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REVIEW

Toxic Effect of Different Neem Formulations against Pests and Mammals

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ABSTRACT

Neem (*Azadirachta indica* A. Juss), prominently known as conventional medication is a local plant in India. Neem is regarded as a promising tree species which can be utilized in variety ways to benefit agricultural communities throughout the world. Neem based insect sprays were productive for the control of different insect species, yet their low lingering impact and absence of normalized definitions are issues for field application. Additionally, neem is fairly have harmful impact in nature. The use of nanotechnology as a mean for nanopesticides is in the beginning time of improvement. All things considered, the nanosphere definition demonstrated upgraded systemicity of the dynamic fixings and made its infiltration better in the plant, because of their little size. Nanoencapsulated pesticides can give controlled discharge energy, while proficiently upgrading piousness, dependability, and solvency. Nanoencapsulation can improve the vermin control proficiency over expanded spans by forestalling debasement of dynamic fixings under ecological conditions. This survey is hence composed to fundamentally evaluate the toxicological impacts i.e to examine the manifestations, systems and identifications of poisoning vertebrates particularly people. The prepared neem nano-plants contrasted with the bulk one have will be assessed on albino mice through two main approaches, i.e. determination of acute oral LD50 and study the toxic effect of sublethal dose (LD10) on some biochemical parameters. The effect of the prepared nano-formulations compared to the bulk one on various biomarkers, i.e. hematological, hepatotoxicity and nephrotoxicity in albino mice after an oral administration of sub-lethal dose during sub-acute treatment were taken in consideration. Hence, this review should thus offer an important guide for building up potential advantages are underlined, while little is known on security or the antagonistic impacts of nano-advances in the agro-foodsystem.

1. Introduction

Chemical synthesized pesticides are extensively used for controlling pests such as insects, mites, fungi, nematodes, rodents, weeds and others. The most basic models in crop production is to diminish decrease in crops and for delivering enough and solid food because of the expanding human populaces overall (7.7 billion) [1]. It has been recorded that around 3 million ton of insect sprays are utilized for crop insurance annually [2]. These inordinate utilization of harmful risky poisonous pesticides which may present expected ecolog-

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ical and natural dangers to human health, non-target living beings and ecosystem[1]. The most widely recognized issues in crop protection are pesticide residues and pest resistance. Likewise, the pesticides deposits influence open health[3]. Insect sprays are utilized to ensure vegetables, plantations, fancy plants and put away item materials against numerous pests during storage and in the field.. In spite of the fact that the fundamental motivation behind these manufactured pest sprays was known, it can make numerous unfavorable impacts individuals,animals, feathered creatures and environment [3]. Also, widespread use of pesticides has led to the development of resistance to pesticides such as "organophosphorous compound, carbamates, pyrethroids, abamectin and indixacarb [4-10]". In this way, the persistent assessments of the plant insect sprays "for security poisonousness are extraordinarily required utilizing diverse creature models since the reactions by these creatures to synthetic operators changes widely [11]". At this moment, new example is going in the state of mind for using the basic plant extracts for instance "neem extracts or EOs [12,13] as regular pesticides to control insects with nanoformulations in green pest management [3]". These green and nanopesticides are safe, low or no mammalian destructiveness and have various goals of noxious action in insect pests, which lead to high selectivity and low restriction development [14,15]. Extracts from the neem tree "*Azadirachta indica,* A. Juss" green growth, organisms, microorganisms and fundamental oils of other plant roots are dynamic against several insect species including vertebrates and useful parasitoids and predators. They are utilized as options in contrast to the customary compound pesticides which lead to ecological unevenness as per their unsafe consequences for normal development, or the event of vermin opposition and the hurtful impact of pesticides deposits in soil or plant.

Low or no mammalian harmfulness and have numerous locales of poisonous activity in pests, which lead to high selectivity and low resistance development [14,15]. Extracts from the neem tree "*Azadirachta indica,* A. Juss" green growth, organisms, microscopic organisms and fundamental oils of other plant origin are dynamic against several pest species including vertebrates and helpful insects. They are utilized as options in contrast to the conventional synthetic pesticides which lead to the occurrence of pest resistance, ecological unevenness as per their hurtful consequences for natural enemies, or the event of harmful opposition and the unsafe impact of pesticides deposits in soil or plant. "As needs be, it is imperative to discover safe choices like plant extracts or basic oils which have demonstrated victories in pest control, as they have insecticidal action, influence the irritation fruitfulness, give antifeedant or obstacle impact just as influence the biochemical procedures inside the insect body". According to another perspective, one of the detriments of the customary organic items is their restricted soundness when applied in the field.

Extracts from the neem tree "*Azadirachta indica*, A. Juss" green growth, organisms, microorganisms and fundamental oils of other plant sources are dynamic against many insect species including vertebrates and valuable biological control species. They are utilized as options in contrast to the customary synthetic pesticides which lead to ecological unevenness as indicated by their unsafe consequences for common foes, or the event of anti-feedant or deterrent effect and the destructive impact of pesticides deposits in soil or plant. Likewise, it is critical to discover safe choices like plant extracts or natural oils which have indicated success in pest control, as they have insecticidal efficacy, influence the insect fertility, give antifeedant or obstruction impact just as influence the biochemical procedures inside the insect body. According to another perspective, one of the detriments of the customary herbal items is their restricted soundness when applied in the field.

