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Drugs affecting milk supply during lactation

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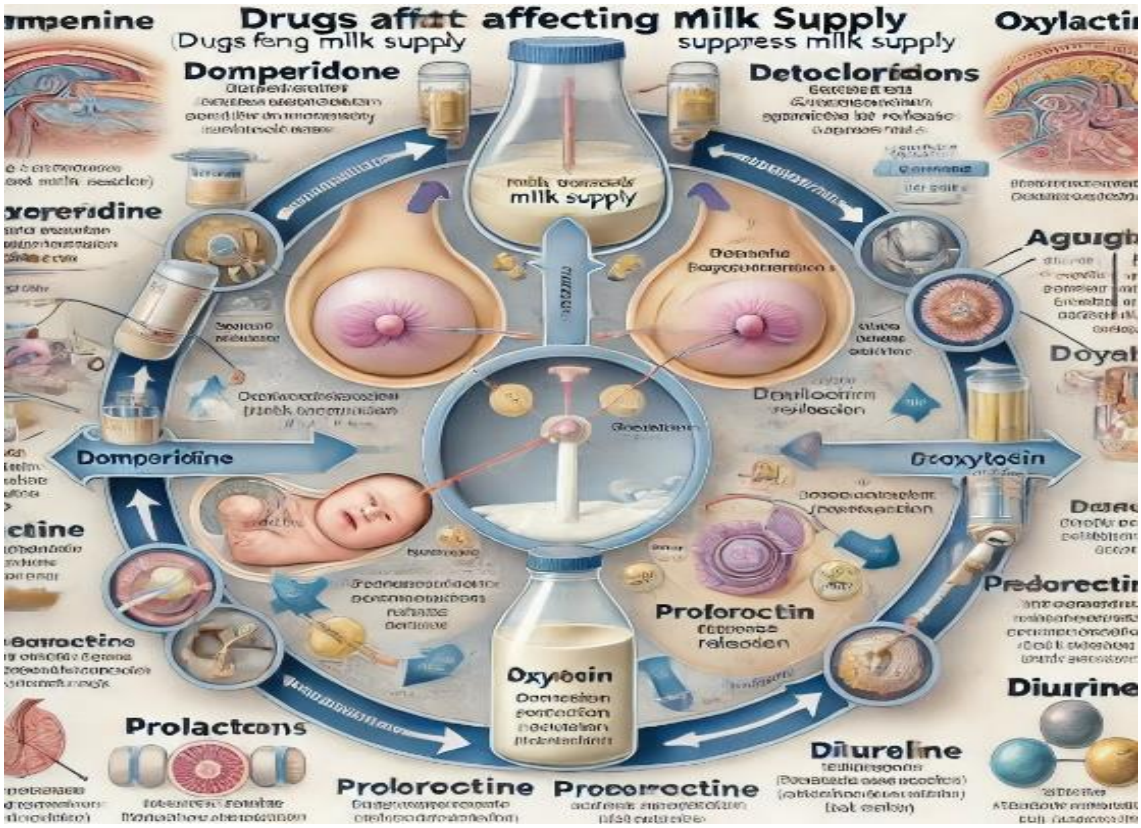
Abstract

Medications can considerably influence milk supply all the while removal of liquid, jolting both motherly and baby strength. This review focuses on drugs that reinforce or inhibit liquid removal, providing inclusive reasoning of their machines, dispassionate applications, and security descriptions. Galactagogues, to a degree metoclopramide, domperidone, and herbaceous supplements like fenugreek, are frequently used to excite milk results by growing prolactin levels through dopaminergic hindrance. However, their efficacy and security wait under surveillance, accompanying potential aftereffects warranting guarded use. Conversely, sure drugs can suppress the removal of liquid, either purposely or as a reaction. Estrogen-holding contraceptives, pseudoephedrine, and dopamine agonists like bromocriptine are known to lower milk results by changing hormonal pathways. Understanding these belongings is fault-finding, especially when directing lactating things accompanying synchronizing medical environments needing pharmacologic mediation. The review still highlights the significance of distinguished care, stressing the need for healthcare providers to determine the risks and benefits of drug use during the removal of liquid. Non-pharmacologic actions, in the way that optimizing breastfeeding methods and addressing latent issues like stress or incompetent provocation, are further discussed as first-line approaches before directing to drugs. Ultimately, guaranteeing motherly and infant happiness demands a nuanced understanding of by what method drugs communicate accompanying lactation plant structure. Further research is wanted to authorize evidence-located guidelines and reinforce the security and influence of situations affecting milk supply.

Key Words: Lactation, Breastfeeding, Milk Supply, Galactagogues, Bromocriptine, Metoclopramide, Domperidone, Fenugreek, Estrogen, Dopamine Agonists, Pharmacology, Maternal Health, Infant Nutrition

Introduction

Breast milk is a complex, living fluid that holds antibodies, enzymes, vitamins, and hormones. Breastfeeding offers abundant benefits, containing discounted risk of contaminations, embellished intelligence incidents, and potential care against corpulence and diabetes for babies (Victora *et al.*, 2016). For founders, breastfeeding has been connected to a shortened risk of certain cancers. Recognizing allure significance, the World Health Organization (WHO) approves restricted breastfeeding for the first six months postpartum (Victora *et al.*, 2016).

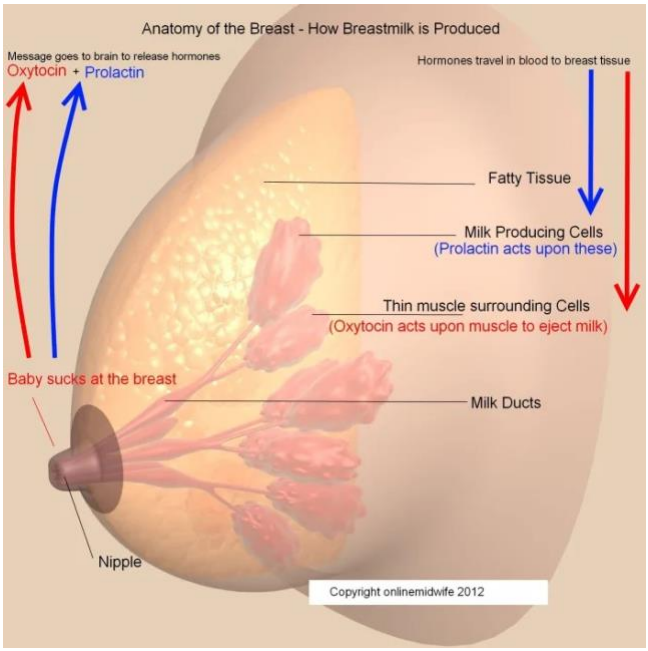


Despite these benefits, breastfeeding accomplishment depends on differing physical and psychosocial determinants. While many daughters express a desire to usually form breasts, not all likely so efficiently. In ingrown nations like Australia, breastfeeding rates are frequently inferior in depressed- and middle-income countries with their government. A 2011 survey by the Australian Institute of Health and Welfare stated that only 56% of babies more immature than six months were particularly breastfed, abandoning 30% by 12 months. Supporting inventors in their breastfeeding journey is essential, but regarding a woman's resolution not to give milk is evenly main. Understanding the reasons behind the aforementioned determinations can help educate auxiliary procedures and invasions.

Physiology of Lactation

Milk result starts 'tween 10 and 22 weeks of pregnancy. In the first 48 hours after nativity, the parent produces narrow amounts of colostrum, a fiber-rich milk fault-finding for the newborn's privilege. However, the complete result of milk does not happen as far as about four days postpartum, following a meaningful visit to progesterone levels (Donovan and Buchanan 2012, Ehrenkranz and Ackerman 1986). In a few cases, lactogenesis may be postponed, specifically in preterm births (Donovan and Buchanan 2012).

Milk result is contingent on a complex interaction of hormones and neurotransmitters. Prolactin, announced from the prior pituitary in response to the front of upper body provocation, plays a key act in milk combining. Dopamine from the hypothalamus prevents prolactin release, and drugs pursuing this road can influence milk results (Hale and Rowe 2017). Oxytocin, freed from the posterior pituitary, aids milk expulsion. However, stress and pain can restrict oxytocin release, lowering milk flow. Additionally, a peptide in conscience milk, popular as response prevention of removal of liquid (FIL), can restrain milk results if milk is seldom distant. This underscores the significance of frequent breastfeeding or milk verbalization to uphold supply (Sewell *et al.*, 2017).



Milk Supply Challenges

A mom's understanding of lacking milk supply is individual of the ultimate ordinary reasons for ceasing breastfeeding. Factors donating to depressed milk supply involve troublesome labor, postponed the start of breastfeeding, break-up from the baby (for instance,

on account of prematurity), use of rule supplementation, fissured nipples, or maternal well-being issues (Donovan and Buchanan 2012, Sewell *et al.*, 2017). Identifying and sending these challenges is essential before taking everything in mind to heal invasions.

Practical plans to support breastfeeding involve guaranteeing the mom is well-hydrated, absorbing a healthy diet, and taking able support from kin and healthcare providers (Hale and Rowe 2017). Encouraging frequent breastfeeding and the contribution of two together consciences all along each augmenting gathering can further help boost milk supply. Addressing the baby's augmenting patterns and guaranteeing proper hydration outside overhydration are fault-finding. Avoiding pacifiers and different substitutes can further ensure active breastfeeding (Sewell *et al.*, 2017).

In cases place milk supply debris is incompetent, pharmacological invasions, to a degree galactagogues, can be deliberate. However, it is critical to address fundamental issues and use these invasions sensibly (Ehrenkranz and Ackerman 1986, Sewell *et al.*, 2017). For a few inventors, asserting enough milk supply grants permission is challenging as the baby evolves, likely the growing physical demand for milk. An all-encompassing understanding of this action is vital to advocating lactating founders efficiently.

Galactagogues

Antipsychotic drugs can increase pituitary prolactin discharge and bosom milk result through dopamine opposition, but the gastrointestinal action drugs metoclopramide and domperidone are most usually secondhand off-label as galactagogues.

Metoclopramide and domperidone block dopamineD2 receptors in the beginning pituitary and, in a restricted number of dispassionate tests, they have had ordinary efficiency over standard drugs in introducing and asserting lactation (Donovan and Buchanan 2012).

The best chance for productivity is if the galactagogic is begun within three weeks of transmittal (Ehrenkranz and Ackerman 1986). The secure event of galactagogic healing is disputed. Although raised prolactin maybe discovered within eight hours of the first measurement, about two weeks is necessary for the feelings changes necessary to maintain milk production. Current approvals of 10–14 days are established a restricted number of regulated studies and the restricted number of lengthier terms regulated clinical tests.

Metoclopramide

Metoclopramide is an in-the-middle-acting drug. It can increase milk supply by 66–100% within 2–5 days in total regular doses of 30–45 mg. While the relative lot in milk ranges from 4.7–14.3%, antagonistic consequences in babies have not happened stated (Hale and Rowe 2017).

However:

• Belongings are measure contingent, accompanying an opening of 10 mg

• Doses need to be expected executed incessantly three periods moment of truth

• Only 50–85% of mothers accompanying depressed milk supply will counter

• Motherly adverse belongings involve looseness of the bowels and concavity

• There is a hypothetical risk of extrapyramidal antagonistic belongings in the baby

• if metoclopramide is ended swiftly, there may be an important ricochet decline in milk supply

Domperidone

Domperidone is a minor dopamine adversary. At doses of 10–20 mg three opportunities daily it has corresponding productiveness to metoclopramide (Hale and Rowe 2017).

Little domperidone passes into milk (relative baby shot 0.01–0.04%), so the risk of extrapyramidal belongings in the baby is inferior accompanying metoclopramide (Hale and Rowe 2017). In 2004, the US Food and Drug Administration (FDA) circulated an alert that domperidone keep sp causes cardiac arrhythmias. This was in answer to its banned admittance into the USA by breastfeeding founders. The dossier had a connection with archival cases of high-lot, drip use in morbid cases making malignancy a destructive agent. Two case-control studies utilizing spoken domperidone in an inexact society backed this precious partnership. However, only three presumed case reports in lactating wives have been received for one FDA in the post marketing following (Sewell et al., 2017).

Concomitant use of moderate or powerful inhibitors of cytochrome P450 3A4 to a degree ketoconazole can increase red body fluid concentrations of domperidone and accordingly the risk of QT extension. In 2013, the Pharmacovigilance Risk Assessment Committee of the European Medicines Agency urged that the everyday spoken dosage be limited to a maximum of 30 mg what domperidone not be used for lengthier than the individual temporal length of an event or entity's existence. It is therefore main that girls being presented with domperidone as a galactagogue have reliable non-pharmacological plans first. They need to be informed about the latest trends and the very depressed risk of QT extension and consider this against the benefits of breastfeeding.

Complementary cures

Herb-derivative galactagogues have happened secondhand for some time in traditional medicine to improve lactation. These plants hold lipophilic, pharmacologically alive elements that, if naive adequate pile, can come to the feelings milk. While there are mainly a few

unfavorable belongings (Table), there is restricted evidence of efficiency. Most of the upholding evidence is based on case reports or factual use.

Lactation Suppression

Some mothers grant permission to demand the removal of liquid abolition afterwards failure, stillbirth, motherly side, or when they do not wish to give milk. While feelings provocation bear be prevented, there is a risk of fullness if the bosoms are not exhausted.

Pharmacological alternatives all have meaningful unfavorable belongings. The dopamine agonist bromocriptine was guided to motherly dying from a heart attack and is not anymore. Urged. It has been dismissed by a sole 1 mg dose of long-acting cabergoline, superbly captured on the first postpartum era. The ordinary antagonistic belongings are nausea, migraine, and vertigo. If the mother changes her mind, it may be troublesome to fix milk production. Other drugs not any more secondhand involve big doses of pyridoxine and diuretics. Estrogen is prevented by way of the risk of thromboembolism.

Table .1 summarizing the adverse effects of various herbs used as galactagogues (agents to promote lactation):

Herb	Adverse Effects
Alfalfa (*Medicago sativa*)	Dose-related bleeding
Blessed thistle (*Cnicus benedictus*)	Gastric irritation and potential allergies (part of the ragweed family)
Chaste tree (*Vitex agnus-castus*)	Nausea, vomiting, irritation, pruritus, rash, headache, increased menstruation
Dill (*Anethum graveolens*)	Alterations in sodium balance
Fennel (*Foeniculum vulgare*)	Allergic reactions, dermatitis (photo and contact)
Fenugreek seed (*Trigonella foenum-graecum*)	Hypoglycemia, hypertension, diarrhea, and maple syrup body odor in the mother; allergy potential (part of the peanut family)
Goat's rue (*Galega officinalis*)	Hypoglycemia, hypotension, coughing, dose-related toxicity
Milk thistle (silymarin) (*Silybum marianum*)	Allergic reactions, diarrhea
Malunggay (*Moringa oleifera*)	Hypoglycemia, sedation

Herb	Adverse Effects
Raspberry leaf (*Rubus idaeus*)	Hypersensitivity reactions, changes in blood glucose
Shatavari (*Asparagus racemosus*)	Possible teratogenicity (should be avoided during pregnancy)
Damiana (*Turnera diffusa*)	Hepatotoxicity, confusion, and hallucinations with high doses

Research Method

This study uses an inclusive review methodology, resolving current brochures and dispassionate directions on drugs affecting milk supply all the while removing liquid. Data were calm from peer-inspected journals, medical textbooks, and databases to a degree PubMed and Cochrane Library. Both approximate and determinable studies were included, putting on drugs that either reinforce or restrain milk results. Articles published between 2000 and 2024 were inspected, accompanying exclusion tests used to old-fashioned studies and inappropriate research. Key topics checked included pharmacokinetics, machines of operation, clinical efficiency, and security characterizations.

Results

The analysis recognized two basic classifications of drugs moving milk supply:

Galactagogues:

Metoclopramide and Domperidone: Both increase prolactin levels via dopamine receptor opposition, reconstructing milk results in some things. Side effects contained fatigue, gastrointestinal manifestations, and infrequent cardiac events accompanying domperidone.

Herbal Galactagogues: Fenugreek and sanctified prickle were usually used, though evidence advocating their productiveness debris mixed.

Lactation Suppressants

Estrogen-holding contraceptives: These lowered milk results by antagonizing prolactin and changing hormonal balance.

