to the normal level thus indicating that the compound is able to act as a good anti-diabetic agent.

Table 11:
Percentage of change (%) in blood glucose level in mice.

Compounds	**Percentage of change (%)**		
	10mg/kg	**30mg/kg**	**100mg/kg**
$C_{19}H_{16}N_4O$ (*L1*)	7.4	18.6	51.2
$Ni(C_{19}H_{16}N_4O)_2$ (*NiL1*)	48.4	30.2	0
$Cu(C_{19}H_{16}N_4O)_2$ (*CuL1*)	18.9	22.0	20.8
$C_{19}H_{15}ClN_4O$ (*L2*)	28.6	22.0	12.5
$Ni(C_{19}H_{15}ClN_4O)_2$ (*NiL2*)	30.4	-6.0	24.5
$Cu(C_{19}H_{15}ClN_4O)_2$ (*CuL2*)	9.0	42.6	-13.8
$C_{19}H_{15}BrN_4O$ (*L3*)	-9.4	-4.3	44.4
$Ni(C_{19}H_{15}BrN_4O)_2$ (*NiL3*)	0	8.2	20.8
$Cu(C_{19}H_{15}BrN_4O)_2$ (*CuL3*)	3.4	13.8	47.1
5% Tween 80		91.1	
Glibenclamide		-22.9	

All compounds exhibited lower percentage of change than that of the negative control group (5% Tween 80) which shows that these compounds are potentially active in reducing blood glucose level in mice. The ability to behave as a potential hypoglycemic compound is the most important criteria in this study. Hence, a decrease in percentage of changes as compared with the group pretreated with the vehicle only is very much desirable. However,

percentage of change which is negative is a sign that the compound at certain concentrations may develop severe hypoglycemic effect. In this case, glibenclamide is an example of sulfonylurea class of anti-diabetic drug which causes hypoglycemia upon longer usage. Glibenclamide may serve as a good anti-hyperglycemic agent but it also introduced severe hypoglycemia as its long term side effect. A negative value of percentage of change in blood glucose level is therefore, not a good sign even though the tested compound could be potentially active for blood glucose lowering.