Considering the real factors implied the massive number of plant extracts, it was found that neem extracts (got from the tree *Azadirachta indica*) is the most well known plant extracts among others. Its insecticidal activity relies upon its strategy for movement as interfering with larval turn of events and improvement, advancement obstacle, development impediment, or enlistment of malformation in the larval stage. "In the grown up adult insects, these concentrates block egg development and may in like cause sterilization in different insect pests and such impacts can likewise be prompted as hormone application [16,17]"

To conquer the inconveniences of the conventional organic extracts for example, instability chemical disintegration and consequently loss insecticide properties their pest spray properties, green nano-innovation is a fruitful way which is as of late used to improve the pesticide properties of plant insect sprays. Nano-innovation has become a significant examination field in all zones. The size, direction and physical properties of nano-particles have supposedly appeared to change the exhibition of any material [18]. Advancement of green procedures for the combination of nano-particles is developing into a significant part of nanotechnology [19]. Be that as it may, phytochemicals, for example, secondary metabolites and fundamental oils face issues of stability and cost adequacy. If there should be an occurrence of fundamental oils, their compound precariousness within the sight of air, light, dampness, and high temperatures that cause quick vanishing and corruption of

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some active components are significant concern. Joining of fundamental oils into a controlled-discharge nano-definition forestalls fast dissipation. Moreover, this sort of definition is required to be more successful than the bulk substances [21,22]. Then again, it has been discovered that pesticide nano formulations indicated less harmfulness towards non target organisms contrasted with bulk or business formulations and subsequently a higher explicitness was observed [23] corruption; prevents rapid evaporation and degradation; enhances stability improves solidness and keeps up the base viable measurements / application because of covering process [20].

To conquer the weaknesses of the customary natural extracts, for example instabiltiy, chemical decomposition and henceforth misfortune their insect spray properties, green nano-innovation is a fruitful way which is as of late used to improve the pesticide properties of herbal pest sprays. Nano-innovation has become a significant examination field in all zones. The size, direction and physical properties of nano-particles have purportedly appeared to change the exhibition of any material [18]. Advancement of green procedures for the blend of nano-particles is developing into a significant part of nanotechnology [19]. Be that as it may, phytochemicals, for example, auxiliary metabolites and basic oils face issues of strength and cost viability. If there should arise an occurrence of fundamental oils, their concoction shakiness within the sight of air, light, dampness, and high temperatures that cause quick vanishing and debasement of some dynamic parts are significant concern. Fuse of basic oils into a controlled-discharge nano-definition forestalls fast vanishing and corruption; improves strength and keeps up the base compelling measurement application because of covering process [20].

Additionally, such an enumerating is depended upon to be more reasonable than the mass substances [21,22]. On the other hand, it has been found that pesticide nano definitions exhibited less poisonous towards non target animals contrasted with mass or commercial formulations and in this manner a higher explicitness was watched. The target of the current survey is to focus on the harmfulness of Neem extracts and Neem nano-items as few is recorded on safety and the antagonistic impacts of the utilization of nano-advances in the agro-food sector [24].

2. Plant Extracts as Biological Control Agents

The characteristic issues achieved by maltreatment of pesticides have been the matter of stress for both scientists and people in recent time. It has been assessed that 3 million tons of pesticides[39] are used on crops each year and the general damage realized by pesticides comes to $100 billion every year. The reasons behind this are the high poisonousness and non-biodegradable properties of pesticides and the development in soil, water resources and harvests that impact open health [3].

Thusly, it must be considered to search for new significantly explicit and biodegradable pesticides to handle the issue of long haul harmfulness to vertebrate creatures, of course, one must analyze the environmental friendly pesticides and make techniques that can be used to decrease pesticide use while keeping up crop yields.

Natural products are a super option in contrast to synthetic pesticides as intends to diminish negative effects on human wellbeing and the earth. Advancing toward green science forms and the proceeding with requirement for growing new harvest security instruments with novel methods of activity makes disclosure pesticides as an alluring and productive interest that is telling consideration.. botanical insecticides are progressively pulling in research consideration as they offer novel methods of activity that may give viable control of pests that have just evolved protection from traditional insect sprays [25]. Neem "*Azadirachta indica*" is one of the most significant limonoid creating plants from Meliaceae family [26] has for some time been perceived as a wellspring of condition agreeable biopesticide. A few constitutions of its leaves and seeds show marked insect control potential and because of their relative selectivity, neem items can be suggested for some Integrated Pest Management (IPM) programs [27]. In any case, there are a couple of occurrences of things got from plant extracts, for instance, neem oil, China berry seed extracts among others that apply diverse method of activities to execute the focus insect pest. From those referenced techniques for activities of such plant extracts, the antifeedant [28,29], (insect development regulators) [30,16] and sterilizers [31]. Most work has focused on azadirachtin and other related compounds luxuriously from neem seed extracts which go about as both solid antifeedants and insect growth regulators. Azadirachtin and its contents has antifeedant effect due to either hydrogenation of - 22 twofold bonds or deacetylation caused any change by impeding of hydroxyl group affected the taking care of inhibitory activity, while acetylation of azadirachtin caused a decrease in the most extraordinary activity [29].

Further the sound framework synthetic structure around hemi acetyl region is critical for antifeedent development. Azadirachtin impacts the insect's reproductive organs, body improvement and other endocrine systems [31] and doesn't impact other biocontrol agent. Neem has impacted more than 300 insect pests [31]. Further neem items are bio-degradable, slight destructive or no noxious to non target living forms, while they are non-hurtful toward people and well evolved creatures [31].

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Neem roots have a capacity to recover sicknesses what's more give a couple of pest spray properties against insect pests [32]. In like way, the perniciousness characteristics of azadirachtin and the component of its insecticidal activity were besides considered [33]. Extracts of various parts of the tree, especially of the seeds, have been appeared to posses feeding prevention properties (antifeedant), repellency, toxicity and growth disruptive properties to different species and phases of insects of different orders "So that, one of the activities of neem is considered as insect growth regulator". A comprehensive review of the entomological properties of neem has been published [16,34,35] Be that as it Neem oil extracted from the seeds of neem tree has been known to contain various bioactive blends significant mixtures are tri-terpenoids of the class of limonoid. Noteworthy mixture as uncovered in published works seem to be (azadirachtin A), salanin, nimbin, 3-tigloylazadirachtol (azadirachtin B), and 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (azadirachtin D) [36].