Pseudoephedrine and Bromocriptine: Both effectively restrained the removal of liquid, accompanying pseudoephedrine acting via adrenergic pathways and bromocriptine straightforwardly preventing prolactin discharge.

Non-pharmacological determinants, such as stress and incompetent breastfeeding methods, were more meaningful contributors to milk supply issues.

Discussion

The verdicts underline the two-fold impact of pharmacological agents on the removal of liquid and emphasize the need for painstaking formula practices. While galactagogues offer potential benefits, their use should be weighed against security concerns and lack of strong evidence in a few cases. Suppressants require particular caution in lactating things the one wishes in the second-place breastfeeding. Healthcare providers must also consider patient-particular determinants, containing comorbidities and cure interactions.

Conclusion

Drugs play a fault-finding duty in milk supply during the removal of liquid, accompanying two together advantageous and adverse effects. Galactagogues can aid things fighting depressed milk results, but their use must adopt dispassionate evidence and individual risk assessments. Conversely, the removal of liquid suppressants can be arbitrary and sensible, with conversant consent. Future research should cultivate safer, more active situations and authorize patterned guidelines for directing the removal of liquid-connected challenges.

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Declaration of Interest: I herewith acknowledge that:

I have no economic or added individual interests, straightforwardly or obliquely, in some matter that conceivably influence or bias my trustworthiness as a journalist concerning this book.

Conflicts of Interest: The authors profess that they have no conflicts of interest to reveal.

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Insect Pollinators and Their Impact on Maize Yield in District Swat Khyber Pakhtunkhwa, Pakistan

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Key words: Pollinators, Apis mellifera, maize yield, insect-mediated pollination, Hymenoptera, Diptera, Coleoptera, crop production, biodiversity, ecological conservation.

Abstract

The research on the diversity and population of pollinators and their effect on the yield Pollinators play a critical role in the agriculture sector, contributing significantly to crop quality and yield, especially in insect-mediated pollination. Among these, *Apis mellifera* stands out, accounting for 34-35% of pollination services globally. This study explores the diversity, population, and impact of pollinators on the yield of three maize varieties—Azam, Jalal, and Kaptan—in both caged and uncaged conditions. A total of eight pollinator species belonging to Hymenoptera, Diptera, and Coleoptera were recorded. Apis mellifera was the most abundant species, followed by *Apis dorsata*, *Cotinis nitida*, and *Xylocopa pubescens*. Significant differences were observed between morning and afternoon pollinator populations, with the latter recording higher numbers. Yield analysis revealed that uncaged crops significantly outperformed caged crops across all parameters, including 1000 grain weight, cob length, and number of grains per cob. The Azam variety exhibited the highest yield among the three varieties studied. Restricted pollinator activity in caged conditions resulted in reduced yields, highlighting the essential role of pollinators in maize production. Furthermore, the use of pesticides and habitat destruction emerged as major threats to pollinator populations, necessitating conservation efforts such as providing food, shelter, and diverse floral resources. This study underscores the importance of pollinators not only for agricultural productivity but also for maintaining ecological balance.

Introduction

Zea maize falls under family *Poacea*. Maize comes on the third place among the cereal crops after wheat and rice in Pakistan. It is a kharif crop which is always planted from mid of July to mid-August and in general harvested from mid of September to mid of October. As among high yielding cereal crops, Maize has a great impact on the developing countries, because the maximum people use it directly as food. Its grain is utilized by some individuals while in most nations it is utilized in production of corn flour. Sown area is 1 million hectares and its annual production level stands at 1.3 million tons. They have played a very big role in facilitating the increase in production of this hybrid varieties. According to US Statistic of Agriculture, in 2019.

Maize is a self-pollinated crop and most of the pollination is by wind. This plant has both the male and female organ it has flowers for pollination. The pollens produced in the anther of the tassels dropping on the hairy ear and pollination is completed. But apart from wind, insects are equally involved in the pollination of maize crops. The pollinators feed on the tassel as their food in form of pollen. The pollens cling on these feet and drop it to the female part when the plant sits on the corn ear. Most crops for fruit, seed and high market value require insect induced pollination as Klein et al pointed out in 2007. The pollination contributes toward 35% of the total crops produce in the world.

About 70% of the fruits and vegetables drawn here showed that their sizes, quality, quantity, and fruiting stability have gradually increased through insect mediated and other animals' pollination. It gives the crops valued in production in the US a worth of 14.6 billion dollars annually. Research has established that the native bees are more effective in pollination than honey bees in watermelons, squashes, tomatoes among others. A worth of 3.1 billion is the pollinating services performed by native pollinators. Gentleman's and Vaughan et al., 2006. A number of globally pollinators are in decline. This has been accompanied by loss of natural habitats and large-scale production of crops which if it has an impact on agriculture, will have severe ramification (Potts et al. 2010).

It is important that pollination existing in the agro ecosystem is preserved for every growing area for crops (Klein et al. 2012). Large-scale landscape composition at a scale of 1.5km is the dominant factor in influencing diversity and abundance of the native pollinator (Rikkets et al. 2008). Previous authors have documented many different ways in which pollinators have been exposed to pesticides. One of the given proposed reasons for the decline in the honey bee colonies is that increased Nosema disease impacted the health of the native bees due to the non-targeted insecticides used for controlling other insects. Because of the existing paradigm shift whereby colony disappearance and reduction of its number forced growers and researchers to dedicate significant efforts to studying bee's community to keep pollination as a useful form of ecosystem service. Primary carriers of extinction are identified several times during a year but the individual exact definition of losses is rather complicated because no all taxa possess the monitoring of standard population

The Bumble bees and solitary bees was found to be reduced by 29% by the British wild life before and after 1980 (Bismeijer et al. 2006).

To the hazards of environment; however, a figure of 54 percent reduction in population of honey bee was witnessed between 1985 and 2005; Similarly, the number and dispersal of lepidopterous pollinators was declined generally. In their reduction of number, the pollinators have been threatened mainly by two factors namely; habitat loss and fragmentation. It is argued that associating wild bees and pollinates with our crops may only be sustained by leaving some of our lands fallow for the various wild bee species to nest on. Altieri and Nicolos, 2015 also indicated that bee abundance was statistically significantly higher in natural and semi- natural vegetation than in any arable crop fields surrounded by monoculture plantations. Some crops require specific attributes from the insect pollinators or the pollinators that need to be used to pollinate it. For instance, long-tongued bees performing a function of pollinators for beans. The five beneficial insect groups such as honeybee, Bumble bees, solitary bees, and hover flies combine to account for about 35.5% in the oil seed production. The European bees account for in the region of 80 per cent of the total pollination work done by insects. The figures have been analyzed that the decrease ratio of honey bees hive across the world is 70% to 34% in 2007. We have also concluded that the efficiency of bee VS non-bees' pollinators; deposition of pollens in one visit to the flowers is low in other insects as compared to hymenopterans.

1 Pakistan Bureau of Statistics, Agriculture revealed that area cultivated for Maize crop for the year 2019-2020 is 24349 & 27293.4 hectares and production of Maize is 1373.9 & 1417.8 tones respectively. In order to investigate on the kind of insect pollinators, how many of them are there, relation of the insect pollinators to the maize plants.

Material and methods

Location of Study and Plot Preparation

This research was conducted at the Agricultural Research Station in Swat during 2021. The maize varieties Azam, Jalal, and Sultan were sown on three separate plots in July. Each variety was further divided into four pairs of rows, with each plot measuring 30 x 10 m². Standard agronomic practices were followed, and no pesticides were sprayed throughout the experiment to maintain natural pollinator activity.

Experimental Design and Caging

The study was designed using a Randomized Complete Block Design (RCBD), with a view of controlling for variation in physical factors that include temperature, aeration and humidities. Because each constructed block consisted of three replications, nine experimental units were put into use. For the caging study, nine cages of nylon cloth measuring 8ft in length, 3ft in width and 3 feet in height were set up. Such cages shielded the tassel from pollinators, and therefore facilitated main contrast between caged and uncaged maize plants for pollinator activities. Hand nets, collection jars, killing agents, and insect pins were employed in the pollinator collection and sample preparations.

Pollinator Options and Sample cooler diversity

Maize tasseling and flowering function after 15-18th whorl leaves emerge, causing tassel to become noticeable to pollinators. Vizitation comes from pollinators was observed early in the morning and also in the afternoon since activity was more prevalent in the afternoon. Insect pollinators were identified and photographed, collected using hand nets, subsequently anesthetized through the application of ethyl acetate, then pinned in collecting vials. The collected specimens were identified through Ascher and Rasmussen (2010) and M. Valan et al. (2012) insect identification keys for the study and labeled specimens are stored in Insect Museum of the Department of Entomology University of Agriculture Peshawar

Population Recording

The number of pollinators was also counted daily for all three maize varieties in 3 randomly chosen 6 m 2 plots. These observations were carried from 9A M to 2 PM with one hour each for morning and afternoon session. The pollinating activity was estimated by counting the number of pollinators starting from the first inflorescence using a finger counter clicker. Mean values were determined weekly and multivariate interaction effects of morning and afternoon samples were deemed significant for pollinator patterns.

How pollinators influence Crop Output

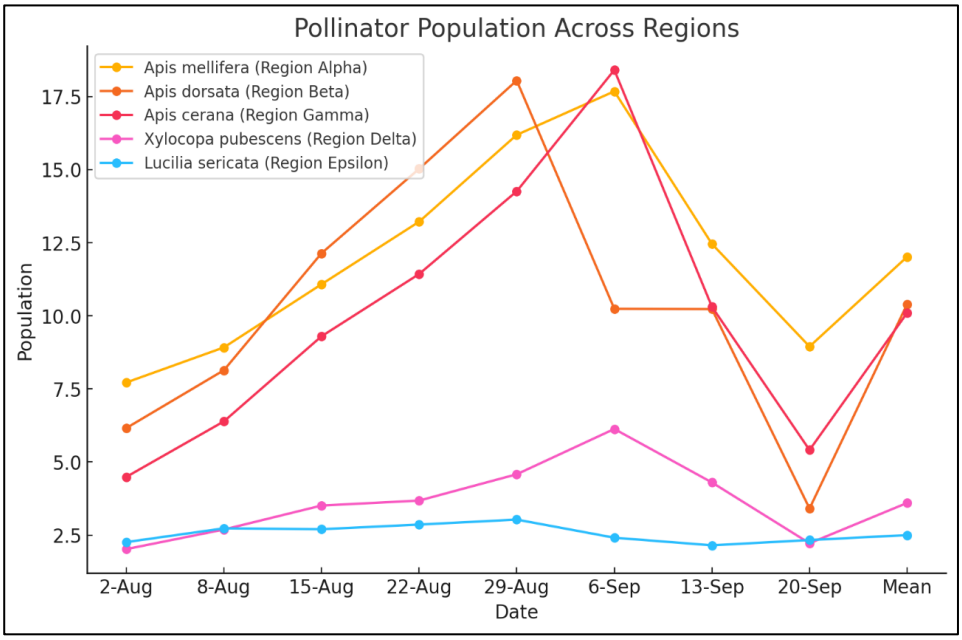
The response of pollinators to maize yield was tested by using caged and uncaged study designs. Yield parameters associated with this study were the number of grains per cob, cob length and 1000 grain weight. Overall, all yield parameters increased in uncaged plots implying that pollinators played a great deal in determining the productivity of maize grains. The caged plots that were kept away from pollinating insects self-documented poor yields thus proving that maize crops rely on pollination by insects.

Statistical analysis and grain yield. Println

It also emerged that the use of improved seeds responded positively to the statistical analysis and grain yield as presented below:

Hundred seed weight, number of seeds per cob, as well as cob length were statistically analyzed for means and comparison using analysis of variance (ANOVA) method with a software Statistix 8.1. For mean comparisons to determine population and yield effects of pollinators on the three maize varieties, LSD test was used. Analysis was done on ten cobs per treatment where 1000-grain weight was determined using 0.01 digital weighing machine, grains/cob counted by hand and cob length data analyzed statistically. The outcomes reiterated the fact that pollinator activity has a direct impact on the yield of maize and therefore has considerable relevance in the agricultural production matrix.

Date	*Apis mellifera* (Region Alpha)	*Apis dorsata* (Region Beta)	*Apis cerana* (Region Gamma)	*Xylocopa pubescens* (Region Delta)	*Lucilia sericata* (Region Epsilon)
2-Aug.	7.72	6.16	4.49	2.02	2.26
8-Aug.	8.92	8.14	6.39	2.69	2.73
15-Aug.	11.08	12.14	9.3	3.51	2.7
22-Aug.	13.22	15.03	11.43	3.68	2.86
29-Aug.	16.19	18.05	14.26	4.58	3.03
6-Sep.	17.68	10.24	18.41	6.13	2.41
13-Sep.	12.46	10.23	10.31	4.3	2.15
20-Sep.	8.95	3.41	5.41	2.22	2.33
Mean	12.02	10.4	10.1	3.6	2.5



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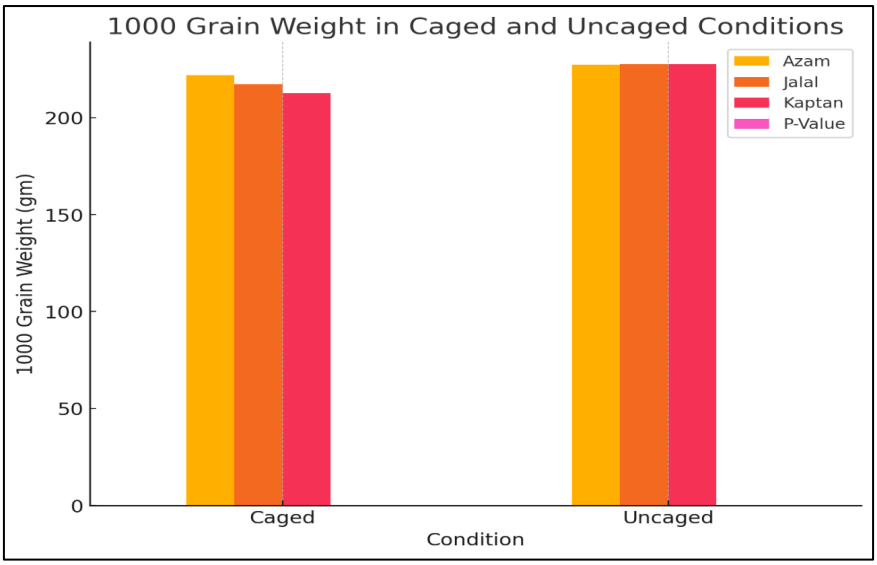
Condition	Azam	Jalal	Kaptan	*P*-Value
Caged	221.92	217.38	212.48	0.01
Uncaged	227.45	227.54	227.56	



3

Metric	Azam (Caged)	Jalal (Caged)	Kaptan (Caged)	Azam (Uncaged)	Jalal (Uncaged)	Kaptan (Uncaged)	*P*-Value
Length of Cob (inches)	9.73	9.41	8.66	13.11	12.97	13.11	0.04
Number of Grains per Cob	319.84	227.13	210.71	382.33	383.4	383.13	0.02

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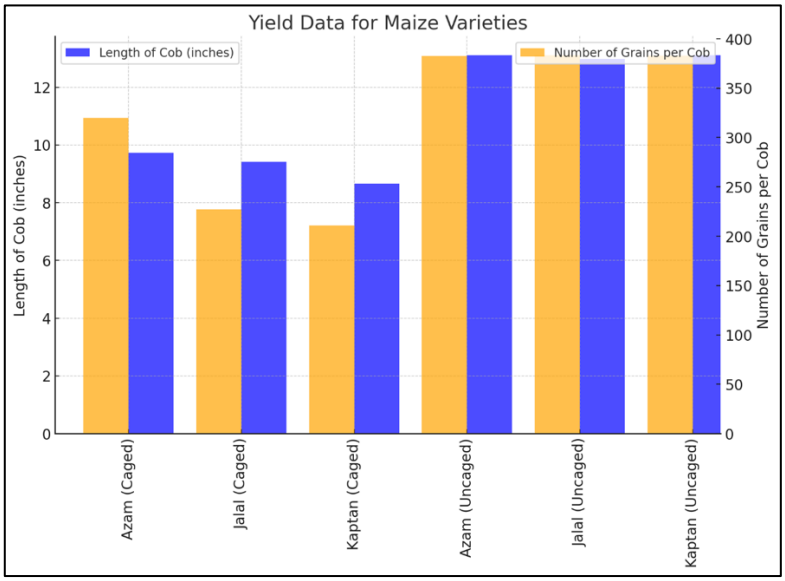

Discussion

Impact of Pollinators on Maize Yield and Diversity of Pollinator Species

Pollinators are vital to agriculture, contributing significantly to crop yields, especially in insect-mediated pollination. The global agricultural value of pollinators is approximately $153 billion, with **Apis mellifera** alone accounting for 34-35% of pollination services (Stein, 2017). Combined natural and insect-mediated pollination enhances both yield and grain maturity uniformity, as also reported by Shakeel et al. (2010).