3.2.4.2 Blood Glucose Level after Pretreatment with L1 and its Complexes

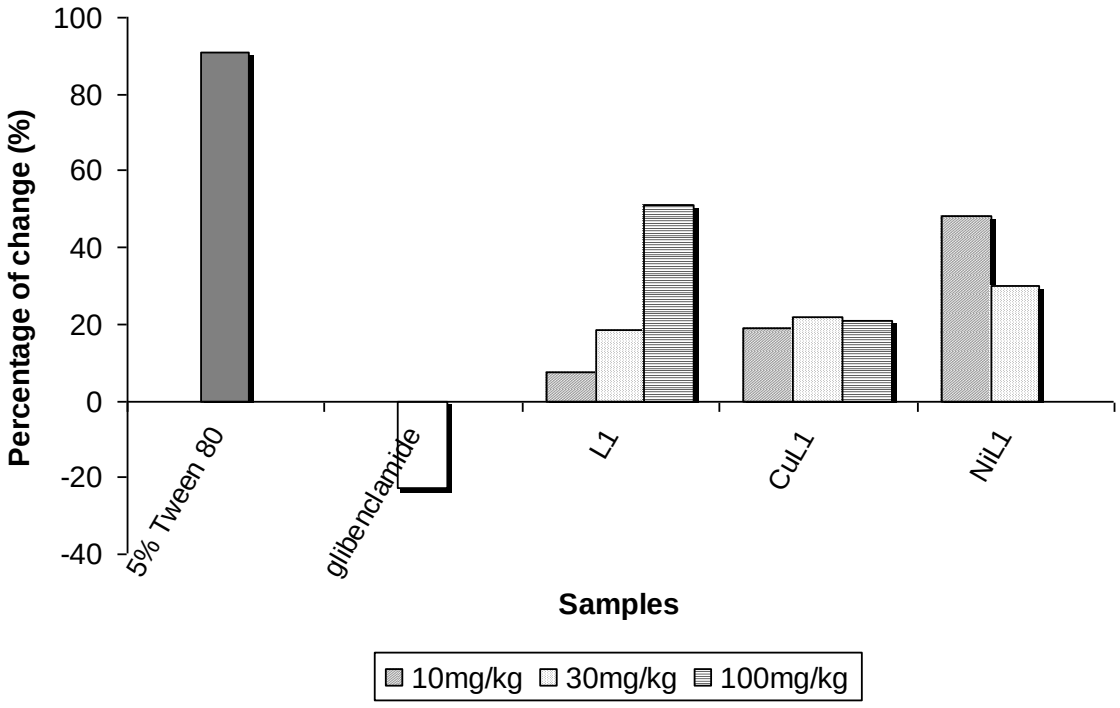


Figure 24: Percentage of change in blood glucose level after pretreatment with indolecarboxaldehyde – indolehydrazide (*L1*) and its complexes

Unsubstituted indole derivatives showed potent activity towards reducing blood glucose level where the activity is concentration dependent (see Figure 24). There is significant difference between the concentrations used for each compound. L1 is potentially active at

10mg/kg but started to decrease its efficacy as the concentration increases from 10mg/kg to 30mg/kg and 100mg/kg. The anti-hyperglycemic activity exhibited by L1 might due to its ability to enhance insulin secretion from pancreas or increases the sensitivity of receptors to sense insulin. However, L1 might inhibit β cells of the pancreas from producing insulin resulting in a decrement in hypoglycemic activity if to be taken excessively. When insulin production is decreasing, the level of glucose in the blood would also increase but the increment is not as drastic as observed in group received the vehicle (5% Tween 80).

Consumption of 10mg/kg of CuL1 was found to reduce the blood glucose level within 90 minutes after glucose loading. The existence of copper(II) ion as the central metal ion, did not however, exhibit any dissimilarity among the concentrations tested. Consequently, further investigation with higher concentration needs to be carried out with the intention of finding suitable dose that ensures CuL1 as a potential anti-hyperglycemic agent. Although the presence of copper(II) ion did not make any difference upon doses used, the complex is still as useful as L1 for it reduced blood glucose level better than L1 at the concentration of 100mg/kg.

NiL1 however, showed its anti-hyperglycemic effect at 100mg/kg where the percentage of change observed is zero indicating that NiL1 is potentially active as an hypoglycemic agent. The presence of nickel(II) ion reduces the activity at 10mg/kg but increases the activity at 100mg/kg. The side effect of L1 at 100mg/kg might be reduced by chelating L1 to the nickel(II) ion while mice pretreated with copper(II) complex of L1 at a range of concentration from 10mg/kg to 100mg/kg showed similar effectiveness as shown by mice pretreated with 30mg/kg of L1.

It seemed that the anti-hyperglycemic activity increases at 100mg/kg upon complexion of L1 with copper(II) and nickel(II) ions. The function or role of having a metal ion in the molecular structure actually helped to improve the effectiveness of the ligand in order to show its ability to reduce blood glucose level. Thus, the ligand which is almost inactive by itself at 100mg/kg, when chelating to the copper(II) and nickel(II) ions, might as well increase the property of anti-hyperglycemic. Therefore, the concentration of 10mg/kg might be insufficient for the complexes to exhibit as anti-diabetic agents but sufficient enough for L1 to act as a hypoglycemic or anti-hyperglycemic agent.