Neem subordinates don't kill insect pests straight forwardly, however behavioural and physiological properties and starvation antifeeding of insects on treated plants [17,37,38]. "The most significant active principal of neem compound is azadirachtin which unequivocally meddles with larval development and improvement. The morphological impacts are growth retardation, development hindrance, molting inhibition, or induction of abnormality."

Insect growth regulatory activity of neem debilitates the cuticle defense system of the larvae causing simple entrance of pathogenic organisms into insect framework. Azadirachtin, a naturally dynamic compound has been advanced as another new pest spray that is viewed as more eco-accommodating than synthetic insecticides. The pesticide adequacy, environmental security and open agreeableness of neem and its items has prompted its selection into different mosquito control programs [22]. Rao et al.,[40] demonstrated that the LC_{50} values for neonate and the second instar hatchlings of *Helicoverpa armigera* were 0.002 and 0.004 %, respectively when ate from Neem Azal-treated cotton leaves constantly. The LC_{50} values were 0.005, 0.02, and 0.03% for the first, second, and third instar hatchlings of *H. armigera,* respectively, when the acquaintance was compelled with 48 hr. Besides, they itemized that the concentration of 200 ppm of Neem Azal on a very basic level diminished larval and pupal weight in assessment with control.

Dimetry, et al.,[41] found that the LC_{50} values for second and fourth instar larvae of *Agrotis ipsilon* were 4.38 and 16.68 ppm individually when given semi-synthetic diet contain various convergences of azadirachtin when the introduction time frame was 96 hours. They included that LC_{90} carried on a similar action request as they were 8.57 and 34.74 ppm for the second and fourth instar individually.

Amin et al.,[42] brought up that neem oil at a sublethal portion (0.75ppm) azadirachtin when added to artificial diet and fed to second instar larvae of *Agrotis ipsilon* expanded the larval length to 22.71 days contrasted and 18.82 days for the control larvae and the level of larval mortalities expanded to 10 % contrasted and 5 % for the control. They additionally included that pupal span was drawn out concerning control treatment, Again, a noteworthy decrease (384.62 mg) was happened in pupal weight in correlation with the control 410.0 mg.

"Neem items have low poisonousness to birds, fish and vertebrates and are more less likely to induce resistance due to their multiple mode of action and their different method of activity on insects".

Generally, it could be concluded that the use of tested compounds with biological insecticides (azadirachtin; pyridalyl and quercetin) could be followed instead of conventional hazards insecticides and these may reduce the environmental pollution and hazard management program using tested compounds looking forward in integrated pest management.

3. Toxicological Evaluation of Plant Extracts against Mammals

Although a ton of work on pharmacological action of neem extracts has been done, very little toxicological assessment has been under taken. Toxicology worried about the investigation of the unfriendly impacts of synthetics on living creatures. The ecological issues came about because of the utilization of synthetic pesticides have been the explanation of worry for every single person and researchers in the ongoing decades. It has been recorded that around 3 million tons of pesticides are utilized on various crops every year. In this way, it must be taken in thought to scan for more safe and biodegradable pesticides to solve the problem of long term toxicity to mammals. Then again, ecological well disposed pesticides and create strategies which can be utilized to lessen pesticide application to get crop yield liberated from pesticide buildups. Herbal pesticides are sheltered option in contrast to synthetic pest sprays as a best mean to diminish negative effects on human well being, creatures, soil, water, *air and condition.* They are more good with the ecological segments than manufactured pesticides[43]. Neem items have low harmfulness to birds, and vertebrates and are more adverse to instigate opposition because of their numerous method of activity on insect pests. Moreover, insect growth regula-

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tor activity weakens the cuticle defense system of larvae causing simple infiltration of pathogenic life forms into insect pest framework. Toxicological examination of *A. indica* leaf extracts at 0.6 - 2.0 g/kg body weight didn't represent any deadly impacts on hematology, protein levels and histopathological boundaries of exploratory creatures though the leaf extracts at 200 g/kg body weight decreased the body weight of the animal and were joined by weakens, anorexia and histopathological defects[44]. The ethanolic root extract has likewise been accounted for to display a portion subordinate hepatotoxicity while the aquous extracts was not harmful to the liver [44].

Destructiveness profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats was explained by Ashafa et al. [11], differentiated and their different beginning body weight of the attempted animal, their last body weight extended ($P<0.05$) all through the introduction time period. The extract moreover extended the incomparable heap of the liver, kidney, lungs and heart of the animals [11]. (Table 1).

The 50 and 100 mg/kg body weight of the extract didn't fundamentally adjust the body weight of the animal, interestingly, the most noteworthy portion (300 mg/kg body weight) expanded the heaviness of the pancreas. So also, all the dosages of the extract expanded the liver-, kidney-, lung-and heart-body weight proportions. The spleen body weight proportion was not significantly different from the control at 50 and 100 mg/kg body weight. Moreover, the 200 mg/kg body weight of the extract diminished the spleen-body weight proportion while the 300 mg/kg body weight expanded it (Table 1). By and large, the adjustments in the biochemical parameters of toxicity effectively affect the ordinary working of the organs of the animals. In this manner, the ethanolic extract of *A. indica* stem bark at the dosages of 50, 100, 200 and 300 mg/kg body weight may not be totally safe as an oral cure and ought to be taken with alert if completely necessary [11].

The alterations in biochemical parameters by the ethanolic extract of *A. indica* stem bark are indications of adverse effects on the various organs of the animals. These will have consequential effects on the normal functioning of these organs. The ethanolic extract of *A. indica* stem barks may not be safe as an oral remedy most especially at 100, 200 and 300 mg/kg body weight. The dose of 50 mg/kg body weight appeared to be relatively safe [11].