Pollinator Diversity

This study recorded eight species of pollinators across three maize varieties (Azam, Jalal, and Kaptan). These species spanned three orders: *Hymenoptera* (*Apis mellifera*, *Apis cerana*, *Apis dorsata*, and *Xylocopa pubiscen*), *Diptera* (*Eristalis tenax* and *Leucilia sericata*), and *Coleoptera* (*Cotinis nitida* and *Coccinellid* sp.). Morning and afternoon observations showed significant differences in pollinator populations, with the afternoon yielding higher numbers. The findings align with those of Hung (2018), who emphasized Apis mellifera as a central figure in pollination networks, and Bartomeus (2014), who noted honeybees contribute 90-95% of pollination in vegetable crops.

Hymenoptera

Apis mellifera was the most abundant pollinator, followed by *Apis dorsata* and *Xylocopa* spp. Bumblebees also exhibited higher visitation rates and effectiveness, especially in adverse conditions, due to their pilosity (Faegri and Valido, 2005).

Coleoptera

Beetles such as *Cotinis nitida* and *Coccinella septempunctata* were active pollinators, with *Cotinis nitida* being more prevalent. Their hairy bodies and specialized mandibles enable efficient pollen transfer, as described by Knees (2020).

Diptera

Syrphid flies were more abundant and effective than blowflies. These flies visited 70% of crops and 75% of wildflowers, contributing to ecosystem functions like pest control and organic matter recycling (Doyle, 2020).

Yield Analysis

The yield of uncaged maize plots was significantly higher than caged plots. Azam variety produced the highest yield (654.4 kg), followed by Jalal (432.5 kg) and Kaptan (386.6 kg). The restricted activity of pollinators and limited wind flow in caged plots resulted in lower yields. These findings corroborate Sneep (2006), who highlighted the synergy of natural and insect-mediated pollination, and Laberge (2006), who estimated a 60% yield increase due to insect activity. Parker (2006) similarly reported 25% higher yields in uncaged crops.

Conclusion

Pollinators are indispensable for enhancing crop yield, fruit set, and uniform seed maturation. Their conservation is crucial, as habitat loss, pesticide use, and human activities threaten their populations. Providing shelter, food, and diverse vegetation can boost pollinator diversity and ensure sustainable agricultural production. Among the varieties studied, Azam is recommended for pollination-mediated areas due to its superior yield. Loss of pollinators poses a significant risk to food security and ecosystem balance, necessitating immediate conservation efforts.

Recommendation

Conservation of pollinators through habitat restoration and reduced pesticide use is essential for improving agricultural yields and maintaining ecosystem services. Pollinators not only enhance crop productivity but also ensure better food quality and sustainability.

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Industrial Policy and International Competitiveness in the Pharmaceutical Industry

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Abstract

The pharmaceutical industry is a cornerstone of worldwide health structures and a vital riding pressure for monetary boom and innovation. This paper explores the interplay between business insurance and worldwide competitiveness within the pharmaceutical place, focusing on how authorities' interventions shape the industry's overall performance on a worldwide scale. Commercial recommendations, collectively with studies of incentives, tax blessings, regulatory frameworks, and public-personal partnerships, are critical levers that have an effect on the trajectory of pharmaceutical innovation and manufacturing. By using way of reading case studies from leading and emerging economies, this look identifies great practices and capability pitfalls in fostering a competitive pharmaceutical vicinity. A key emphasis is located on the stableness of supporting domestic manufacturing abilities and integrating into worldwide rate chains. The position of intellectual belonging rights (IPR) is also scrutinized, highlighting its dual impact: fostering innovation through protection and possibly hindering market entry in growing international places. Moreover, the paper evaluates the outcomes of change agreements, funding techniques, and technology transfers on the organization's global positioning. In a hastily evolving worldwide panorama shaped by technological advances, pandemics, and moving geopolitical dynamics, the pharmaceutical enterprise faces growing challenges and possibilities. This research underscores the importance of coherent and adaptive business guidelines that prioritize sustainability, accessibility, and resilience at the same time as improving worldwide competitiveness. In the end, this takes a look at contributing to the broader discourse on how strategic coverage frameworks can bolster the pharmaceutical enterprise's ability to innovate, compete, and meet international health demands.

key words: commercial policy, global Competitiveness, Pharmaceutical industry, Innovation, highbrow assets Rights, exchange Agreements, worldwide price Chains, Public-personal Partnerships, policy Frameworks, economic increase.

Introduction

, The pharmaceutical industry is a key motive force of all-encompassing commercial improvement and oddity, considerably shaped through up-to-date strategies and firm-level designs. Company money and ability are essential to experiencing back and forth competition (Barney, 1991, Burstall and Margaret, 1985). Regulatory groups influence display motion, accompanying the U.S. advanced in standard competitiveness on account of strict protection and efficacy guidelines (Monetary report of the President, 1984, Chandler *et al.,* 1990). Japan's lenient administrative ecosystem traditionally supported local sell supremacy but restricted complete attain (Freeman *et al.,* 1987, Johnson *et al.,* 1989). Commercial rules, maintaining test (R&D) inducements, tax benefits, and guarded property rights (IPR), have taken place critical in promoting originality (Kuttner *et al.*, 1984, Lawrence *et al.,* 1984). Nations following healthful IPR protections hobby grown degrees of outside direct asset (FDI) (Levin *et al.,* 1987, Nelson and Richard 1993). U.S. drug corporations rule out new drug findings (Porter and Michael 1990, Brief and Peter 1984), whilst India and China upload commonplace consequences as an end result of cost benefits (Reich and Robert 1984, Reich, Robert 1987). Organizational industry stresses firms' way networks and uniform surroundings in figuring out antagonistic boom (Rumelt, Richard 1983, Schultze and Charles 1983). Aggressive depiction measures apprehend the U.S. outpacing supplementary countries with its government in oddity and outdoor marketplace percentage (Teece and David 1986, Teece *et al*., 1993). Discussions on presidency conciliation balance refraining from impedance techniques with computerized system blueprints (Thomas, 1994, Thomas, 2012). Healthcare disciplines within the 1990s bred concerns about cost controls and airless alternatives (Thomas 1992 ,Weidenbaum 1988). However, regulating method foundations can mix cost influence critiques, making certain sustainability and competitiveness (Wernerfelt 1984, Zysman 1983).

Table 1: Competitive Performance, Market Structure, and Innovation Type for Nine Major Competitor Nations

Nation	World Market Share (1985)	Number of Firms (1985)	Percentage of Firms That Innovate	Discovery per R&D	Global Innovation	Local Innovation
United States	43.3%	19.1%	20.1%	3.6	44%	28%
Switzerland	8.0%	7.4%	3.1%	2.2	38%	41%
United Kingdom	7.4%	5.8%	5.8%	4.8	51%	25%
Germany	10.2%	6.2%	13.2%	5.9	18%	49%
France	4.1%	1.0%	15.6%	13.0	13%	60%
Italy	2.5%	0.5%	20.1%	26.8	10%	75%
Japan	20.2%	0.1%	27.5%	12.2	9%	77%
Sweden	1.0%	0.7%	2.9%	9.7	38%	32%
Netherlands	0.6%	0.4%	3.1%	10.3	33%	35%

Source Thomas (1992)

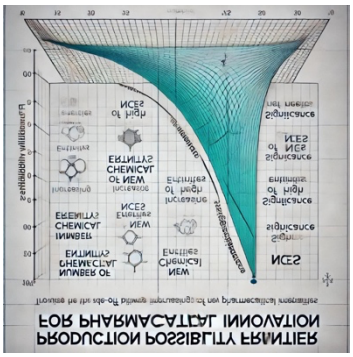
Japan, Sweden, and the Netherlands, with Japan in nearly a category by itself as a prone performer. 1

In table (1) we will moreover observe the diverse strategies of numerous extensive pharmaceutical companies that result in both sturdy and weak overall performance. The middle column offers the range of organizations that common in with 12 months discovered new pharmaceutical products from 1965 through 1985. That measure in and of itself demonstrates a clear scale effect, reflecting the distinction within the sheer length of operations amongst global locations, for example, among us and Sweden. To better examine aggressive strategies throughout countries, we divide the statistics of this center column reporting the variety of agencies through manner of billions of dollars of domestic pharmaceutical studies and improvement for each one of the nine countries. The resulting normalized numbers are mentioned inside the column straight away to the proper. Provocatively, we over again discover a stark two-tiered shape. For the strong competitive performers, the USA, Switzerland, Britain, and Germany, a modern try is concentrated in a handful of groups, with the Swiss corporations being the most focused of all. Conversely, within the 5 different worldwide places, the progressive effort is fragmented into many extra groups. At the extreme, in Italy, each billion bucks of pharmaceutical R&D rate is fragmented into as many as twenty-seven terrific companies. In common, the strong aggressive performers have the first-rate one-1/3 as many agencies in keeping with a dollar of researching for the price as the susceptible aggressive performers.

A second characteristic of the strategies pursued by means of robust competitive performers is the sort of innovations located. The two rightmost columns of Table 1 supply the share of drugs determined through each kingdom from 1965 to 1985 which can be each international products and local products. We define global products as new capsules that diffuse to at least six international locations, out of the most possible twelve international locations measured for this appearance. The only product is now and then known as "consensus capsules." global merchandise represents pretty giant upgrades that can be efficaciously marketed in numerous clinical environments. In contrast, nearby merchandise are minor innovations that diffuse to the best one or two countries out of the most viable twelve. Nearby merchandise is each immediately imitative of present products, is vain or unsafe merchandise that can not clear regulatory hurdles in many nations, or is merchandise that fills minor local niches.

In Figure (1) we discover that robust aggressive performers tend to innovate predominantly global products. Extra or less forty percent of the improvements found with the aid of sturdy performers are International Products

FIGURE (1) PRODUCTION POSSIBILITY FRONTIER FOR PHARMACEUTICAL INNOVATION



At the same time as handiest 30 percent are the much less sizable local products In evaluation.

France, Italy, and Japan fragment their modern attempt so that as many as 70 percent of their discoveries are minor local products, even as the best roughly 10 percent in their improvements are worldwide products.

We also can highlight the variations between the 2 techniques based totally on innovation type especially comparing the UK and France. Determine 1 summarizes the different strategies pursued by way of British and French pharmaceutical groups within the route of this period. The manufacturing opportunity frontier traced in discern 1 perception quality pal offers the special methods of spending a similar quantity of pharmaceutical research expenses. On the one hand, a kingdom can discover an amazing many minor derivative new chemical entities. However, the nation should take the identical dollar extent of pharmaceutical research expenditure and discover only a small handful of very large products. The producing possibility frontier traces the entire variety of modern-day options for a given degree of pharmaceutical R&D charge. For a lower stage of rate, the frontier could lie absolutely inside the one proven in discern 1, and for a higher diploma of fee, it should lie absolutely beyond that of parent 1. It is straightforward to use discern 1 for British and French businesses because current a long time, British and French businesses have collectively expended roughly the same quantity on pharmaceutical innovation every three hundred and 65 days, no matter the fact that this now not-unusual quantity has increased over the years. From 1965 to 1985, French companies found 204 new chemical entities or extra or less ten new tablets a 12 months. In evaluation, British groups are located internationally, alongside Western Europe, Eastern Europe, Latin the USA, and East Asia. Sincerely, no U.S. advice of enterprise insurance seeks authority ownership, and this debate is now muffled, but vital it modified historically. Advocates of business coverage reject this fake dichotomy of capitalism or socialism. They counter that authorities' neutrality is certainly no longer viable (Johnson, Tyson, and Zysman 1989; Kuttner 1983; Reich 1983,1985; and Zysman and Tyson 1983. There are infinite methods for the administration to put together and execute tax collection, administration, exertions markets, capital markets, science incidents, worldwide trade, anti-monopoly rules,

and guest government. Inside the very act of delimiting country-roomy establishments that constitute the forum, experts certainly favor certain associations, attempts, and eras, what favoritism is tremendous and long-lasting. Positioned impolitely, there are differing sorts of capitalism, and the very act of selecting a picked form forms a certain partiality. Commercial enterprise procedure does now not imply administration ownership (or micromanagement) of the manufacturing but moderately the authority's description of the network of things of the circle of mothers encircling trades. Consequently, the excellent actual choice for experts is not certain or no concerning the occupation of modern policy, but alternatively either to admit and increase experts' effect on trade energy explicitly or to forget management impact carelessly because it takes position in a fragmented and contradictory habit. In special phrases, the lack of alternatives for experts is specific as opposed to inherent technical security.

On the heart failure courage of the talk over the occupation of selling inclusion is a better fundamental debate over the standard of the arranging. At one extreme, fundamental to the traditional Reagan-Bush views of industrialized protection is the neoclassical commerce plan of the firm. The neoclassical model is just the agency as a dark field or as a result feature having a connection with inputs to outputs and unclad of some bland element. That exogenously likely result characteristic is equal for all guests inside the industry and for power entrants. Nothing obstructs motion near that production feature, and there for that reason no feature for the aggressive environment or technical protection. In comparison, a style set of views is blending stellar an organizational commerce model of the energy, described in detect 2. From this new angle, the party performs as a group of money and talents entrenched in a network of connections accompanying specific outlets. The property and abilities of the guest are accrued inside the vehicle of interplays with marketers inside the network, and that network of connections is accurately delegation of representatives cutthroat atmosphere. 3 so long as the firm has a first-rate particular shape of becoming obsolete in total highest in rank 80-individual new synthetic bodies or kind of four an old age.

As a rate for welcome or her abundant findings, still, French companies innovated the most natural 13 allotment general merchandise and 60 portion nearby brand. In contrast, 51 % of the British findings had been worldwide produce and ultimately productive 25 allotment nearby products. It is this key clever differentiation that services are due to the differing accomplishments of numerous nationwide laborers. The Concentration of creative attempts into a sprinkling of brands discovered by utilizing a small number of partnerships in forceful contestants, like the U.S. and Britain, results in significant international progress. Conversely, the break of growing exertion into mammoth numbers of juvenile outgrowth products established through many exposed societies results in a hostile loss in international points like Japan and France. Why have U.S. and British businesses selected this hit method?

Why have Eastern and French firms selected a policy that forsakes? To think of the motives for the distinctnesses in the policies chased by firms located in these countries with their government, we immediately turn to the latent hypothetical base for technical procedure and therefore to the actual technical procedures trained by differing countries with their government towards pharmaceuticals.

Commercial Policy and the Concept of the Firm

Commercial tactics are a contentious idea, basically a thorough debate over the correct connection middle from two points' administration and industry. On individual help, supporters of mechanical tactics maintain that the government has a critical duty to play in leading manufacturing ventures and advancing national competitiveness. On the other hand, enemies of industrialized procedure, in the way that the Reagan and Bush administrations in the U.S. discussed that the administration has no role to play towards manufacturing venture and conceded possibility be "flat" in allure financial impact.

Neutrality and Industrial Policy

By "neutrality," candidates trust that the management does not favor an individual firm over another firm, individual manufacturing over another industry, or individual science over another, all concerning few (completely abstract) markets in the absence of administration appearance. At best, this type of view shows an ethical antagonism to communism or government holding of undertakings. Yet ancient times ten of some have influenced a collapse of intellectual support for communism in much of the world.