3.2.4.3 Blood Glucose Level after Pretreatment with L2 and its Complexes

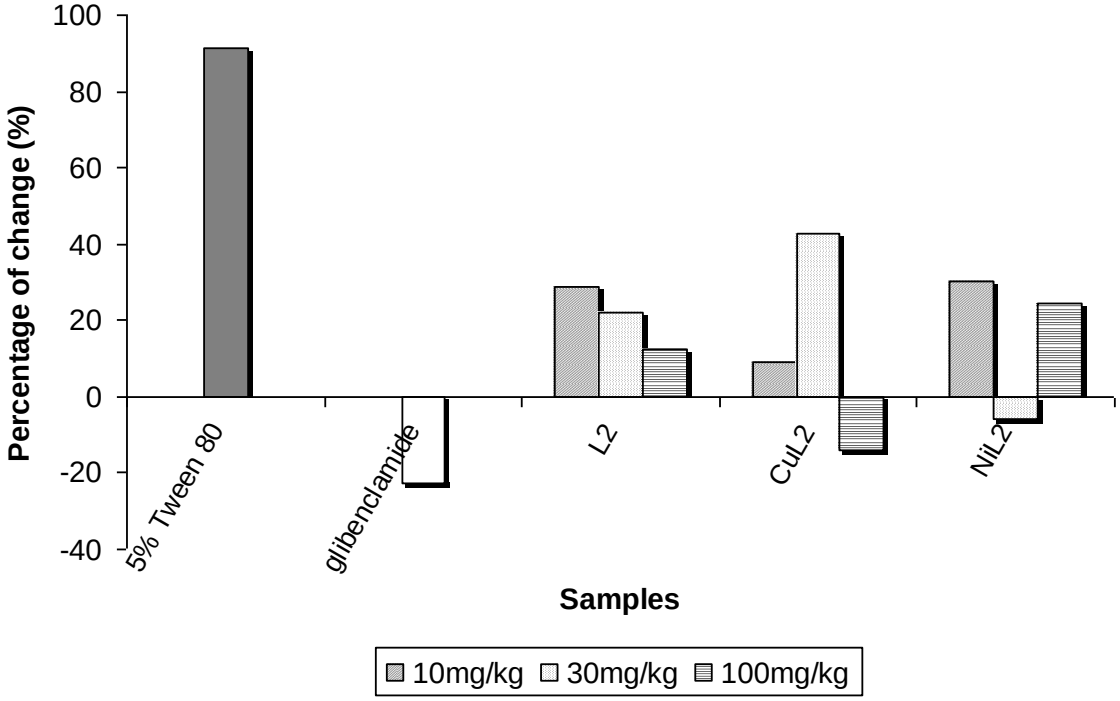


Figure 25: Percentage of change in blood glucose level after pretreatment with chloroindolecarboxaldehyde – indolehydrazide (*L2*) and its complexes

There is an increment in anti-hyperglycemic activity when mice were pretreated with L2. The activity increases with the concentrations used. Significant change in blood glucose

level was observed in Figure 25 when mice were pretreated with 10mg/kg, 30mg/kg and 100mg/kg of L2. It seemed that in the presence of chlorine atom on the 5th carbon of indole-3-carboxaldehyde, the concentration required is increased in order to increase the activity of L2. Chlorine atom also improve the activity by reducing the side effect of L1 at 100mg/kg hence, suggesting L2 as a safer compound than L1 if L2 is required to be consume for a longer period.

Besides, CuL2 showed a small difference in its activity. The percentage of change increases as the concentration increases to 30mg/kg indicating its ineffectiveness than 10mg/kg but when 100mg/kg of the compound was administered, the level of blood glucose dropped drastically and introduced hypoglycemia in mice. This phenomenon can be explained in such a way that CuL2 enhances the pancreas to generate more insulin so there are excess of insulin secreted into the blood stream thus, glucose is drastically reduced. If that is the case, then mice that were given 100mg/kg of CuL2 would experience severe hypoglycemia if this compound is to be taken upon longer time of medication.

Nickel(II) complexes of L2 did not show any significant change in blood glucose level after mice were pretreated with 10mg/kg and 100mg/kg. The percentage of change shown after pretreatment with 10mg/kg and 100mg/kg of NiL2 showed similarity as shown by L2. This suggested that the presence of nickel(II) ion did not change the anti-hyperglycemic activity of L2. From Figure 18, it is clear that nickel(II) ion did not contribute in improvement of L2 as copper(II) ion. The role of copper(II) ion in L2 is seen to increase the hypoglycemic activity when the mice were pretreated with CuL2 at the concentration of 10mg/kg and 100mg/kg.