Table 1. Efficacy of the stem bark of *A. indica* ethanolic extract on some organs of male rats (n= 10)

Parameters	Extract (mg/kg body weight)				
	check	50	100	200	300
Initial body weight (g)	185.00±8.93^a	188.00±5.26^a	184.00±7.11^a	187.00±4.44^a	184.00±7.74^a
Final body weight (g)	205.00±10.00^b	218.00±9.15^b	214.00±7.54^b	207.00±8.09^b	210.00±9.00^b
Weight of liver (g)	5.56±0.58^a	7.05±0.93^b	8.86±0.55^c	7.27±0.28^d	8.58±0.76^c
Weight of kidney (g)	0.80±0.05^a	1.03±0.07^b	1.09±0.09^b	1.07±0.20^b	0.98±0.07^b
Weight of lungs (g)	1.16±0.05^a	1.59±0.11^b	1.95±0.81^c	2.03±0.28^c	1.49±0.66^b
Weight of spleen (g)	0.56±0.03^a	0.57±0.02^a	0.54±0.06^a	0.27±0.07^b	0.71±0.07^c
Weight of heart (g)	0.51±0.01^a	0.77±0.04^b	0.72±0.04^b	0.74±0.09^b	0.73±0.03^b
Liver-body weight (%)	2.71±0.02^a	3.23±0.05^b	4.14±0.03^c	3.51±0.01^d	4.08±0.05^c
Kidney-body weight (%)	0.39±0.01^a	0.47±0.08^b	0.51±0.02^b	0.52±0.01^b	0.47±0.03^b
Lung-body weight (%)	0.57±0.03^a	0.73±0.02^b	0.91±0.01^c	0.98±0.02^c	0.71±0.07^b
Spleen-body weight (%)	0.27±0.02^a	0.26±0.04^a	0.25±0.05^a	0.13±0.01^b	0.34±0.01^c
Heart-body weight (%)	0.25±0.01^a	0.35±0.01^b	0.34±0.03^b	0.36±0.02^b	0.35±0.01^b

Note:
Mean±SD values carrying different superscripts from the check for each parameter are significantly different ($P<0.05$).
After Anofi Omotayo Tom Ashafa, Latifat Olubukola Orekoya, and Musa Toyin Yakubu (2012).*

In another examination done by Yun-xia Deng et al.[45], they found that in the acute toxicity test, the LD_{50} estimations of neem oil were seen as 31.95g/kg. "The 28 days subacute treatment with neem oil failed to change body weight gain, food and water consumption. Histopathological tests indicated that the objective organs of neem oil were testicle, liver and kidney. Serum biochemistry investigation demonstrated no significant contrasts in any of the boundaries inspected under the portion of 1600mg/kg/day".

They included that in no-watched antagonistic impact level (NOAEL) of presentation and target organs of neem oil for building up security standards for human exposure, the subchronic toxicity extract with neem oil in mice was assessed. The mice (10 for each sex for each portion) was orally managed with neem oil with the dosages of 0 (to fill in as a control), 177, 533 and 1600 mg/kg/day for 90 days. After the treatment time frame, perception of reversibility or perseverance of any poisonous impacts, mice were constantly taken care of without treatment

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for the accompanying 30 days. During the two trials, the serum biochemistry, organ weight and histopathology were inspected. The outcomes demonstrated that the serum biochemistry and organ coefficient in exploratory gatherings had no significant difference compared with control group. At the 90th day, the histopathological assessments demonstrated that the 1600 mg/kg/day portion of neem oil had differing degrees of harm on every organ with the exception of heart, uterus and ovarian. Following 30-day recovery, the level of sores to the tissues was decreased or even reestablished. The safe dose of neem oil was 177 mg/kg/day for mice and the objective organs of neem oil were resolved to be testicle, liver and kidneys [89].

Toxicity to other Mammals and Birds

New neem leaves given to Goat and Pigs for 7 days in 200mg/kg portion caused passing of creatures at 5 th day and posthumous revealed clog in brain [47]. SDS PAGE examination of heart proteins of the Bioneem treated chick embryo didn't show any noteworthy contrast in protein profile when contrasted with that of the control.

In study done to broiler chicks by Nety et al. [88] who announced that the hydro-alcoholic extract of *Azadirachta indica* (Neem Leaves) as an alternative in contrast to anti-biotic growth promoter- Bacitracin methylene diasalicylate (BMD) was assessed in broiler chicks. The extract was prepared by 50 % methanol and 50 % water AIE, 0.4 g/L) and was placed in drinking water and given to the chicks of particular treatment for 42 days. 90 broiler chicks (day-old) were arbitrarily allocated to 3 groups and each group with 3 replicates with 10 chicks of each.

They found decreased body weight, body weight gain and decreased feed conversion in (AIE) *Azadirachta indica* extract supplemented birds suggest that *A. indica* leaf extract contains toxic substance. The results of this study are in accordance with the findings of previous workers in acute toxicity study on neem leaf aqueous extract in chicken. The elevated activity of serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) in the serum of AIE supplemented birds for 6 weeks indicated severe liver damage and increase level of uric acid and creatinine concentration indicated hepato-nephro toxicity, significant lower level of packed cell volume and lymphocyte count was observed in AIE supplemented group. Nety et al. [88] concluded that supplementation of hydro-alcoholic extracts of *Azadirachta indica* leaves (AIE) in broiler birds caused death of 20 % of birds during the 4th and 5th weeks and toxicity is mainly associated with the hepatotoxicity, nephrotoxicity, suggesting that AIE should be used with caution in ethano-veterinary practice. Rafeeq Alam Khan& Maryam Aslam [48] expressed that acute oral poisonousness (LD_{50}) was seen in albino mice utilizing standard conventions where as sub-chronic, hematological and histopathological contemplates were surveyed on 24 albino rabbits after giving herbal formulations for 60 days in two dosages (20 and 60 mg/kg) against control gatherings.