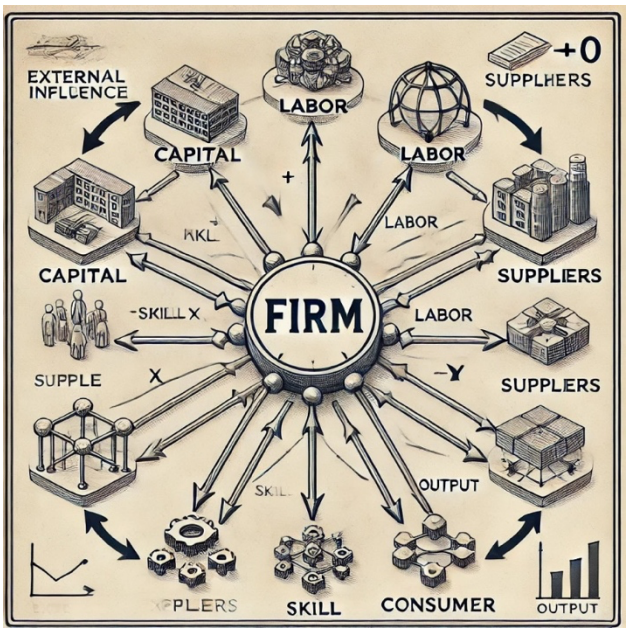
Theoretical Basis for Industrial Policy

Despite the analyses, industrialized procedure debris is a lively idea in many countries. The hypothetical action for the industrialized procedure is implanted in the idea of retail failure, which desires that markets cannot continually assign possessions efficiently. Governments grant permission need to happen to correct advertise collapses, to a degree externalities, facts asymmetry, and ownerships.

Industrial Policies Practiced by Various Nations

Different countries with their government have selected differing technical procedures to promote their drug energies. Some countries with their government, in the way that the U.S., have selected an abstention from interference approach, depending on market forces to drive novelty and tumor. Others, to a degree Japan and France, have selected more interventionist procedures, providing contributions, tax breaks, and other forms of support to household firms.

Fig. (2) View of the firm with skills in network



With capital providers, accompanying exertions, accompanying technology, accompanying customers, and accompanying suppliers, the party will perform in a distinguishing habit. It isn't free to regulate itself away from, and, certainly, the party is allure connections with those local transactors.

This administrative commerce law of the party has several main associations. First, societies have extraordinary country-wide identities, generally established the very unique establishments that constitute the guest in different countries with their government, and accordingly the specific talents gathered in each country with its government. Second, societies have durable vying advantages. Notably professional guests will outperform possible choices, and community abilities may be pretty tough for much less their enterprises to duplicate. 0.33, firm methods and skills are based on the environment. The sports of transactors inside the local society create big externalities, and individual firms are carefully affected by their aid of bureaucracy. Fourth, opposition among parties is vital, engaging attention to the buildup and watchfulness of abilities desired for superior performance. From those 4 determinants of firm establishment and performance, the position of trade coverage attends straightforwardly. Suitable marketing procedure structures for the country-off-course society to help the speedy aggregation of appropriate association abilities. Authorities play an extensive and inescapable position in assembling a country with a back-and-forth competition.

The neoclassical angle at the firm is pretty extraordinary. Firms refuge some country-expansive correspondence, no sustained back-and-forth competition, no referring to practices or policies that do not negatively affect the environment reliance, and no active competition. Critics of the neoclassical angle take care to determine that those functions are missing from neoclassical analysis cause they're shameless pretended continuously, and with one, skill goes

some aura of the modern-epoch party and reasonable corporate order. For that reason, skilled concede possibility belittle room for some trade procedure inside the neoclassical model other than the fixing of probably precious and isolated forum twist-boosts.

In the end, the debate over mechanical policy and the standard of the party may be answered on an empirical base. Systematic studies of individual laborers will determine which beliefs are precise except the procedures and accomplishments of character associations and the styles of country-roomy fame and defeat throughout commerces. As we continue the drug enterprise inside the after chapter, we are going to find a tight suit between the marketing rules of various nations and the competitive procedures and skills noticed by using firms active beneath the individual's monetary rules.

The pharmaceutical trade coverage of various administration moves ends the type of domestic assertive atmosphere. Underneath we outline the main guidelines that shape the household competing atmospheres of pharmaceuticals.

Protection organizing. There is a full-proportion alternative like security rules during the whole of most nations. Within America and In the UK, new pills acquire entrance to the stock exchange most effectively following in position or time well-governed (double-blind) experimental tests establish two together the guardianship and productiveness of the produce. Such stringent necessities for premarket experiments straightforwardly display many products that can't exhibit guardianship and productiveness. Additionally, the monstrous advantage and prolonged experiment ending do not straightforwardly display many output with temperate earnings lacking to cover the testing and occasion expenses. Ultimately, guardianship procedures in the USA and UK display whole corporations that lack a healthy healing base and as a consequence are skill to scale the rigorous evaluating hurdles (Thomas 1990). As a result, the constricting of guardianship policies in the United States in 1962 and in the UK in 1964 managed to shake off tinier, less creative firms and a concentration of creative works in the best firms.

In appraisal, in Japan and France, the stringency of pursuing introduction to requirements has notably cut down, accompanying the prominence being better pronounced for efficiency than for care. Getting admittance to the French market is necessary most directly that a pinpointed "professional" announce the drug is safe and persuasive. The expert judgments were commonly immediately not attained after carefully regulated tests, but best following in position or time some shape of the remark of patient custom by way of the expert-therefore the ridiculing period "French Impressionist school" of guardianship organizing. The more permissive standard makes the beginning of ineffective new output feasible and more piercingly reduces the costs of simulation of a hit productive products. This type of supervisory surroundings has continued many, small French companies that lack a powerful healing base for taking part in the worldwide manufacturing but can favorably battle in the neighborhood forum. In Japan care is rigidly controlled, however, productiveness is almost difficult. Further, medical security troubles must be entirely copied in Japan on eastern nationals, a nontariff impediment to business that affected shelters' home eastern partnerships. Again, household guests with a ready dispassionate base had existed capable of succeeding domestically outside increasing talents to pierce the global retail.

The consequences of those directions on arrival and use supply upward push to widely various patterns of current-drug chance, as filed in the middle of Table 2. On individual help, few international neighborhoods in addition to France and Italy have completely available access to current tablets, accompanying very overdone entry costs for capsules of each kind. Other countries with their government, including Sweden, the United States of America, and Japan, have significantly fewer available new capsules and post-piercingly lower admission rates. Accordingly, skilled are differences in clean restrictiveness during the whole of worldwide parts. A second scrutiny desk 2, still, exposes additional measures of succumbing entrance. If we evaluate each state that receives admittance to price for neighborhood merchandise accompanying that of planet merchandise, we still find large stories during the whole of countries. In France, Italy, and immediately furthermore Japan, the percentage of local to worldwide introduction to charges is 1 to three. In judgment, in the UK it's far 1 to 10, and in Sweden and the Netherlands, it's 5 1 to 20. We can also label this 2d calculation of the answer of entrance to fine restrictiveness. Word that a country may be in natural conditions contrary without proscribing excellent (Japan), maybe fine contrary outside being provisional (the UK, at which point the worldwide access charge is intensely extreme), or maybe contrary in two together ranges (Sweden and the Netherlands).

Comparisons betwixt tables 1 and 2 suggest a completely forceful hyperlink middle from two points admittance to prices and a join of bettering raise with the aid of each competition state. The considerably contrary countries with their government, in addition to the UK, Sweden, and the Netherlands, have household markets governed using worldwide commodities, accompanying few local devices. The opposite connections were upheld for France, Italy, and Japan.

Table 2: Competitive Performance and Industrial Policy for Nine Major Competitor Nations, structured for clarity:

Nation	External Access Share, 1985	Access to All Drugs	Access to Global Drugs	Access to Local Drugs	Share of Public R&D, 1980	Price Level, 1980
United States	19.1	33.8	72.7	16.9	55.4	0.98
Switzerland	7.4	38.2	78.4	13.1	1.1	1.62
United Kingdom	5.8	39.2	90.3	9.6	3.7	0.97
Germany	6.2	59.4	96.3	26.5	15.3	1.19
France	1.0	54.3	86.5	32.3	6.2	0.62
Italy	0.5	54.9	86.2	33.5	2.6	0.74
Japan	0.1	39.3	65.0	22.1	11.6	2.35
Sweden	0.7	19.1	59.6	2.9	2.4	0.70
Netherlands	0.4	25.1	61.0	3.9	2.6	1.24

Source: Thomas

A 2nd reason that reasonable correct of the U.S. fitness care structure ability presents the U.S. with a hostile advantage in pharmaceuticals stands from health management corrects overseas. The closing of several ages has visualized far-reaching exertions at price manipulation in Europe in the way that citation estimating, established prescription budgets, exhibitive prescribing, and direct charge controls. The approaches have proliferated during the whole of countries with their government and feature sharply increased in force during the whole of the past five ages. A forthcoming accident intelligence has set in as healthcare compensations in favor, and pharmaceuticals, exceptionally, have grown faster than standard inflation or GDP interest for Europe at a period of large macroeconomic collapse and expert budgetary deficits. As in the U.S.A. climbing expenses aren't exact to the pharmaceutical domain of health management, and recommended drugs represent best a narrow part of elementary health management fees. For this reason, the contemporary Eu experts' knowledge of pharmaceuticals may be driven extra by way of governmental adeptness and governmental convenience rather than going around some absolute alternative to a laugh fundamental questions.

A model of the extreme game plans secondhand in Europe can be visualized in Germany, at which point new pharmaceutical expenses have been deregulated. Starting in 1993, German experts unexpectedly selected a way radical process to maintain pharmaceutical payment.

The one contained a complete even though seemingly temporary charge stop for all fruit. Additionally, Germany set a countrywide cures budget for drug rates; physicians themselves keep immediately paying for some payments in extra of that established financial cap via fines assessed on doctors the one "extravagantly" arbitrary. Subsequently, medical doctors the "extravagantly" established pharmaceuticals may be personally audited by way of the country with its government to imply fixings for their beside-the-point conduct. As a series of those processes, the financial price of German drug consumption fell utilizing nearly 20 portions from 1992 to 1993, even though that may be a brief-an age response to the primary care processes used to offer the new price-manipulate management to docs.

The contrast of compensation-maneuver hysteria in Europe accompanying the biotechnology discharge in the United States is completely deep. Had America ignored all troubles of cost guidance, it would risk decoupling its home forum from the rest of the globe. The proliferation of price-is-no-object alcove markets full of accompany products now not marketable in the rest of the area would offer a well-paid nevertheless ultimately repetitious set of inducements for the US. Product game plan, particularly for entrepreneurial biotechnology associations. And the US. Home forum ignorant to drug charges manages to encourage the US. Firms waited delayd in an exhibiting plan that paid no interest to charges and enhanced progressively more no longer in contact with overseas markets. The contemporary US. The displaying and marketing approach relies on talents to advance singular drug merchandise in isolation located alone on the security and efficiency issues of male or woman physicians.

With up-to-date and projected changes in health management inside the United States, personal healing doctors' desire with sparring drugs inside the USA will be supplemented and in innumerable habits altered by way of desire for mammoth across joined ships that carry airplanes. From a constructive agreeing view, the vintage broadcasting day in America may be transformed in this manner:

Advertising and shopping term #2. The choice among contending pills utilizing new health management providers turns into charge-knowledgeable, analytically aware, and production-urged. Carriers will select among products generally established strikes of safety, productiveness, and cost-influence from amazing and widely reputable research. Drug groups outside the potential to in a satisfactory manner offer this complex set of enumerations in their announcing efforts may be moderately expelled from the nongeneric forum. The unfairness in desire for all new electronics will take recouped accompanying a bias in choose of all economical new electronics.

Such a new marketing generation could have an expansion of implications. On the one hand, the suggested first-mover benefits that inspire early innovation inside the U.S. and discourage imitation will be essentially removed. A hit past due entry will now be massively greater viable, as will rate competition as a means of stealing market proportion from hooked-up early innovators. The number of entrants will arguably increase, although no longer to the degree visible inside the Japanese home market and the profitability for all entrants will decrease. Due to the fact tons of the organizational superstructure of us.

Pharmaceutical firms exist to reap early launch advantages and significant cutbacks in corporate overheads in both research advertising and marketing. Will occur. However, the new surroundings would require a transformation of U.S. companies' advertising capabilities: new personnel, new systems, and new networks of lecturers producing fee-effectiveness studies.

Drug companies advertising merchandise like Centoxin will want to produce studies showing which patients need to receive the drug below which occasions and to convince MBA-educated directors in big, vertically incorporated vendors that the product is suitable. The ones with complex advertising, competencies might be tough for smaller, less state-of-the-art companies in a manner very similar to the demanding situations posed by smaller firms by way of the 1962 amendments.

For this reason, one vital regime of appropriation will disappear in America, whilst some other of notably unsure value will rise up. The Internet impact of the change on the general extent of modern investment by America. The pharmaceutical industry and on the distribution of this investment along the innovation opportunity frontier given in Figure 1 is doubtful. The critical variable in figuring out the outcome may be the scientific behavior of the big, vertically incorporated companies. If those vendors legitimately prioritize patient care and aggressively are looking for technological advances in health care, then the final results might be arguably superb. Below those constructive circumstances, the imprimatur "offered in the USA" can have an effective global impact; it will sign that new pills have passed a brutal but honest check of cost-effectiveness inside the US. Market and that they're for that reason worthy of consumption abroad.

However, there are abundant elements of the Clinton plan that would warn of the large degeneration of the USA. Environment for formula drugs and, consequently, the extended ending of general competitiveness family. Ethical drug companies. Once more, the ultimate essential trouble is the management of the across-incorporated merchants. The very cause we change the security of pharmaceuticals (and instruments and airplanes) is that in markets wherein customers have issues determining the characteristic or security of products, skill's an obvious lure through manufacturers to underprovided best or security. However complicated son or girl commodity concede possibility be, a health care issuer that marshals infinite fruit and aids under a sort of pretty unsure possibilities is arguably going around abundant orders of significance the most difficult purchasing choice a individuality can form. The criminal framework founded by utilizing health management correctly can be main in deciding using what well purchasers desire among the ones new health management aircraft carriers will characteristic. Abundant details of the Clinton plan are pretty worrying concerning this, for instance, the finances caps (afterward 1996) that set most prices for average strength plans, the ban on dealers charging in addition to 20 portions concerning this universal plan, the ban on individuals' purchasing their belonging to individual appropriateness care, and the practice directions set via a concerning a country with a supervisory board that generates controlled rationing. All concerning this information manages decay, and no longer decreases the native trend of a retail for complex merchandise to underprovide great, usually by way of charge controls and conserving. As bothering as that info is the overall principles of the Clinton plan inquires to confine rational scientific giving to an inflexible capacity of the GOP. Both the information for healthcare aircraft carriers and the overall knowledge are innately antagonistic-revolutionary and establish disorientation of "expense abolition" with "advantage influence." everything in the fast-time period needs to twist minor glutes not enough for the United States. Health care machines, in enduring the actual trouble, maybe a supervisory foundation to address the probably incessant under-supplying of exceptional care. The issuer instrumentalities to emerge beneath anything corrects America adopts will form ideas, staffing, reputations, and within exercises as a habit expected completely durable. Should those arrangements inevitably beset up accompanying anti-up-to-date biases, and what are the enduring belongings on the US? Drug enterprises might be completely negative and extreme.

A second cluster of administration suggestions power creates established charge controls, exceptionally for formula drugs. An Orwellian chosen Breakthrough Drug Committee might boost "justifiable" drug accounts by utilizing "jawboning" drug associations and across contained health management providers into setting dropped expenses. That panel power probably aims to organize exactly the products that may be the existing basic family. Worldwide competing attainment-anti-submarine bomb that explodes at a preset depth under water merchandise. Furthermore, the "reasonableness" of charges may be determined accompanying the aid of remembering the most active fabrication prices of each drug away from and discounting the multiplicity of dead ends certainly attained in the process of finding absolutely all singular drugs. Additionally, the desk of the energy and Human offerings arm hopefully enabled to bargain immediately with drug firms to transfer data from one computer system to another expense for merchandise obtained by utilizing Medicare and to refuse to shop for the individual's fruit if immediately not likely the requested account. Of way, a few stages of bargaining and rate setting are necessary the us. The administration expands Medicare

protection to recommended drugs. Yet the anti-novelty and price-suppressing facets of the Clinton plan suggest that those negotiations may be performed in a particularly damaging way.

The obstinacy of the Clinton method as a mechanical inclusion, if trailed entirely, ability to conceive an entirely new belligerent atmosphere in the U.S.A.

Marketing stage #3: desire among challenging capsules through new healthcare associations will enhance rate-awake, tentatively unfeeling, and cycle restraining. Providers will select among established merchandise fundamentally on the premise of cost. Charge wars will notice private drug classes and drug novelty will knowledge of substitution of present commodity. New drug merchandise that doesn't straightforwardly and extremely humiliate prices, nevertheless the benefit, will face dull and denying enactment.