3.2.4.4 Blood Glucose Level after Pretreatment with L3 and its Complexes

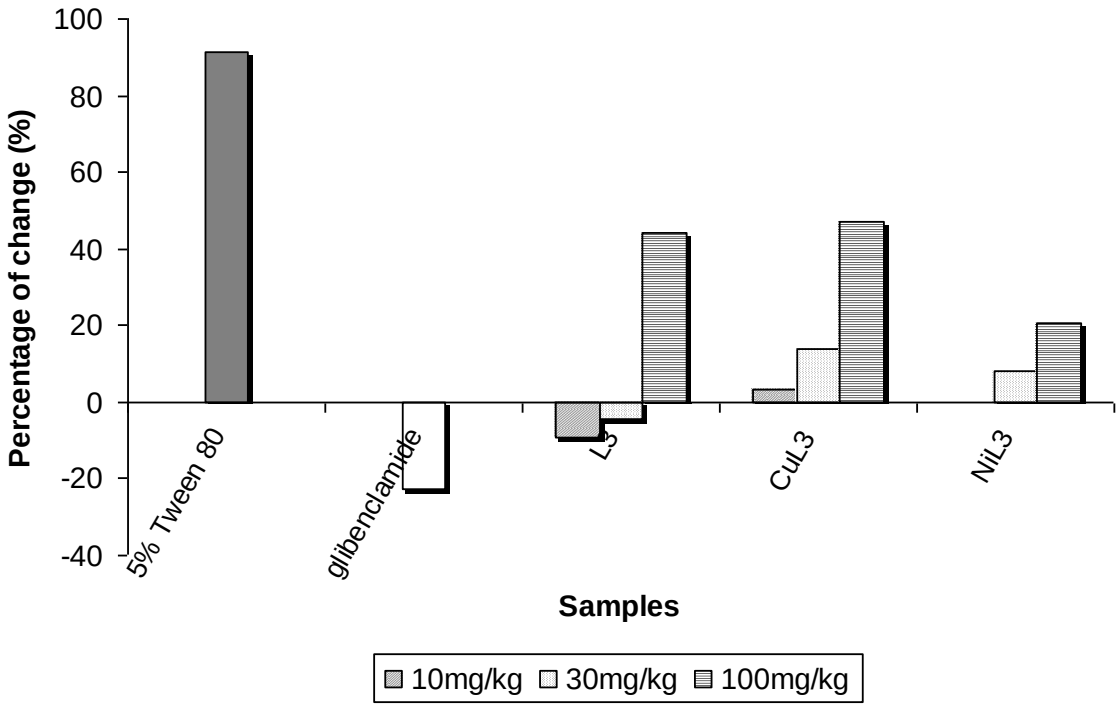


Figure 26: Percentage of change in blood glucose level after pretreatment with bromoindolecarboxaldehyde – indolehydrazide (*L3*) and its complexes

Bromo-substituted indole derivatives demonstrated promising activity according to their respective concentrations as shown in Figure 26. Blood glucose level in mice is reduced below its normal value when the mice undergo pretreatment with L3 at the concentrations of 10mg/kg and 30mg/kg but the anti-hyperglycemic activity was slowed down after pretreatment with 100mg/kg of L3. From the observation, 30mg/kg of L3 is seen to perform better activity since the blood glucose level after pretreatment is found to be approximate to that of the fasting blood glucose level. Nonetheless, pretreatment with 100mg/kg of L3 might cause damage on the receptor hence reducing the sensitivity of those receptors to sense the presence of insulin or it might also cause damage on the pancreatic β cells which then led to the inhibition on insulin secretion (Abraham, 2003).

Meanwhile, CuL3 and NiL3 also showed similar effect like L3. Anti-hyperglycemic effect is seen when pretreatment was carried out by applying 10mg/kg instead of 100mg/kg of test compounds. The anti-hyperglycemic activity reduces as the concentration increases. Even so, upon complexion to copper(II) and nickel(II) ions, the percentages of change obtained were reduced than that obtained after pretreatment with 10mg/kg and 30mg/kg of L3. Despite showing a negative value in percentage of change, the central metal ion played a role in determining the efficacy of the complexes.

Mice pretreated with NiL3 showed excellent reduction in blood glucose level at 10mg/kg. The difference between the latter and fasting blood glucose level is zero, indicating that NiL3 can become a potential anti-hyperglycemic agent at a minimum concentration of 30mg/kg. L3 and CuL3 are unlikely to behave as anti-hyperglycemic agents at 100mg/kg but NiL3 showed such promising activity at all concentrations tested.

The nickel(II) and copper(II) ions decreases the severe anti-hyperglycemic activity of L3 so that the blood glucose level will not be reduced lower than the normal blood glucose level. These ions also ensure that the reduction of blood glucose level in mice reach to the nearest level from the normal blood glucose level. Therefore, NiL3 and CuL3 can be consumed at a minimum concentration of 10mg/kg but are able to be promising anti-hyperglycemic agents.