Likewise, It is accounted for that leaves of neem brought about harmful impacts on sheep[49], goats and guinea pigs [50]. A portion higher than prompting passing in guinea pigs. In any case, 200 mg/kg in a similar course was seen as non-poisonous to rabbits [51]. Ethanol neem containing 3000 ppm azadirachtin (±10%) is recorded by the Environmental Protection Agency (EPA), USA. The information submitted on intense oral harmfulness in rodents showed no negative impact up to a portion of 5ml/kg (National Academy Press, Washington D.C., 1992) [71].

In another assessment, methanolic leaf and bark extracts showed an oral LD50 (Lethal segment, half) of about 13g/kg in acute harmfulness assessment on mice's. Creature gave general signs of wiped out prosperity and disquiet, gastro-intestinal spam, lack of concern, refusal of water.

4. Nanotechnology in Pest Control

Pesticides denotes a wide range of agro-chemicals, those are extensively used in agriculture for protection of crops from diseases, pests and weeds. Pesticides are exploited both extensively and intensively to minimize these losses and around 3 billion tons of pesticides are employed for the same[2]. The biggest hurdle that lead to the failure of these pesticides was the water-insolubility of these formulations. Purchaser familiarity with the health hazard from the residual harmfulness and the issue of most efficient protection from pesticides has contend the researchers during the most recent two decades everywhere throughout the world to look for progressively safe methods [52]. One of the most effective options is utilizing nanotechnology [20]. Nanoemulsions are emulsions whose minuscule size is uniform and incredibly little with the size ranges that of bulk materials and can be figured without the utilization of organic solvents [54]. In excess of 1300 business nanomaterials with wide spread of potential applications are as of now available [55-58]. Kamaraj, et al. [59] orchestrated a neem gum nano-plan (NGNF) as antifeedant, larvicidal and pupicidal exercises against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) at 100 ppm. The NGNF showed significant (100%) antifeedant, larvicidal and pupicidal practices against *H. armigera* and *S. litura.* The LC50 estimations of 10.20, 12.49 and LC90 estimations of 32.68, 36.68 ppm on H. armigera and S.

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litura, exclusively were resolved at 100 ppm.

At a time when the conventional pesticides pose an immense threat to the quality of environment and the health of the organisms, more emphasize should be given on development of nano-formulation of pesticides, which proves to be more potent and selective over the conventional formulations. Though these formulations have not reported to cause any acute toxicity in the non-target organisms till date, more thrust should be given on this aspect to come up with ways to nullify the short comings before its ingression in agriculture[90]. Dimetry, et al.[60] found that the utilization of nanomaterials will bring about the advancement of productive and expected methodologies towards the management of insect pests. They arranged the planned neem oil nanoemulsion just as loaded neem was described by Transmission electron microscopy. Likewise, the harmfulness of neem oil free as bulk, nano and loaded neem have been evaluated against the second and fourth larval instar of *Spodoptera littoralis* under lab conditions. The toxicity of the tried oil dependent on LC_{50} of loaded neem was lower 2.22 ppm contrasted and neem oil nano emulsion and bulk neem (5.09 and 6.71 ppm separately for the second larval instar. A similar pattern was found concerning the fourth larval instar.

Dimetry et al. [41] assessed the harmfulness of neem and peppermint oil nano plans against *Agrotis ipsilon* hatchlings. They revealed that the LC_{50} estimation of loaded neem or pepper mint were lower (0.62 and 36.47 ppm) contrasted and neem or pepper mint oil nano-emulsion and bulk neem for the second larval instar. They included that the various definitions of neem are more strong than if there should be an occurrence of peppermint oil, as LC_{50} and LC 90 qualities were essentially lower.

Amin et al. [42] considered the capability of nano-details of neem and peppermint oils on the bionomics and enzymatic potancy of *Agrotis ipsilon* hatchlings. They exhibited critical stretching of the larval span, rate mortalities were extended similarly as larval malformations. Aftereffects of enzymatic potency indicated significant impacts of the three formulations of neem and pepper mint oil. Essential oils increase in the activities of cuticle phenoloxidase and chitinase were viewed, in any case, noteworthy restraints were recorded for amylase, invertase, trehalase, protease and alkaline phosphatase. Potential uses of nanotechnology in agribusiness are: transport of nano-pesticides epitomized in nano-materials for controlled release; alteration of biopesticides with nano-materials; slow arrival of nano material helped fertilizers, biofertilizers and micronutrients for capable use; and field employments of agrochemicals, nanomaterials helped movement of nano material for crop improvement. Nano-sensors for plant pathogen and pesticide disclosure, and NPs for soil protection or remediation are various zones in agribusiness that can benefit by nano-technology [20].

The utilization of miniaturized scale and nano-emulsions as carriers of pesticides diminishes the utilization of natural dissolvable and expands the dispersity, wettability, and penetration properties of the droplets and may prompt improvement of the organic viability of pesticides [61]. Papanikolaou, et al. [62] expressed that expanding insecticidal action of nano-detailed pyrethrins in combination with the nonappearance of antagonistic consequences for non-target aphid predators make them compatible plant protection items in natural cultivating and IPM procedures in different crops. Additionally, they added that their outcomes confirmation to the utilization of nano-innovation in improvement of pest spray definition for the advancement of solid and decreased natural hazard plant insurance items.

4.1 Toxicity of Nano-particles

Little information is available in relation to the toxic effects of the prepared nano-particles on the mammalian organisms. From the available information and some facts related to the toxicity of the nano-particles is that a large number of scientific evidence such as increasing the heart, pulmonary and neurological diseases caused by the nano-particles, were pushed many international scientific organization to recognize the harmful effects of nano-particles even the emergence of Nano-toxicology. Furthermore, the long term exposure to the nano-structures agents may lead to new/unforeseen harmful effects. Also, it is not known how the ingested nano-particles will behave in the body. In addition, Absorption, distribution, metabolism and Excretion profiles of nano-materials are different from bulk equivalents. So that, the long term health consequences of ingested bio-persistent nano-particles are not known. Based on such facts, FAO/WHO Expert Meeting on the Application of Nano-technologies decided to carry out a risk assessment of each nano-particles based products[63]. Dimetry and Hussein [64] called attention to that there is an extraordinary concern with respect to the nano-materials which can possibly apply unsafe consequences for nature and human wellbeing and when we have a nano-pesticide, it turns out to be twofold edged weapon". Bayoumi,[65] mentioned the important points that have to be in consideration when testing the nano-formulations which could be summarized as the following: Exposure route and exposure period (acute and chronic) of the tested organisms, Existence of impurities and agglomeration

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of nano-materials.