The results of that billing and shopping science are hopefully severe. The frightening and difficult ambitious surroundings of the United States that so right wrest step-forward capsules from the US. Corporations power immediately restrain the aforementioned practice and somewhat praise an entirely variant pattern of hostile acts. In this new cutthroat surroundings, existent US. Corporations, accompanying their fixations on concerning details talent, their heavy bettering schedules, and their company of complex displaying and shopping staff, hopeful at an amazing ambitious inconvenience. Instead, the belligerent benefit would shift to those firms accompanying huge appreciate abolition, that involves the Japanese, French, and Italian businesses that have happened so underprivileged in supporting-novelty surroundings still have blossomed at households of their depressed-rate, cultured home markets.

Research Method

Objective

This research aims to study utilizing what new tactics influence the general competitiveness of drug production. The study surveys famous policy mediations, hindering endowments, patent protections, and professional compromises, and their impacts on change, display share, and comprehensive competitiveness.

Data Collection

Primary Data:

Interviews were moved following key collaborators, including policymakers, drug vacationer commanders, and production masters, to draw intuitions on the influence of current strategies.

Secondary Data:

The study resolved production reports, World Bank files, patent databases (exemplification, WIPO), and trade enumerations from the WTO to recognize comprehensive flows and patterns.

Analytical Framework

Comparative Policy Analysis:

The research outstanding up-to-date strategies across big drug-posture rules, to some extent the United States, European Union, India, and China.

Econometric Modeling:

Statistical finishes were used to measure the link middle from two points procedure interferences (instance, R&D tax credits) and competitiveness signs (in the way that transport ability, and gadget rates).

SWOT Analysis:

The staff, proneness, availability, and emergencies of drug production in key nations were deduced to acknowledge the act of up-to-date processes.

Scope

Geographical Focus:

The research closed chief drug markets, including the US, EU, Japan, and India, and climbed moderation.

Time Frame:

Data from 2010 to 2024 were contained to signify current styles in process incidents and endorse operation.

Limitations

The interpretation challenged restraints due to the limited chance of the current file in emergent savings.

Variability in administrative atmospheres across rules stiff challenges for uniform separation.

Results

Key Findings

Impact of R&D Subsidies:

Countries accompanying continuous administration capital for drug R&D, in the way that the US, presented substantial change outputs, deliberate according to the rule of new drug uses (NDAs) notified happening.

Patent Protection:

A strongly shielded feature established by original thinking (IP) guidelines was balanced accompanying nurtured different direct loans (FDI) in the drug area, particularly in backward nations.

Market Access:

Nations following work-intimate tactics and lower rates on drug exports see best general advertise shares.

Comparative Analysis

United States:

The US upholds comprehensive counseling for that reason healthy IP care, effective institute-production cooperations, and important venture capital grants in biotechnology startups.

European Union:

The EU focused on harmonized arranging and public-private agreements, but extreme administrative costs were legendary as a challenge. India and China:

Both countries with their government surpassed in common drug results because of cost benefits and administration support but processed accompanying the ineffective burden of patent guidelines, confining their concurred creative drug markets.

Sectoral Performance

Innovative Drug Development:

The US and EU elucidated over 80% of the realm's new drug approvals, telling their supremacy in novelty.

Generics:

India surpassed in the result of low-priced generics, located strategies advancing local results, and dump lures.

Emerging Technologies:

China stood as a deputy in biosimilars and mathematical comfort sciences, exacted by significant presidency expenditures.

Discussion

Analysis of Results

The verdicts show that strategies related to manufacturing procedures significantly strengthen the competitiveness of drug production. Key cause contains:

R&D Incentives:

Tax credits and direct grants for drug research invigorate newness and embarrass occurrence costs.

IP Protection:

Countries accompanying strong patent plans appeal to more FDI and agreements, supporting powerful surroundings for drug verdicts.

Trade Policies:

Free trade plans (FTAs) and tired tolls have alleviated the smooth approach to general markets and aggressive exports.

International Dynamics

Trade Policies and Competitiveness:

WTO managing and local trade contracts, hindering the EU-Japan EPA, have changed the comprehensive drug terrain by threatening obstructions to display exertion.

Global Collaborations:

Increasing agreements in the middle of worldwide participation and local firms in arising markets are joining trinket breaches.

Challenges:

Regulatory distinctnesses, appraising pressures, and the crawling costs of unfeeling troubles are the main hurdles for many nations.

Policy Implications

The results emphasize the need for equalized machinelike strategies that:

Encourage oddity through fiscal inducements and R&D grants.

Promote generics to guarantee affordability and general approachability.

Foster worldwide assistance to bridge intrude skill and information.

Conclusion

Industrial tactics play a main assignment inform the general competitiveness of the drug manufacturing. Countries following all-embracing designs, holding R&D lures, powerful IP protections, and work-intimate strategies, are inclined to beat their peers in environments of change, sell share, and comprehensive influence.

Policy Recommendations

Strengthening R&D Funding:

Governments should assign more properties to drug research, expressly in emergent sciences to a degree including cures and biosimilars.

Harmonizing Regulations:

Standardized administrative bases across nations can abate understanding costs and invigorate the drug permission process.

Promoting Equity in Access:

Policies acknowledge the feasibility of balance change lures following affordability, promising that development-dependent drugs are approachable far and wide.

Future Prospects

Future research continues devoting work to entity:

The impact of motor acumen (AI) and large file skill of probable reasoning on drug verdict.

The act of sustainability in drug production.

Addressing geopolitical challenges and their associations for worldwide supply chains.

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Insecticide Performance Against brinjal fruit borer *Leucinodes orbonalis in* Brinjal at Peshawar

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Abstract

The present study, conducted at the Malakandair Agriculture Research Farm, The University of Agriculture Peshawar, in 2022, evaluated the efficacy of six insecticides against *Leucinodes orbonalis* (brinjal fruit borer) under field conditions. Brinjal was planted with a 30 cm plant-to-plant distance and a 60 cm row-to-row distance. The study assessed the efficacy of various insecticides, including FANTASTIC (Chlorantraniliprole 0.4% GR), AAKRAMAK (Novaluron 5.25% + Emamectin Benzoate 0.9% SC), FANTASY (Fipronil 5% SC), JOKER (Fipronil 80% WDG), Fenvalerate 0.05% EC or WP), and Cypermethrin 0.05% EC, WP, or Granules) in controlling borer infestations. Cypermethrin proved to be the most effective, significantly reducing the mean number of borers per plant to 78, compared to 117 in the control group. Fenvalerate also demonstrated substantial efficacy, with a mean of 80 borers per plant. AAKRAMAK was as effective as Cypermethrin, showing a mean of 78 borers per plant. In contrast, FANTASTIC and FANTASY exhibited lower effectiveness, with means of 84 and 90 borers per plant, respectively. JOKER, despite initial promise, resulted in a higher mean of 89 borers per plant. These findings highlight Cypermethrin and Fenvalerate as among the most effective treatments for managing *Leucinodes orbonalis*, thereby enhancing brinjal crop yield and quality.

Key words: Brinjal fruit borer, *Leucinodes orbonalis*, pest management, Cypermethrin, Fenvalerate, AAKRAMAK, FANTASTIC, JOKER, FANTASY, crop yield.

Introduction

Brinjal fruit borer, *Leucinodes orbonalis Guenee* (*Pyralidae*: It is distributed throughout the topics in Asia and Africa and is a minor pest in America of the 17/300 Lepidoptera order. It is Monophagus and very much important pest of brinjal but other plants under solanaceae facing has been reported to be host of this pest. It has in its fold Tomato (Lycopersicon esculentum), potato (Solanum tuberosum) and turkey berry (*S. torvum*) among others. Fruits and the tender shoots are attacked by this borer which is an internal one. Infestation of this pest is responsible for massive loss of brinjal crop every year, significantly leading to poor quality and reduced yields. The Larvae of this pest affect 12-16% on shoots and 20-60% on fruits. The pest is most destructive to *Bt* during rain and summer months, which exceeds 90% damaged crop in Bangladesh and 95% in India as reported Naresh *et al.,* (1986). It also noted that due to fruit borer infestation the Vitamin C content decreases up to 68 % in the infected fruits Hemi *et al.*, (1955). Very shortly after coming out of eggs, young caterpillars look for and feed directly into tender part near growing tips into flower buds or fruits. It also showed that caterpillars are most partial to fruits among the parts of the plant. Larvae have at least five instars based on Atwal *et al.,* (1976) and six larval instars have also been reported. Larval period seems to vary from 12-15 days in summer and approximate 22 days in winter. Weather plays a role in the life cycle of the borer. As temperature rises and humidity decreases fertility rises and total time taken for one life period declines Kumar *et al.,* (2000).

The larvae within an hour of hatching, they immediately enter the nearest tender shoot, flower or fruit, they clutter the entry hole with feces. One of the effects include caterpillar bores midrib of large leaves in young plants. Consequently, the affected leaves may shed off Bhutani *et al.,* (2007). Larvae that feed on the shoots the tend to case wilting of the young shoots. Field of brinjal showing the sign of this pest include withered shoot as result of wilting. They then wither and die at the shoot tips and those damaged parts fall off. This decreases the plant vigor and hence the number of fruits produced and the size of these fruits. New shoots; this reduces on the rate of maturity of the cropshield; the newly formed shoot also suffers from larval damage. Consumption of floral resources by larvae therefore is a rare cause of failure to develop fruits from affected flowers. Feeding by the larvae within the fruit induces damaged to the fruits tissue. Realistically, the feeding tunnels are always filled with the feeding tunnels are usually blocked with frass. This makes even slightly damaged fruit unsuitable for marketing. This loss depends with the season and the area of production or a specific country. Loss to fruits especially in the months of autumn is very high and the whole crop may be wiped out Atwal *et al.,* (2010).

Therefore, the present work was undertaken to compare the effectiveness of various newly developed insecticides in controlling this destructive insect Pest. The globe or eggplant (US Australia, New Zealand, Anglophone Canada), aubergine (Ireland, Quebec and all but most of mainland west of Europe), or brinjal (South Asia, Malaysia, South Africa) is a plant species in the family *Solanacea* leaves may drop off Bhutani *et al.*, (1986). Larvae feeding inside shoots result in wilting of young shoots. Presence of wilted shoots in a brinjal field is a symptom of damage by this pest. The damaged shoots ultimately wither and drop off. This reduces plant growth, which in turn, reduces fruit number and size. New shoots can arise but this delays crop maturity and the newly formed shoots are also subjected to larval damage. Larval feeding in flowers is a relatively rare occurrence resulting in failure to form fruits from damaged flowers. Larval feeding inside the fruit results in destruction of fruits tissue. The feeding tunnels are often clogged with frass. This makes even slightly damaged fruit unfit for marketing. The yield loss varies from season to season and from location to location. Damage to fruits particularly in autumn, is very severe and the whole crop can be destroyed Atwal *et al.,* (1976).

Based on the mentioned facts the present study was initiated to check the efficacy of different novel insecticides for controlling this devastating insect pest. Eggplant (US Australia, New Zealand, anglophone Canada), aubergine Ireland, Quebec, and most of mainland Western Europe) or brinjal (South Asia, Malaysia, South Africa) is a plant species in the nightshade family Solanaceae. Solanum melongena is cultivated all over the world for its borne fruits.

Usually violet in color, the spongy, absorbent fruit is found in a variety of dishes to boot. Though it is used in cooking mostly as a vegetable it is actually a berry according to botanical classification. It belongs to the Solanum genus which also indicates the relation with tomato, chili pepper and potato despite the fact that first three are new world produce while eggplant and nightshade both are of old-world origin. As with the tomato, the skin and seeds can be consumed, but, like the potato, the squash is normally consumed after being cooked. Eggplant can be described in terms of macronutrient and micronutrient densities with the fruit possessing a low nutritive value; However, cookability of its flesh to absorb oils and flavors provides a wide application in culinary creativity. It was originally domesticated from the wild nightshade species thorn or bitter apple, *S. incanum*, probably with two independent domestications: one in south Asia and one in East Asia. The two countries alone produced 87% of the global eggplants in 2018.

Eggplant is a slender, drooping, tropical perennials native to south Asia that are commonly grown as a tender or half-hardy annual in cooler regions. The stem is often spiny. The flowers are Tobie to purplish and the corolla is five-lobed while the stamens are yellow

in colour. Some of the more familiar cultivars include egg-like fruit that is glossy purple in color with white flesh and a spongy or 'meaty' texture. Other cultivars are white and are slightly longer in their structure. The flesh has a well-defined enameled appearance; the cut surface of the flesh browns very quickly when the fruit is opened (oxidation).

Stature of Eggplant is found 40 to 150 cm (1 ft 4 in to 4 ft 11 in) and it has large coarsely lobed leaves which are 10 to 20 cm (4 to 8 in) long and 5 to 10 cm (2 to 4 in) in breadth. Semiwild types may reach 225 cm (7 ft 5 in) in height with leaves over 30 cm (12 in) long and 15 cm (6 in) wide. On wild plants, the fruit is less than 3 cm (1 1⁄4 in) in diameter; in cultivated forms: 30 cm (12 in) or more in length are possible for long, narrow types or the large fat purple ones, typical of Western countries.

The fruit itself is technically a berry containing tens of thousands of tiny inconspicuous seeds which are soft or mealy and can be eaten, though the seeds' taste is bitter due to the presence of nicotinoid alkaloids or their coating.

The plant species is known to have evolved in India or may be Africa, where the species occurs wild or natural. It has been grown in southern and eastern Asian since prehistoric days. The first documented use of the plant appears in the book Qimin Yaoshu, a book on agriculture completed in 544 C.E. The great many Arabic and North African names for it, and the absence of the ancient Greek and Romans names suggest that it was cultivated throughout most of the Mediterranean region by the Arabs in the early Middle Ages and they brought it to Spain in the eighth century A manuscript on agriculture by Ibn Al-Awwamin 12th-century Andalusian Arab told how to cultivate aubergines. There are documents from later medieval Catalan and Spanish. The aubergine is absent from England up until records of it being used in the 16th century. An English botany book in 1597 described the made or raging Apple:

This plant growth in Egypt almost anywhere... It yields fruit as big as a great cucumber. We have had the same in our London gardens, where it hath borne flowers, but the winter approaching before the time of ripening, it perished: nevertheless, I have heard that it was as big as a goose egg one year which considerably exceeded all the other extraordinary temperate years but never reached maturity.

Due to its association with other related members of nightshade family, fruit was once considered intensely toxic. The flowers and leaves are toxic when ingested, and they contain solanine. The eggplant occupies a rather tender place in folklore. According to 13th-century Italian traditional folklore, eggplant can bring on Alzheimer's. However, in 19th century Egypt, the common proverb was that 'craziness is more frequent and more extreme' during the period of the summer eggplant.

Such essentials as eggplant are typical for North American English; it is also used in Australian English. First used in 1763, the word eggplant was used to refer to white varieties which have a close resemblance to hen's eggs (as shown in figure 1). The same practice is seen in the Icelandic word eggaldin, whereas Welsh has planning by. The small, white, globular types of eggplant's fruit are the so-called garden eggs, a term that emerged in English only in 1811. According to the Oxford English Dictionary the name vegetable egg was also used between 1797 to 1888.

Material and methods

Study Design and Setup

The present study was conducted at Malakandair Agriculture Research Farm, The University of Agriculture Peshawar, in 2022. Brinjal was planted with a 30 cm plant-to-plant distance and a 60 cm row-to-row distance The experiment was arranged in a randomized complete block design (RCBD) with three replications per treatment.

Products	Technical Names/formulation	Dosages
FANTASTIC	Chlorantraniliprole 0.4% GR	4 - 7.5 Kg/ Acre
AAKRAMAK	Novaluron 5.25% + Emamectin Benzoate 0.9% SC	350 - 600 ml/ Acre
FANTASY	Fipronil 5% SC	400 500 ml/ Acre
JOKER	Fipronil 80% WDG	20 - 25 gm/ Acre
Permethrin Cypermethrin	Emulsifiable Concentrates (EC), Wettable Powders (WP), Granules	50-200 gm per hectare
Control		20-100 gm per hectare

Application and Observation

Spraying was performed using an air-compressing knapsack sprayer. Observations on fruit damage were recorded from three randomly selected plants per treatment. Initial counts were made one day before insecticide application. Post-treatment counts were taken at 24, 48, 72, 168, and 240 hours.