Also, Mossa et al. [66] called attention to that no indications of harmfulness or mortality in male rodents acquainted with nanoemulsion of camphor or the EO. Biochemical cutoff points likewise show insignificant changes in all liver biomarkers in serum of male rats. The liver is the basic organ in the body, expect a colossal action in xenobiotic detoxification. "It is the basic goal to harmful xenobiotic and their metabolites. Accordingly, changes in liver cutoff biomarkers are conventionally utilized as biomarkers for liver noxiousness and damage [67, 68]. It has been represented that the development in the potency of liver synthetic compounds and change in grouping of protein, albumn and globulin can be direct result of cell injury [68, 69]" hepatotoxicity and change in proteins

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute harmfulness tests were done. They found that subacute treatment with neem oil did not succeed to change body weight increase, food and water utilization. intense poisonousness depicts the horrible effect of a substance that result either from a solitary presentation [70] or from various exposures in short space of time (for the most part under 24 hours). While, sub acute harmfulness, can be delineated as the negative effects should occur inside 14 days of the association of substance. Intense oral poisonousness of ethanol neem extracts containing 3000 ppm azadirachtin (± 10%) is enrolled with the Environmental Protection Agency (EPA), USA. The data submitted on acute oral harmfulness in rodents showed no negative effect up to a doze of 5ml/kg[71]. In another assessment, methanolic leaf and bark extracts showed an oral LD50 of about 13g/kg in intense poisonousness concentrates on mice [45].

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute poisonousness tests were completed. In the acute poisonousness test, the LD50 estimations of neem oil were seen as 31.95g/kg. The subacute treatment with neem oil did not affect to change body weight gain, food and water utilization. Serum natural chemistry investigation indicated no huge contrasts in any of the boundaries analyzed under the dose of 1600mg/kg/day. Histopathological tests indicated that the objective organs of neem oil were testicle, liver and kidneys up to the portion of 1600mg/kg/day.

"Raizada et al. [74] communicated that a single oral dose of azadirachtin (5000 mg/kg) to male and female rat didn't convey any sign of harmfulness nor demise in the treated animal. The LD50 regard along these lines is more than 5000 mg/kg both in male and female rodents"

Dorababu et al. [75] exhibited that acute similarly as sub acute harmfulness mulls over demonstrated no mortality with 2.5 g/kg dose of *Azadirachta indica* extract in mice.

4.1.1 Acute Toxicity

"Acute harmfulness depicted the adversarial effect of a substance that result either from a solitary presentation [70] or from a several exposures in short space of time (commonly under 24 hours)". While, sub acute harmfulness, can be portrayed as the troublesome effects should occur inside 14 days of the organization of substance. "Acute oral poisonousness of ethanol neem extracts containing 3000 ppm azadirachtin (±10%) is enrolled with the Environmental Protection Agency (EPA), USA [71]. The data submitted on acute oral poisonousness in rats showed no negative effect up to a portion of 5ml/kg[71]".

In another examination, methanolic leaf and bark extracts demonstrated an oral LD50 (Lethal portion, half) of about 13g/kg in acute poisonousness evaluations on mice[47]. Animal gave general indications of sick wellbeing and uneasiness, gastro-intestinal fit, unresponsiveness, refusal of water and feed and hypothermia. Mice passed on under terminal spasms. No gross tiny injury was found on autopsy[91]. The information submitted on acute oral harmfulness in rats showed no negative impact up to a portion of 5ml/kg [71]. Target organs of poisonous impacts were the central nervous system and lungs[92]. Be that as it may, methanol solvent and insoluble parts, from an equeous leaf extracts were not harmful inside 24hr at an oral portion of 200mg/kg in mice [93]. In this way, all the above examination is done on the rodents, rabbits and guinea pigs and they indicated their response [47].

Upon acute presentation, nimbidin, disconnected from neem seeds,dose conditionally decreased intense paw oedema in rodents. The medium compelling dose (ED50 esteem) was 79.4 mg/kg body weight in rats [72]. Once more, the LD 50 values for Neem Azal (Neem based pesticidal item) were higher than 2g/kg body weight in mice [73].

The consequences of the accessible oral poisonousness tests demonstrated that, intense harmfulness at high dosages may occur [76,77] depending upon the particle size, covering and synthetic chemical composition of the nano particles. "Raizada et al.,[74] conveyed that a solitary oral dose of azadirachtin (5000 mg/kg) to male and female rats didn't make any indication of hurtfulness nor passing in the creatures. The LD50 value in this manner is in excess of 5000 mg/kg both in male and female rats". Dorababu et al. [75] showed that acute also as sub acute noxiousness examines demonstrated no mortality with 2.5 g/kg portion of *Azadirachta indica* extracts on, hematological profile and distinctive liver and kidney work tests in rats when rewarded for 28 days with 1 g/kg portion of *Azadirachta*

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indica extract. Acute, subacute and subchronic destructiveness following oral introduction have been explored in rats for a few nano particles.

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute poisonousness tests were investigated. In the acute noxiousness test, the LD50 estimations of neem oil were exhibited as 31.95g/kg. "The subacute treatment with neem oil did not change body weight increment, food and water use.

Youssef [78] pointed out that the toxic effect of bulk, nano-emulsion and loaded nano-emulsion of neem extract on the Swiss albino mice clearly determined acute oral $LD_{50,}$ values were 113.33, 134.83 and 140.90 mg/kg body weight for loaded nanoemulsion, nano-emulsion and bulk neem extract respectively. This result revealed that the nano formulations were more toxic than the bulk one. In addition, the sub-acute administration of sub-lethal dose (LD_{10}) during 14 days showed significant alterations between induction and reduction in the selected biomarkers, *i.e*. hematological toxicity (hemoglobin increased to reach +107.53% after 3 days from continues treatment of bulk oil, RBCs was +343.89% after 3 days in loaded nano-emulsion treatment, WBCs was +150.00 after 5 days in bulk treatment), hepatotoxicity (GOT) +57.27% after 3 day in bulk treatment, GPT -51.96% after 14 days in loaded nano-emulsion, glutathione S-transferase +241.38 after 5 days in bulk oil, reduced glutathione +86.85% after 5 days in nano-emulsion and bilirubin + 355.88% after 20 days in bulk oil), nephrotoxicity (creatinine +330.56 after 20 days in loaded nano-emulsion), total ATPases +8.56% after 3 days in nano-emulsion and total protein in liver samples -74.60% after 7 days in loaded nano-emulsion and in brain samples was -74.16% after 3 days in loaded nano-emulsion

An another report was completed to explore the toxic effect of the prepared neem nano formulation on albino mice as mammalian model. Human adventitious ingestion of 20ml neem oil declared the harmful encephalopathy [72]. Fresh neem leaves offered to Goat and Pigs for 7 days in 200mg/kg portion caused demise of creatures at 5 th day and after death revealed obstruct in cerebrum [73]. SDS PAGE examination of heart proteins of the Bioneem treated chick embryo didn't exhibit any valuable effect in protein profile when contrasted with that of control. Diminished body weight, body weight gain and diminished feed transformation in the *Azadirachta indica* extract (AIE) enhanced supplemented birds propose that *A. indica* leaf extracts contains poisonous substance. The after effects of this investigation are as per the discoveries of past laborers in acute toxicity on neem leaf water extracts in chicken 5,9. The raised movement of ALT and AST in the serum of AIE supplemented birds for about a month and a half demonstrated extreme liver harm and increment level of uric Most of neem based products might be toxic. The assorted neem formulations regulated once or chronically conflictingly sway animal health and once in a while now and again even reason demise with medium deadly concentration LC50 values varying in go from 1.6 to 16 ml/kg body weight.

Treatment of mice had no influence on liver, spleen, thymus or body weight records and an update of macrophage relocation prevention and foot cushion thickness [79]. The non-hepatotoxic nature of Neem Leaf Preparation was illustrated. The level of serum urea remained not changed and run of the mill designing of the cortical and medullary bits of kidney were also observed after neem leaf arrangement treatment corrosive and creatinine related with nephrotoxicity [79].

4.1.2 Chronic Toxicity

"A large portion of neem based products may be harmful. The diverse neem formulations regulated once or chronically alternately sway creature wellbeing and now and then once in a while even reason demise with medium lethal concentration (LC_{50}) values changing in go from 1.6 to 16 ml/kg body weight".

Treatment of mice had no influence on liver, spleen, thymus or body weight records and an overhaul of macrophage movement deterrent and foot cushion thickness[79]. The non-hepatotoxic nature of Neem Leaf Preparation was illustrated. The degree of serum urea remained not changed and regular building of the plant planning of the cortical and medullary bits of kidney were moreover seen after neem leaf preparation treatment.

Panda and Kar [80] nounced that dose dependent impacts were seen in mice treated with neem fluid leaf extract. Neem application diminished tri-iodothyronine (T3) what's more, extended serum thyroxine (T4) centers and hepatic lipid peroxidation and decreased glucose-6-phosphatase development while improving the activities of super oxide dismutase and catalase. "Khosla et al. [81] found that Sub-chronic organization of neem leaf extracts caused a decrease in glucose levels in regular and diabetic rabbits. The concentrate was more convincing than seed oil." "Abdel Megeed et al. [82] indicated that when rats were treated with azadirachtin, expanded serum SGOT and SGPT activities and bilirubin content were seen". Histopathological thinks about exhibited over the top changes in the liver to the degree blockage, hydropic degeneration, defilement and lymphocytic infiltration. The eventual outcomes of the activities of liver and serum AST, ALT, ALP, S-bilirubin, S-albumn, S-cholesterol and S-protein of an-

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alyzed and control rats displayed no large changes in the clinico-engineered cutoff points of creatures treated with various convergences of azadirachtin [74].

Dehghan et al. [83] observed a decline in ATPase activity in caput and cauda epididymis of sperm of mice when treated with neem seed alcoholic extract. The vast majority of the neem-based products are poisonous. For Praneem and Nimbokil-60 effects on reproduction and fertility are accounted for. Every single other agent, regulated once or chronically, contrarily influence animal health and sometimes even reason demise with medium deadly focus (LC_{50}) values changing in the range from 1.6 to 16ml/kg of the neem-based items are noxious. For Praneem and Nimbokil-60 impacts on reproduction and fertility are represented. Each and every other agent, controlled once or chronically, oppositely influence animal health and once in a while even explanation downfall with medium destructive center (LC50) values changing in the range from 1.6 to 16ml/kg.