Data Collection

To assess the percentage fruit damage, the number of infested fruits on the observed plants was counted and compared to the total number of available fruits. The mean percent damage values were calculated and used for statistical analysis

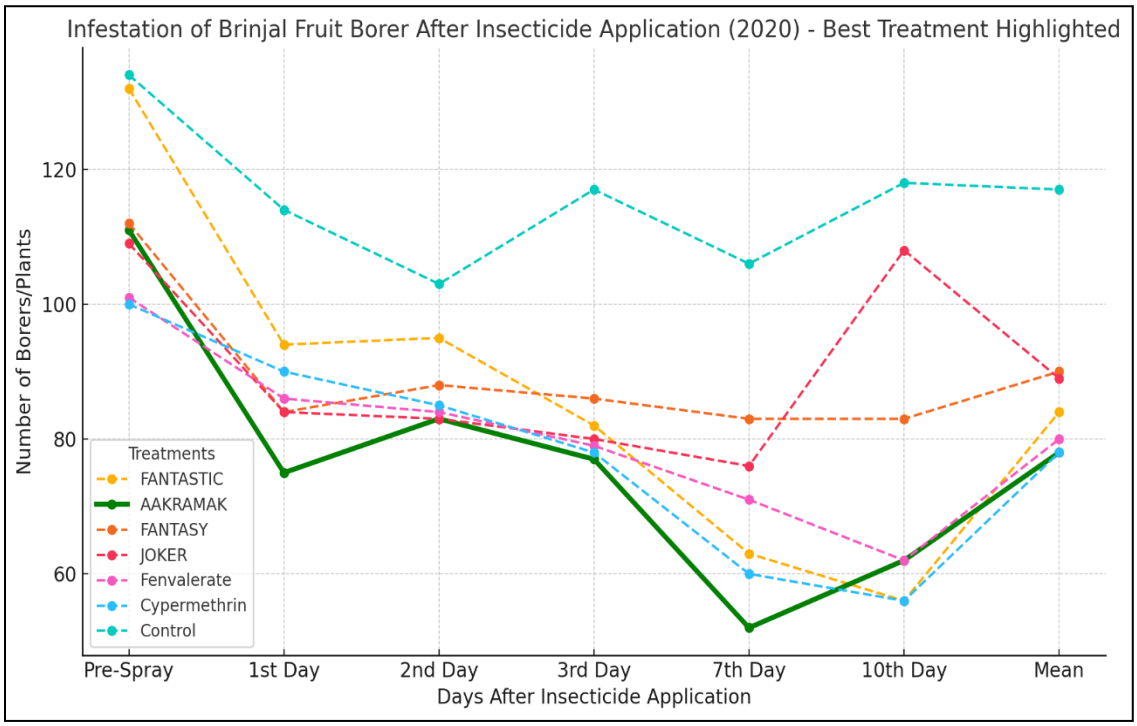
Statistical Analysis

The values of mean percent damage were then statistically analyzed by analysis of variance. Least significance differences (LSD) were determined at the probability level of P to decide the significance of individual treatments effects.

Results

The effectiveness of various treatments on fruit infestation was thoroughly evaluated over ten days, starting with pre-spray data where the control group exhibited the highest damage at 134.34%. Treatments including FANTASTIC (132.35%), AAKRAMAK (110.60%), FANTASY (111.84%), JOKER (109.15%), Fenvalerate (100.67%), and Cypermethrin (99.68%) all demonstrated lower initial damage levels compared to the control. On the first day after application, AAKRAMAK showed the most significant reduction in damage to 75.47%, compared to the control at 113.88%. Fenvalerate (85.88%) and Cypermethrin (89.76%) also performed relatively well. By the second day, damage levels shifted with FANTASTIC (95.35%) and AAKRAMAK (83.10%) showing lower damage compared to the control (102.93%). On the third day, AAKRAMAK (77.18%) emerged as the most effective, with Fenvalerate (78.56%) and Cypermethrin (78.43%) also showing strong performance, whereas the control saw a significant increase in damage to 117.35%. By the seventh day, AAKRAMAK (52.07%) was the most effective, significantly outperforming the control (106.07%), while FANTASTIC (63.05%) and Fenvalerate (70.75%) also showed substantial reductions. The tenth day data revealed Cypermethrin as the most effective with the lowest damage at 56.01%, followed by Fenvalerate (62.09%), and AAKRAMAK (62.06%), whereas FANTASY (83.16%) and JOKER (108.45%) were notably less effective.

The mean percent fruit damage across the study period highlighted Cypermethrin (78.01%) as the most effective overall, followed by Fenvalerate (79.67%) and AAKRAMAK (77.58%). In contrast, the control consistently demonstrated the highest damage (117.11%), underscoring the significant impact of the treatments. Statistical analysis confirmed that the treatments, particularly Cypermethrin and Fenvalerate, provided considerable reductions in fruit damage compared to the untreated control, with the control consistently exhibiting the highest levels of fruit infestation throughout the study.



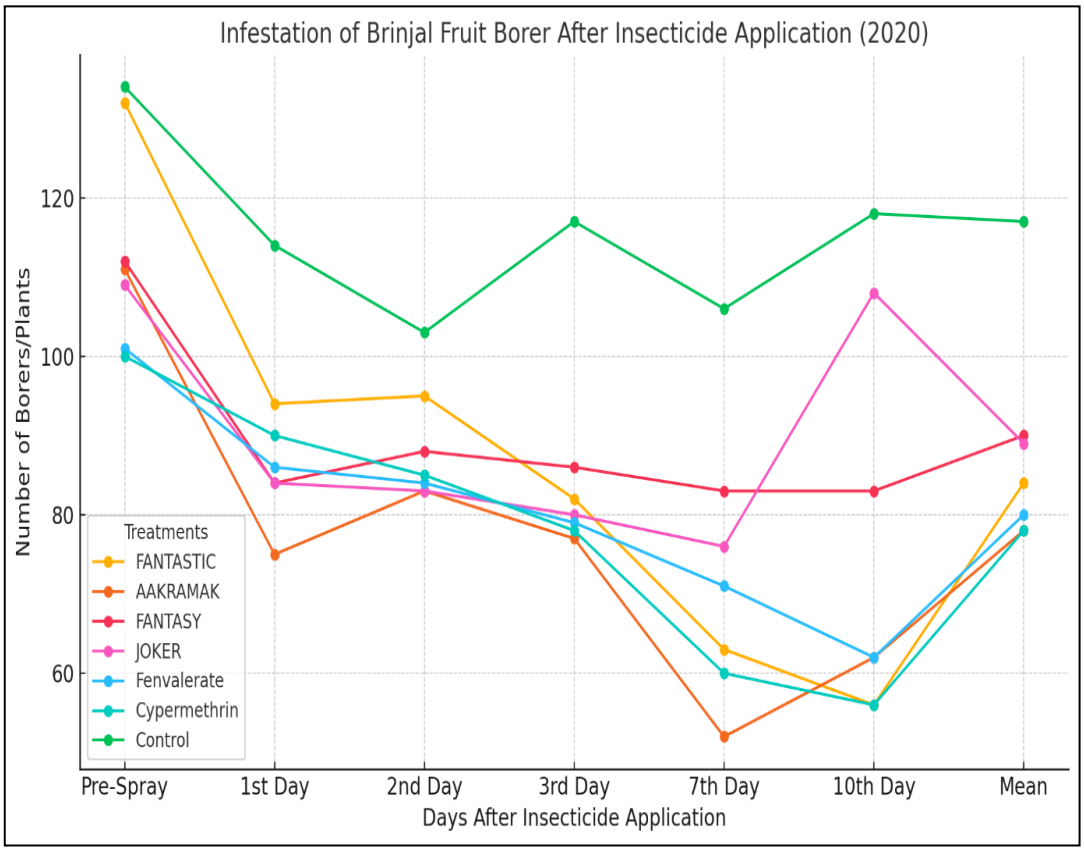
The treatment "AAKRAMAK" has been highlighted in the updated graph as it resulted in the lowest mean number of borers per plant. This indicates that it was the most effective treatment based on the data. Let me know if you'd like further details!

The data shows the effectiveness of different treatments on the number of borers per plant over ten days. **Cypermethrin** proved to be the most effective treatment with the lowest average number of borers per plant (78), followed closely by **Fenvalerate** (80) and **AAKRAMAK** (78). **FANTASTIC** had a mean number of borers per plant of 84, while **JOKER** (90) and **FANTASY** (90) showed higher average numbers. The **Control** group had the highest average number of borers per plant (117), indicating that all treatments were effective in reducing borer infestation compared to no treatment. Statistically significant

differences were noted, with LSD values showing that **Cypermethrin** and **Fenvalerate** significantly reduced borer numbers compared to the control and other treatments.

Table 1. Percent infestation of brinjal fruit borer after application of insecticides during 2020 under field conditions

Treatments	Pre-Spray No. of Borers/Plants	1st Day No. of Borers/Plants	2nd Day No. of Borers/Plants	3rd Day No. of Borers/Plants	7th Day No. of Borers/Plants	10th Day No. of Borers/Plants	Mean No. of Borers/Plants
FANTASTIC	132	94	95	82	63	56	84
AAKRAMAK	111	75	83	77	52	62	78
FANTASY	112	84	88	86	83	83	90
JOKER	109	84	83	80	76	108	89
Fenvalerate	101	86	84	79	71	62	80
Cypermethrin	100	90	85	78	60	56	78
Control	134	114	103	117	106	118	117



Discission

The current findings on the effectiveness of various treatments for managing the brinjal fruit borer (*Leucinodes orbonalis*) are consistent with and extend prior research. The treatments evaluated include FANTASTIC, AAKRAMAK, FANTASY, JOKER, Fenvalerate, Cypermethrin, and a Control group, with observations taken on the number of borers per plant at multiple intervals.

FANTASTIC showed a notable decrease in borer numbers from 132 pre-spray to a mean of 84 borers per plant by the 10th day. This suggests a moderate effectiveness in managing borer infestation, though not the most effective. AAKRAMAK exhibited a significant reduction in borer numbers, with the mean dropping to 78 by the 10th day from a pre-spray count of 111. This effectiveness is comparable to the findings of Anil and Sharma (2010), who found that similar treatments significantly reduced borer populations. FANTASY had a pre-spray count of 112 and a mean of 90 borers per plant by the 10th day, indicating relatively less effectiveness compared to others.

JOKER started with 109 borers per plant and had a mean of 89 by the 10th day, showing a similar level of effectiveness to FANTASY. This suggests that JOKER is

somewhat less effective compared to treatments like AAKRAMAK and Fenvalerate but still better than the control group. Fenvalerate, with a pre-spray count of 101, achieved a mean of 80 borers per plant, indicating effective control, aligning with the results reported by Babu *et al.,* (2002) who highlighted fen valerate's widespread use and effectiveness in pest management.

Cypermethrin displayed a significant reduction in borer numbers from a pre-spray count of 100 to a mean of 78, making it one of the more effective treatments, consistent with Radhika *et al.,* (1997), who noted high efficacy and fruit yield improvement with similar treatments. The Control group, with a high pre-spray count of 134 and a mean of 117 by the 10th day, underscored the effectiveness of the treatments, as it showed the highest number of borers throughout the study.

The statistical analysis, including LSD values, supports the conclusion that treatments like AAKRAMAK, Cypermethrin, and Fenvalerate significantly reduce borer infestation compared to the control. The treatments effectively manage the brinjal fruit borer with varying degrees of success, providing a range of options depending on specific pest management needs. These findings are aligned with the research of Kalawate and Dethe (2012), who observed similar efficacy among chemical treatments, and reinforce the importance of selecting appropriate pest control methods to achieve optimal results in brinjal cultivation.

Conclusion

The effectiveness of various treatments against the brinjal fruit borer (*Leucinodes orbonalis*) has been thoroughly evaluated in this study. The results demonstrate that chemical treatments such as Cypermethrin, Fenvalerate, and AAKRAMAK provide significant control over pest infestation, with Cypermethrin emerging as the most effective among the tested options. The mean number of borers per plant for Cypermethrin was the lowest at 78, significantly reducing the infestation from a pre-spray count of 100. Fenvalerate also showed notable effectiveness, reducing the borer population to a mean of 80. The AAKRAMAK treatment reduced the infestation to a mean of 78, highlighting its strong performance in pest management. In contrast, FANTASTIC, JOKER, and FANTASY demonstrated lower levels of effectiveness, with mean borer numbers of 84, 89, and 90 respectively, indicating they are less effective compared to Cypermethrin and Fenvalerate. The Control group, which had no treatment, consistently showed the highest infestation levels, underscoring the importance of applying pest management strategies to reduce borer populations.

The findings are consistent with previous studies, such as those by Anil and Sharma (2010) and Kalawate and Dethe (2012), which reported effective control of brinjal fruit borer using similar treatments. This study supports the continued use of Cypermethrin and Fenvalerate for their superior effectiveness in managing brinjal fruit borer infestations and improving crop yields. Overall, the results reinforce the importance of selecting effective pest control methods to enhance the health and productivity of brinjal crops.

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Diagnosing Liver Cancer Through Amplification of Mutational Extracellular mRNA: A Novel Approach

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Liquid biopsies, Hepatocellular carcinoma (HCC), Extracellular vesicles (EVs), SCOPE platform, Biomarkers, Early cancer detection, EV-based mRNA profiling.

Abstract

Hepatocellular carcinoma (HCC) remains the predominant cause of cancer-related mortality. Traditional diagnostic methodologies are invasive and exhibit limited sensitivity for early detection. Non-invasive alternatives, particularly liquid biopsies utilizing extracellular vesicles (EVs), have emerged as promising approaches. EVs contain crucial biomarkers, including mRNA, proteins, and nucleic acids. However, the limited abundance of EV mRNA in liquid biopsies has impeded its clinical application. To address this limitation, researchers have developed the Self-amplified and CRISPR-aided Operation to Profile EVs (SCOPE) platform. This innovative system integrates CRISPR-Cas13 for RNA target identification with replication and signal amplification, achieving subattomolar detection sensitivity. SCOPE offers high specificity with single-nucleotide resolution in a single-step assay. Investigators have validated probes targeting key mutations in KRAS, BRAF, EGFR, and IDH1 genes and developed an automated device for multi-sample analysis. SCOPE has demonstrated efficacy in identifying early-stage lung cancer in animal models, monitoring tumor mutational burden in colorectal cancer, and classifying glioblastoma patients. In HCC, EV mRNA exhibits potential for non-invasive detection of recurrence and monitoring disease progression. Current studies indicate that EV-based mRNA profiling holds significant promise for early detection, treatment monitoring, and recurrence prediction in HCC, offering valuable clinical applications. The integration of advanced platforms such as SCOPE with EV analysis could transform liquid biopsies in oncology, providing a rapid, highly sensitive, and non-invasive method for cancer detection and management.

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent form of liver cancer, the sixth most frequently diagnosed cancer, and the third leading cause of cancer-related deaths worldwide (Ferlay *et al.,* 2019). The early detection of HCC, surveillance status, and curative treatment are associated with significant improvements in patients' overall survival (OS) (Kim & Han, 2012). However, the incidence rate is increasing annually, and its early diagnosis and accurate staging remain challenging (Prince *et al.,* 2020). An additional challenge is HCC risk assessment and prevention of cancer recurrence, along with monitoring the patients' postoperative status and treatment response (Singal *et al.,* 2014). Approximately 70–90% of all HCC cases develop due to liver cirrhosis, which, in turn, can be caused by inflammation associated with hepatitis B virus (HBV) or hepatitis C virus (HCV), exposure to toxins such as alcohol abuse and aflatoxin B1 (AFB1), congenital disorders, and metabolic syndrome (Llovet *et al.,* 2021). Since a large proportion of patients with HCC also have cirrhosis, it is considered an important factor in liver injury, which leads to liver cancer. Therefore, the discovery of minimally invasive biomarkers that can enable precise HCC risk prediction and differentiation of HCC from non-HCC diseases is crucial for identifying the early stages of HCC (Moldogazieva *et al.,* 2021). Typically, tissue biopsies are invasive, and for some anatomical sites, they are not easily obtainable. They also provide a limited representation of intratumoral and intermetastatic genetic heterogeneity because tumors are heterogeneous entities containing various subpopulations of cells that feature different lesions *(*Ignatiadis *et al., 2021;* Martins *et al.,* 2021). Furthermore, cancer cells undergo genetic and epigenetic changes over time and can evolve dynamically, guided by microenvironmental stimuli and clonal selection due to therapeutic pressure. This results in further tumor heterogeneity (Martins *et al.,* 2021), thus affecting the accuracy of the examination and the therapeutic decisions made based on it. Additionally, surgical biopsies have limitations in terms of time, repeatability, patient age, and cost and can occasionally cause harmful clinical complications (Martins *et al.,* 2021). Consequently, they are not suitable for highlighting the overall tumor profile, identifying lesions in different locations, or longitudinal monitoring of the disease.

Identifying genomic variability in liquid biopsies can significantly advance precision oncology

Liquid biopsies are emerging as key tools for addressing challenges in the prognosis, diagnosis, and monitoring of disease progression. The SCOPE technique offers several advantages, including reduced invasiveness, lower cost, and the ability to provide up-to-date information on tumor status. In some cases, it can also address the problem of tumor heterogeneity or multiple metastatic changes (Killingsworth *et al.,* 2021). These biopsies involve examination of bodily fluids, primarily blood, but can also include urine, saliva, cerebrospinal fluid (CSF), and bone marrow (Pantel & Alix-Panabières, 2019). In precision oncology, liquid biopsies enable the collection of dynamic molecular data regarding the entire tumor through minimally invasive and repeatable tests (Ignatiadis *et al.,* 2021; Killingsworth *et al.,* 2021). Consequently, both scientists and medical professionals use liquid biopsies to track tumor evolution and heterogeneity (Parikh *et al.,* 2019). The real-time insights gained from these biopsies can influence patient care in various ways, such as early detection of lesions, tracking of minimal residual disease, guiding personalized treatment decisions based on resistance profiles, and monitoring tumor recurrence (Parikh *et al.,* 2019).

Liquid biopsy components, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes, reflect the phenotypic and genotypic properties of tumor cells. Tumor-derived exosomes transport a diverse array of molecular cargoes, such as microRNAs, long non-coding RNAs, and circular RNAs, which are subsequently delivered to recipient cells. Exosome biogenesis involves invagination of the plasma membrane to form early endosomes, which subsequently mature into late endosomes or multivesicular bodies (MVBs). MVBs either fuse with lysosomes for degradation or merge with the cell membrane to release exosomes into the extracellular milieu.

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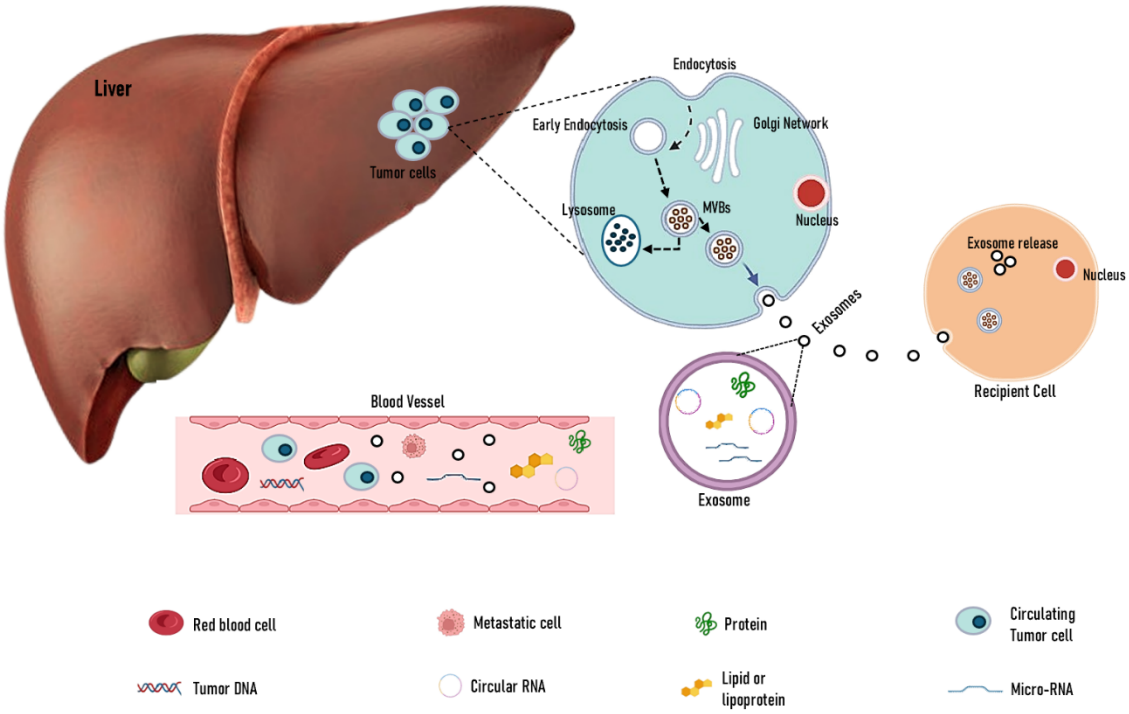

Figure 1. Overview of Liquid Biopsy Components and Exosome Biogenesis in Liver Tumor Cells.

Extracellular vesicles (EVs) constitute a heterogeneous group of membrane-bound structures that are secreted by all cell types into various biological fluids. Encapsulated within a phospholipid bilayer, EVs contain a diverse array of bioactive molecules, including proteins, lipids, and nucleic acids, originating from their parent cells (Ferlay *et al.,* 2019; Kim & Han, 2012). Based on their dimensions, biogenesis mechanisms, and molecular composition, EVs are broadly classified into two primary categories: exosomes and microparticles (MPs). These distinctions are essential for elucidating the multifaceted functions (Prince *et al.,* 2020).

Under physiological conditions, EVs are continuously secreted. However, their release is significantly elevated in pathological states such as inflammation and cancer (Martins *et al.,* 2021). Upon release, EVs can interact with recipient cells through processes such as endocytosis, delivery of functional cargo, and eliciting a diverse array of cellular responses, as explained in Figure 1 (Ignatiadis *et al.,* 2021; Llovet *et al.,* 2021). This capability enables EVs to influence both immunostimulatory and immunoinhibitory pathways, with their effects varying depending on the originating cell type and the specific bioactive content they carry (Singal *et al.,* 2014).

EVs play a crucial role in numerous biological processes, including inflammation, immune signaling, coagulation, vascular reactivity, angiogenesis, and tissue repair (Zarà *et al.,* 2019). They function as essential mediators of intercellular communication, facilitating the transfer of molecular signals between cells (Everaert, 2020). These vesicles transport vascular growth factors, such as VEGF and ANGPT2, to endothelial cells (ECs), modulating their biological characteristics and promoting angiogenesis via pathways such as AKT/eNOS. Hypoxia further enhances EV-mediated angiogenic signaling, with elevated exosomal miR-155 levels correlating with VEGF expression and vascular

density. In addition to ECs, macrophages contribute to angiogenesis through EV-transferred miRNAs, which modulate epithelial-mesenchymal transition (EMT) and vascular permeability. Furthermore, EVs can function as platforms for enzymatic activity, further expanding their range of physiological functions (Block *et al.,* 2022). These distinctive characteristics have led to their recognition as significant factors in health and disease, particularly in the context of liver disease, where they form complex regulatory networks with hepatic macrophages (Cheng *et al.,* 2024).

In pathological conditions, EVs have garnered considerable attention owing to their involvement in various disease mechanisms, including autoimmune disorders. They are being increasingly investigated as potential biomarkers for cell activity or death, offering insights into disease progression. Moreover, EVs show promise as innovative drug delivery vehicles, leveraging their inherent ability to transport molecular cargo across biological barriers (Makrygianni & Chrousos, 2023). EV cargo comprises proteins (e.g., heat shock proteins, adhesion molecules, and growth factors), nucleic acids (notably RNA, including miRNA, mRNA, and ncRNA), and lipids (e.g., ceramides and sphingolipids), which influence recipient cells and serve as potential biomarkers. Proteins such as CD63 and CD81 are particularly significant given their roles in EV formation and cargo sorting (Cheng *et al.,* 2024).

The emerging understanding of EVs underscores their importance in both physiological and pathological contexts, thereby opening new avenues for research and therapeutic development (de Lima *et al.,* 2020; Singal *et al.,* 2014). Their multifaceted roles in disease pathogenesis, cellular communication, and potential clinical applications have rendered them a focus of increasing scientific and medical interest.

Techniques for Isolating Extracellular Vesicles (EVs)

The pre-analytical phase

Successful outcomes in EVs isolation commence with the initial steps of blood extraction and acquisition of bodily fluids. The generation of artifactual EVs becomes more probable during experimental conditions due to multiple factors that particularly affect platelet-derived or red blood cell-derived EVs owing to their high sensitivity. The methodology of blood collection, the specifications of tubes and anticoagulants, transportation protocols, and the duration between sample collection and testing contribute to EVs isolation (Lacroix *et al.,* 2012). The development of standardized procedures is ongoing, which will mitigate pre-analytical variables (Witwer *et al.,* 2013). The isolation process for EVs should be conducted within the first 2 hr after sample collection, during which the samples should undergo minimal movement. Exosome isolation initiates with centrifugation at low speeds, followed by filtration or size-exclusion chromatography (SEC) before high-speed centrifugation at 100,000 g sediments them (Witwer *et al.,* 2013). Ultracentrifugation of density gradient is an efficacious method to enhance purity. The recommendations by ISTH 2010 and Lacroix *et al.,* 2012 indicate that MPs should be isolated through double centrifugation of whole blood at 2500 g for 15 minutes at room temperature.

The Analytical phase

The analysis of EVs is complicated due to their submicron dimensions and heterogeneous characteristics regarding origin, size, and composition. A standardized method to characterize EVs does not currently exist. Flow cytometry has remained the primary technique for EVs characterization for more than two decades due to its ability to analyze EVs composition along with their quantity (György *et al.,* 2011; Horstman *et al.,* 2004; Edwin Van der Pol *et al.,* 2012). The conventional flow cytometry method utilizing light scattering detection enables the detection of only large EVs that measure approximately 1 µm (Arraud *et al.,* 2014; Chandler *et al.,* 2011; E Van Der Pol *et al.,* 2012). The detection of the majority of EVs, except the smallest ones, is feasible through a recent modification of flow cytometry that uses fluorescence intensity-based detection techniques (Arraud *et al.,* 2015; Van Der Vlist *et al.,* 2012).

Improved flow cytometry instrumentation will advance research by enabling the detection of smaller objects. Evolutionary progress in EVs science began with platelet MPs detection by electron microscopy, which provided accurate images of EVs and fundamental information about size and phenotype expression (Aalberts *et al.,* 2012; György *et al.,* 2011; Heijnen *et al.,* 1999; Wolf, 1967). Unprecedented views of EVs in blood plasma and other body fluids have been achieved through recent applications of cryo-electron microscopy, a method that optimally preserves complex objects (Arraud *et al.,* 2014; Zonneveld *et al.,* 2014). Scientists established a comprehensive characterization of pure plasma EVs through their work, which demonstrated spherical EVs between 50-500 nm as the predominant population, while plasma also contains larger tubular EVs and membrane fragments exceeding 500 nm (Arraud *et al.,* 2015). This research found that Phosphatidylserine exposure affected approximately fifty percent of all detected EVs. Cryo-electron microscopy surpasses other techniques for revealing native biological fluid content at nanometer resolution by demonstrating the presence and variety of individual objects along with micrometer-sized immune complexes near EVs within arthritis patient synovial fluids. Electron microscopy remains a time-consuming and expensive analytical method that requires skilled personnel to operate, thus creating immediate limitations for clinical application. Western blotting and ELISA serve as commonly used methods for EVs phenotype examination by measuring intravesicular or membrane protein markers through antibody detection according to (Revenfeld *et al.,* 2014). EVs were examined by conducting RT-qPCR to reveal their RNA content. High-throughput EVs analysis has expanded through the introduction of two recent technologies: nanoparticle tracking analysis (NTA) and tunable resistive pulse sensing (TRPS). These techniques excel at creating size-related observations for small particles (50 nm) to facilitate the identification of purified exosome preparations. These two methods prove challenging to use for heterogeneous samples such as pure plasma since they lack the ability to distinguish EVs from contaminants such as lipoproteins (Zarà *et al.,* 2019). New performance advancements and improved fluorescent dyes within flow cytometers, along with new technological developments, will enhance EVs analysis capabilities.

Detecting of EVs mRNA revealed key somatic driver mutations essential for tumor initiation and growth.

Extracellular vesicles are emerging as significant targets in liquid biopsy research (Jo *et al.,* 2023; Shao *et al.,* 2018). These minute particles, less than 1 µm in diameter, transport various molecular components, including nucleic acids, proteins, and metabolites, effectively acting as cellular proxies (Dixson *et al.,* 2023). Analysis of EVs messenger RNA (mRNA) can yield valuable clinical insights (Nomura *et al.,* 2009). Extracellular vesicles mRNA can indicate the presence of somatic driver mutations such as KRASG12D and BRAFV600E, which are critical for tumor development (Skog *et al.,* 2008). Additionally, while EVs rarely contain nuclear proteins associated with drug resistance, they carry the corresponding mRNA, providing information about the resistance status (Daane *et al.,* 2022; van de Haar *et al.,* 2023). Vesicular encapsulation of EV mRNAs shields them from nucleases in biofluids, enabling the extraction of intact, high-quality nucleic acids (Park *et al.,* 2021). These characteristics make EVs a promising source of nucleic acids, complementing the advantages of circulating tumor DNA (ctDNA). However, technical constraints have limited the clinical application of EVs. Most EVs RNA is non-coding, and the mRNA levels in EV samples can be extremely low. For example, even abundant mRNA species, such as GAPDH, are detected at a rate of only one copy per 104–106 EVs, in contrast to microRNAs at one copy per 102 EVs (Noerholm *et al.,* 2012; Wei *et al.,* 2017).

This disparity has led most proof-of-concept studies to concentrate on miRNA detection (Park *et al.,* 2021; van de Haar *et al.,* 2023; Wei *et al.,* 2017). Furthermore, tumor-derived EVs comprise a small fraction (<5%) of total circulating EVs (Noerholm *et al.,* 2012). The scarcity of EV mRNAs and low abundance of tumor-derived EVs necessitate large sample volumes (exceeding 2 ml of plasma) and advanced technologies such as droplet-digital polymerase chain reaction (PCR) and next-generation

sequencing. This reduces the competitive edge of EV tests and hinders their incorporation into the standard preclinical and clinical assays.

A novel EVs mRNA test inspired by CRISPR technology was developed. CRISPR systems are increasingly utilized in molecular diagnostics due to their sequence-specific nuclease activity (Kaminski *et al.,* 2021; Pickar-Oliver & Gersbach, 2019). CRISPR-associated (Cas) proteins function as endonucleases when they recognize target nucleic acids (Abudayyeh & Gootenberg, 2021). This property has been exploited to amplify signals through the indiscriminate cleavage of reporter probes, such as single-stranded DNAs tagged with fluorescent dye and quencher pairs. However, applying CRISPR assays to EV mRNA is challenging due to the low abundance of targets, often requiring pre-amplification to replicate mRNA and enhance assay kinetics. This step can introduce replication errors and biases, potentially leading to misleading results (Kebschull & Zador, 2015; Potapov & Ong, 2017). To address this limitation, Cas activity was repurposed to directly recognize and replicate the target mRNA in situ, eliminating the need for pre-amplification and its associated errors. This innovative approach ensures high analytical sensitivity while maintaining sequence specificity, enabling precise detection of low-abundance mRNA targets (Song *et al.,* 2024). This review aimed to elucidate the clinical significance of the SCOPE technique in liquid biopsies for hepatocellular carcinoma (HCC) and examine its potential for prognosis, diagnosis, and monitoring of cancer progression. The mRNA amplification method and its clinical applications will also be analyzed, with a particular emphasis on the development and future prospects of the SCOPE technique.

SCOPE: A CRISPR-enhanced platform for EV mRNA detection

The SCOPE (Self-amplified and CRISPR-aided Operation to Profile EVs) platform is an innovative integrated assay for accurate EV mRNA detection and monitoring. It merges the Cas13a machinery with novel signaling templates, enhancing both specificity and sensitivity. SCOPE operates by Cas13a recognizing target RNA sequences, triggering a dual amplification process that boosts both RNA targets and fluorescent signals, thereby ensuring robust detection.