Haque et al. [84] "announced that essential obstruction of progress of Ehrlich's carcinoma was watched following prophylactic treatment on Swiss albino mice with Neem Leaf Preparation (NLP-1 unit) when consistently for about a month. "Harmful impacts of this specific part (1 unit), close by 0.5 unit and 2 units of NLP portions, were assessed on various murine physiological frameworks. 100% of mice could drive forward through 4 infusions of 0.5 and 1 unit Neem leaf course of action (NLP) dosages". "Body weight, particular organ-body weight extents and physical conduct of treated mice remained absolutely unaltered during treatment with different NLP doses. These Neem Leaf Preparation doses were seen to animate hematological systems as confirm by the expansion in total account of RBC, WBC and platelets and hemoglobin percentage". As histological changes also as climb in serum alkalinee phosphatase, SGOT, SGPT were not found in mice treated with three distinct dosages of NLP, the non-hepatotoxic nature of NLP was represented. The degree of serum urea stayed unaltered and normal plan of the cortical and medullary bits of the kidney were in like way safeguarded after NLP treatment. Increased immunizer creation against B16 melanoma antigen was perceived in mice inoculated with 0.5 unit and 1 unit of NLP. Number of splenic T lymphocytes (CD4+ and CD8+) and NK cells were in like way observed to be stretched out in mice infused with 0.5 unit and 1 unit of NLP. Regardless, NLP portion of 2 units couldn't show such safe stimulatory changes.

NLP safe incitement was associated well with the improvement restriction of murine carcinoma. In end, tumor advancement impediment was watched precisely when mice were injected with immuno stimulatory dosages of NLP (0.5 unit and 1 unit).).

"Portion subordinate effects were found in mice treated with neem aquous leaf extract. Neem application diminished tri-iodothyronine (T3) and expanded serum) glucose-6-phosphatase activity while overhauling the activities of super oxide dismutase and catalase".

4.1.3 Toxicity of Sub-lethal dose (LD_{10}) of Neem Bulk and Nano-formulations on Some Biochemical Markers

The sub-lethal. dose (LC10) impact of neem bulk and nano formulations were done to Swiss Albino mice orally during a week and the exposure procedure was stopped during fourteen days as a recovery period the presentation dependent on the information got from Hayes and Youssef [85,78]. To investigate the harmful impacts of the various formulations utilized in examination with the bulk form, some biochemical markers were estimated during the subacute treatment (heamatological, hepatotoxicity and renal biomarkers. Additionally, acute and sub choronic toxicity were evaluated by Rafeeq Alam Khan et al.[48] who researched Acute oral harmfulness (LD50) in albino mice using standard shows though sub-interminable, hematological and histopathological looks at were reviewed on 24 albino rabbits after giving herbal formulations for 60 days in two dosages (20 and 60 mg/kg) against control bunch. The results of this investigation showed that the medication is sheltered up to 5000 mg/kg body weight following acute oral poisonousness test and no mortality was seen during sub chronic harmfulness contemplates. Consequences of sub-chronic poisonousness didn't show any significant changes in biochemical, dosage (LC10)) effect of neem bulk and nano extracts were done to Swiss Albino mice orally during a week and the introduction strategy was quit during fourteen days as a recuperation period the introduction subject to the data got from Hayes and Youssef [85,78], ematological and histopathological boundaries. In any case,some markers, for instance, urea, creatinine, hemoglobin, and RBC check were changed, yet these progressions don't compare with the histopathological results and may be identified with intra singular varieties.

NLP interceded resistant incitement was related well with the advancement restriction of murine carcinoma. All things considered, tumor advancement confinement was observed exactly when mice were infused with immuno stimulatory dose of NLP (0.5 unit and 1 unit).

Dose subordinate effects were found in mice treated with neem watery leaf extract. Neem treatments diminished tri-iodothyronine (T3) and extended serum

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) glucose-6-phosphatase activity while upgrading the exercises of super oxide dismutase and catalase

4.2 Long Term Toxicity

"Information from harmfulness evaluations with various courses of presentation show that several systemic effects for different organ structures may occur after long term introduction to Nano-Particles, including the invulnerable system, provocative effects and cardiovascular framework. Effects on the safe framework may fuse oxidative pressure or enactment of master fiery cytokines in the lungs, liver, heart and cerebrum. Effects on the cardiovascular system may fuse pro- thrombotic impacts and negative outcomes on the cardiovascular capacity (intense myocardial localized necrosis and unfavorable consequences for the pulse."

In addition, genotoxicity, and conceivable carcinogenesis and teratogenicity may occur, for the going with endpoints, further investigates are expected to insist. In an overview on the health evaluation of Neemazal TM-T/S (Neem-based item), this item didn't have any impact on reproduction and didn't cause skin or eye disturbance. No cancer-causing nature was watched and 100ppm didn't have any impact after 90 days administration in rats [73].

4.3 Conclusions and Future Prospective

Worldwide market patterns towards crops protectants were progressively focused on items gotten from natural sources. As these biopesticide framework, plant extracts contain at least one or more chemical compounds, the safety evaluation to human being become more necessary, so as to guarantee security to man. A considerable lot of the botanicals have not been completely explored for their mammalian toxicity.

Once more, a deficiency of the toxicological data of various arranged nano-particles, studies on the toxicity of nano-particles demonstrated that there is a requirement for future exploration about the synthesis of new materials and assessment of their harmfulness. There is a solid thought that biological activity of nano particles will rely upon physiochemical studies and don't considered in toxicity screening contemplates. These limits that may be important in understanding the unsafe impact of the attempted materials consolidate particle size and size appropriation, shape, crystal structure, chemical arrangement, surface zone, surface chemistry and agglomeration state [86]. "The expanded surface reactivity predict that NSPs display more biological activity prominent natural movement per given mass contrasted with bigger particles, they ought to be taken into living beings". This increased biological activity can be either positive as for example cancer prevention agent action or negative for example harmfulness (subsequently its utilization in huge sums may demonstrate risky [87] or mixture of both "Data from toxicity evaluation demonstrate that several systemic consequences for various organ system may happen after long term exposure to nano-particles (NPs), including immune system, inflammatory effects and cardio-vascular system". Likewise, genotoxicity and possible carcinogenesis and teratogenicity may happen. These will affect the normal functioning of these organs. Likewise further investigates are needed to affirm the safeness of these neem or nano - formulations for warm blooded creatures with unique reference to human beings and their animals.

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