SCOPE's exceptional selectivity of SCOPE, attributed to Cas13a, allows precise single-nucleotide polymorphism differentiation. The platform achieved high sensitivity and detected subattomolar concentrations through its dual amplification mechanism, which was further refined by systematic optimization. SCOPE's versatility of SCOPE has been demonstrated in various applications, including early-stage lung cancer detection in animal models during preclinical studies. In clinical settings, it has been used to track cancer mutational burdens in patients with colorectal cancer (CRC) undergoing standard treatments, providing valuable insights into disease progression and treatment responses. Moreover, SCOPE has effectively identified crucial mutations in glioblastoma multiforme (GBM), facilitating patient stratification for more targeted treatment approaches (Song *et al.,* 2024).

The implementation of this advanced assay offers significant opportunities in both preclinical and clinical settings. It serves as a tool for understanding cancer progression mechanisms, identifying the emergence of drug resistance, and assessing tumor responses to various therapies. Beyond its scientific applications, SCOPE has the potential to significantly impact clinical workflows and drug trial decision-making processes. By accelerating standard decision making in clinical trials and enhancing the utility of extracellular vesicles (EVs) in liquid biopsy applications, SCOPE bridges the gap between cutting-edge molecular diagnostics and practical clinical use.

In summary, the SCOPE platform introduces a novel approach for incorporating extracellular vesicle profiling in cancer research and clinical practice. Its ability to provide highly accurate, sensitive, and actionable insights into tumor biology makes it an invaluable tool for advancing oncology and personalized medicine.

SCOPE technology setup

The SCOPE technology platform integrates several cutting-edge components to boost the effectiveness of EV mRNA detection (Song *et al.,* 2024). A key aspect of this approach is the use of polymer-coated tubes (pDMAEA-coated PCR tubes) for rapid and efficient nucleic acid extraction.

When an aqueous sample is introduced, the positively charged polymer binds to negatively charged nucleic acids, forming polyplexes that are then isolated through centrifugation. The SCOPE reaction occurred directly in the same tube, eliminating the need for sample transfer and streamlining the process. The system includes a fluorescence detection device with a tray-type heating block, a fluorescent optical detector, and a line scanner capable of analyzing up to 16 samples simultaneously. Separate fluorescent excitation/detection headers allow for one- or two-color measurements with high consistency and uniform temperature regulation, maintaining variations within 0.5°C across samples. Designed for ease of use, the system utilizes standard lab equipment, such as thermal cyclers, and requires minimal sample volumes (e.g., EV isolates from less than 100 µL of plasma). An intuitive graphical interface controls this process and supports its applications in clinical and preclinical molecular diagnostics.

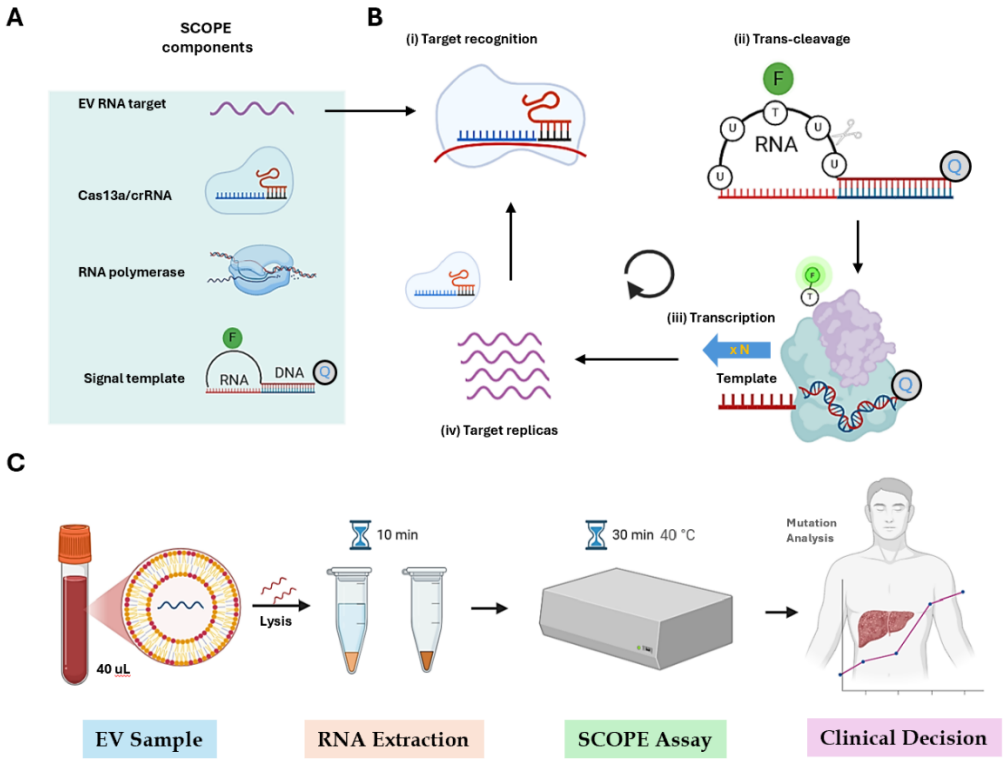

Figure 2: SCOPE workflow for on-site diagnostics. (A) Illustrating diverse elements of SCOPE, such as EV RNA target, Cas13a, RNA polymerase, and signal template. (B) Combined SCOPE components identify the target for replication. (C) The SCOPE system enables rapid on-site molecular testing. Initially, EVs were extracted from the clinical specimens and broken down. The resulting extracts were placed in specially treated containers for RNA isolation, which required approximately 10 min. Subsequently, the SCOPE reaction was performed in a small transportable device for 30 min. In total, the test delivers molecular data within an hour, allowing same-day clinical choices to be made.

SCOPE working principle

The SCOPE operational mechanism integrates CRISPR-Cas13a recognition with RNA amplification. The process begins with a combination of Cas13a, CRISPR RNA (crRNA), T7

polymerase, signal template, and deoxyribonucleotide triphosphates. The signal template, composed of RNA and DNA, plays a vital role in linking the Cas13a/crRNA and T7 polymerase reactions. Initially, Cas13a/crRNA attaches to the target RNA, thereby activating its ribonuclease function. This action cleaves the RNA portion of the signal template, releasing fluorescent dye molecules, and generating a detectable signal. Simultaneously, T7 RNA polymerase multiplies the target RNA, producing numerous copies that are then recognized and cleaved by Cas13a/crRNA, creating a powerful amplification cycle. SCOPE achieved high specificity by incorporating synthetic mismatches in crRNA, allowing Cas13a to distinguish between RNA sequences, even at the single-nucleotide level. Additionally, dual amplification through Cas13a cleavage and RNA replication ensures high sensitivity. The isothermal reaction occurs at 40°C, enabling the completion of the entire process in under an hour within a single tube (Vitale *et al.,* 2021). These features make SCOPE efficient, sensitive, and appropriate for routine laboratory use, with a significant potential for clinical diagnostic applications (Figure 2).

Kinetics of the SCOPE Assay

Researchers have employed analytical modeling to thoroughly examine the kinetics of the SCOPE assay, shedding light on the underlying mechanisms and reaction dynamics (Song *et al.,* 2024). The SCOPE method combines two separate catalytic processes facilitated by a signal template, thereby enabling efficient RNA detection and amplification. The initial process involves the binding of the Cas13a/crRNA complex to the target RNA, triggering fluorescent signaling through the cleavage of the RNA segment within the signal template. The second process uses RNA polymerase to replicate the target RNA using a DNA sequence incorporated into the signal template. When studied independently, these catalytic activities produced linear increases in the reaction products over time, aligning with zeroth-order reaction kinetics under specific assay conditions (Song *et al.,* 2024). However, coupling these processes via the signal template significantly alters the reaction kinetics, approximating a first-order reaction rate and substantially enhancing the efficiency. The SCOPE signals exhibited exponential growth, reaching a plateau within 30 min.

A key feature of the SCOPE assay is its ability to suppress off-target RNA amplification. The amplification process requires the Cas13a/crRNA complex to first recognize and bind to the target RNA, activating Cas13a to cleave the RNA segment in the signal template and initiate RNA polymerase activity. If the RNA segment remains intact, polymerase activity is inhibited, likely because the intact loop configuration of the signal template physically obstructs the polymerase from accessing the promoter region and initiating transcription. To enhance the performance of the SCOPE assay, researchers have refined the signal template design and optimized the reaction conditions for maximum signal intensity (Figure 3A). The validation experiments (Figure 3B) demonstrated that the optimal analytical signal was achieved only when all crucial assay components—target RNA, Cas13a, crRNA, and T7 RNA polymerase—were present (Song *et al.,* 2024). Excluding RNA polymerase from the reaction significantly reduced the signal intensity by halting additional RNA target generation. These findings highlight the robustness and efficiency of the SCOPE assay and confirm its reliability for RNA detection and amplification. The ability of this assay to combine rapid signal generation with high specificity makes it a valuable tool for various molecular diagnostic applications.

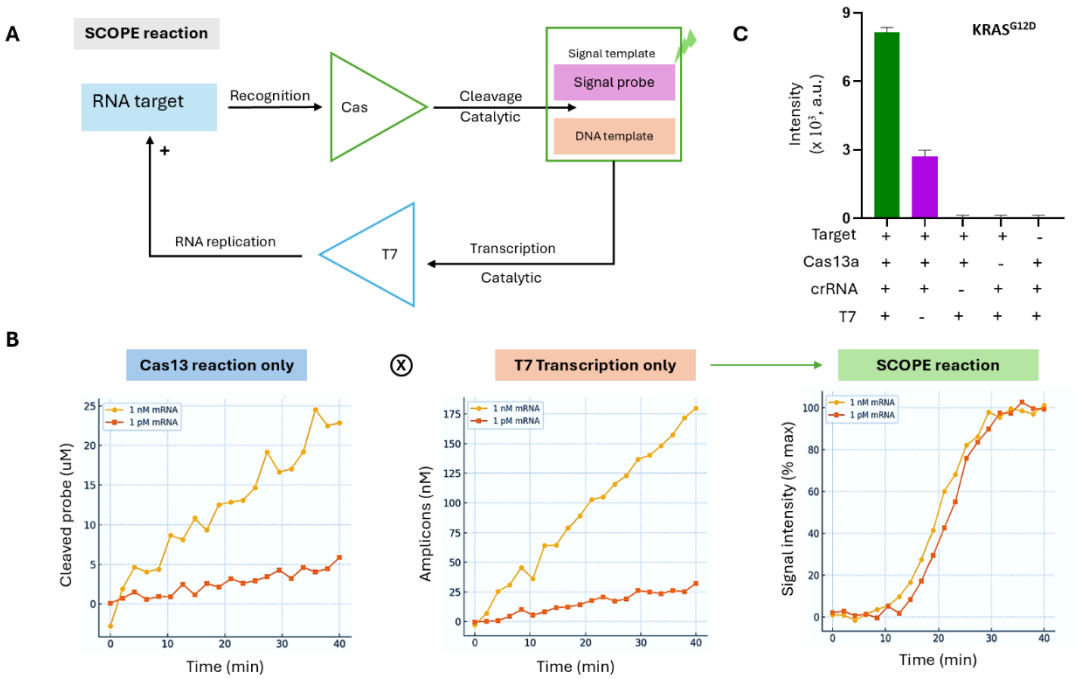

Figure 3: SCOPE assay dynamics. (A) The SCOPE method integrates two enzymatic processes: Cas13a/crRNA produces fluorescence by breaking down RNA in the signal template, whereas T7 polymerase multiplies RNA targets. (B) When operating independently, Cas13a/crRNA and T7 reactions displayed linear product increases over time. However, when combined with SCOPE, they result in exponential signal enhancement, achieving a plateau within 30 min. Experimental confirmation demonstrated a peak signal when all components were present; eliminating T7 polymerase or impeding mRNA recognition diminished signal strength. (C) The starting KRASG12D RNA concentration was 1 nm.

Conclusion and Future Prospects

Self-amplified and CRISPR-aided Operation to Profile EVs (SCOPE) technology represents a significant advancement in liquid biopsy applications and offers exceptional sensitivity, specificity, and accessibility. Its capacity to detect genetic mutations at subattomolar concentrations and differentiate single-nucleotide variations surpasses conventional diagnostic tools. By facilitating the analysis of EV mRNA, SCOPE addresses critical challenges in early cancer detection and monitoring, particularly in malignancies such as liver cancer, where existing diagnostic approaches often fail to identify the disease at an early stage. Furthermore, the versatility of this technology extends to monitoring treatment responses in real time, assessing minimal residual disease, and tumor subtyping. With its low cost (less than $4 per marker), rapid assay times (approximately 30 minutes), and compatibility with standard laboratory equipment, SCOPE has the potential to revolutionize clinical and research practices by rendering liquid biopsy both accessible and practical for routine use (Daane *et al.,* 2022).

While SCOPE shows significant promise, several obstacles must be overcome to fully harness its potential. One major issue is the unintended isolation of other extracellular RNA carriers, including lipoproteins and EVs, from platelets during the sample preparation process using size exclusion chromatography (SEC). These unwanted components can increase background noise, make data analysis more complex, and potentially decrease the diagnostic precision. To tackle this problem, advanced techniques, such as single-vesicle imaging, can be employed. This method involves labelling and monitoring specific proteins on vesicle surfaces, which can greatly improve the accuracy of target identification and enhance the reliability of SCOPE diagnostic results.

Future research should focus on validating the SCOPE across a broader spectrum of cancers and diverse treatment settings. Integrating the analysis of EV mRNA with circulating tumor DNA (ctDNA) could provide a more comprehensive molecular tumor profile by capturing both transcript-level changes and unique genomic alterations, such as promoter mutations and methylation patterns (Everaert, 2020). This dual approach would bridge the gap between transcriptomics and genomics, ensuring improved diagnostic precision and enabling a deeper understanding of tumor biology. Furthermore, optimizing SCOPE for multimodal treatment strategies and establishing cancer-specific timelines for EV analysis after surgery or therapy initiation will enhance its clinical relevance. For instance, EV mutational loads observed in patients with colorectal cancer (CRC) demonstrate fluctuations after surgery and during chemotherapy, highlighting the importance of defining optimal timeframes for sample collection to refine prognostic predictions and guide adjuvant therapy decisions (Daane *et al.,* 2022).

Self-amplified and CRISPR-aided Operation to Profile EVs (SCOPE) exhibits considerable promise in preclinical drug development, offering swift and accurate insights into tumor biology that can accelerate the assessment of therapeutic responses. Its capacity to identify point mutations with minimal interference, even at low variant allele frequencies (such as 0.01%), outperforms many advanced techniques including digital PCR and BEAMing PCR, making it an invaluable tool for drug testing. These attributes can streamline the drug discovery processes, enhance experimental therapies, and promote clinical translation.

The revolutionary aspect of SCOPE lies in its ability to provide same-day results and enable real-time clinical decision-making. This rapid turnaround, combined with its high sensitivity and cost-efficiency, makes it particularly well-suited for applications such as early cancer detection, monitoring recurrence, and personalizing treatment. When integrated with complementary methods, such as the analysis of EVs mRNA and ctDNA, SCOPE offers a comprehensive molecular profile of tumors, ensuring more precise diagnoses and customized treatment plans.

Addressing current limitations, such as improving EVs specificity, expanding the range of cancers analyzed, and exploring its utility in multimodal treatment contexts, will be critical to realizing SCOPE's full potential of SCOPE. For instance, in cases such as EGFRvIII deletion, in which designing a specific ctDNA assay is challenging, the detection of EV mRNA through SCOPE provides a more feasible and effective alternative. Additionally, incorporating new imaging techniques to study the origin of EVs-associated mRNA will further enhance its utility as a diagnostic tool.

Building on these advancements, SCOPE is well-positioned to redefine cancer diagnostics and treatment monitoring. Its ability to provide a reliable, accessible, and comprehensive molecular analysis platform will undoubtedly enhance precision oncology, improve patient outcomes, and establish a new standard for liquid biopsy in both academic and clinical settings.

Data and code accessibility

The authors confirm that the data presented in the article and additional data can be provided by the corresponding author upon request.

Author contributions

A.H., and M.A., have gathered data, outlined and finalized the initial manuscript draft. M.K., G.M., Z.U.A., A.H., M.Q., Y.R., and M.B., helped to analyze data. W.S., provided revisions and finalized the manuscript. All authors have reviewed and approved the final manuscript.

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